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Meta-analysis of immune epitope data for all *Plasmodia*: overview and applications for malarial immunobiology and vaccine-related issues

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Summary

We present a comprehensive meta-analysis of more than 500 references, describing nearly 5000 unique B cell and T cell epitopes derived from the Plasmodium genus, and detailing thousands of immunological assays. This is the first inventory of epitope data related to malaria-specific immunology, plasmodial pathogenesis, and vaccine performance. The survey included host and pathogen species distribution of epitopes, the number of antibody vs. $CD4^+$ and $CD8^+$ T cell epitopes, the genomic distribution of recognized epitopes, variance among epitopes from different parasite strains, and the characterization of protective epitopes and of epitopes associated with parasite evasion of the host immune response. The results identify knowledge gaps and areas for further investigation. This information has relevance to issues, such as the identification of epitopes and antigens associated with protective immunity, the design and development of candidate malaria vaccines, and characterization of immune response to strain polymorphisms.

Keywords

malaria; epitope; *Plasmodium*; vaccine

Malaria, the Immune Epitope Database (IEDB) and Meta-Analysis

Human malaria is a mosquito-borne disease caused by protozoa of the *Plasmodium* species (*Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*), of these, *P. falciparum* is responsible for the most significant morbidity and mortality in humans. Each year, this parasite is responsible for more than 200 million infections, which result in nearly 1 million deaths globally (1). Those most vulnerable for severe and complicated malaria and death are children under the age of five, pregnant women (primigravidae) and immunocompromised individuals, such as those with HIV/AIDS (2,3).

Immunity to malaria is slow to develop and often incomplete (4,5). While anti-disease immunity does exist in endemic areas as a result of repeated infections, memory and protection appear to be short-lived in the absence of continuous parasite exposure. The increase in drug and insecticide resistant strains of *P. falciparum* renders standard anti-

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malaria drugs increasingly ineffective against *falciparum* malaria in disparate geographical regions (6,7). This phenomenon further heightens the sense of urgency for the development of a malaria vaccine, and also emphasizes that parasite variation (mutants within a strain) should be considered in the design of malaria vaccine candidates.

The *Plasmodium* parasite has a large genome encoding approximately 5300 proteins (8) and a complex multi-stage life cycle. The complexity of the plasmodial life cycle presents both challenges and opportunities for vaccine design. On one hand, this complexity presents many potential target antigens to incorporate into different prophylactic or therapeutic modalities. Indeed, candidate vaccines have targeted all life cycle stages (sporozoite, liver, blood and sexual stage), a number of which are currently in clinical trials (9–11). However, with the exception of RTS,S (12), candidate vaccines to date have been not completely efficacious (13) and the recent field trials of promising recombinant protein vaccines (11,14,15) suggest that current understanding and vaccine development strategies may be suboptimal. It is also thought that different immune mechanisms target different stages of the parasite life cycle (16–19), adding an additional challenge to vaccine development.

Malaria vaccine development may therefore benefit from a combined approach to assessing malarial immunobiology. Both traditional and bioinformatic approaches can enhance our current vaccine efforts and understanding of pathogenesis. Indeed, several bioinformatic resources incorporate information related to malaria and *Plasmodium* species (20). Among these, the Immune Epitope Database and Analysis Resource (IEDB) provides scientists with a comprehensive repository of immune epitope data and associated analysis tools <www.immuneepitope.org>. The data in the IEDB, which is captured from the peer-reviewed literature (PubMed), is updated quarterly. The database contains antibody and T cell data from human, non-human primate and rodent hosts, and targets epitopes derived from a broad range of organisms, including infectious (bacteria, viruses, fungi, parasites), as well as non-infectious (allergy, autoimmunity, transplant/alloantigen) agents. The database has been designed to capture the immunological and experimental details associated with each epitope.

To enhance the utility of the IEDB for scientific community, we have initiated meta-analyses of all epitope data related to pathogens of interest. To date, the IEDB has completed meta-analyses for several high-profile pathogens – influenza A, Mycobacterium (TB and related species) and Anthrax/Botulinum toxins (21–23). These analyses provide a comprehensive inventory of pathogen-specific epitope data, while at the same time, identify knowledge gaps and highlight potential areas for further research. We report here a comprehensive analysis of all malaria immune epitope data as of 31 March 2008. Our literature queries were designed to retrieve all relevant data from the published literature, including all B cell and T cell data, covering all host systems for the pathogen of interest. However, occasionally our queries do miss papers. We encourage the scientific community to help us correct any oversight, by the contacting us through ‘Support’/‘Provide Feedback’ function of the IEDB webpage.

The Nature of *Plasmodium* Epitopes

A total of 4497 epitopes (unique molecular structures) were retrieved by our search of epitope data related to the *Plasmodium* genus. Here, an epitope is defined as any structure (peptidic or non-peptidic) interacting with a B cell or T cell receptor, and the term unique molecular structure reference to the total number of non-redundant structures. This total includes 1566 structures associated with positive data (referred hereafter as ‘epitopes’) and 2337 molecular structures only associated with negative data (immunologically un-reactive). The curation of negative data is relevant, as it provides for the identification of non-epitopic

(non-immunogenic/antigenic) regions. Overall, the large number of *Plasmodium* epitopes described in the literature and curated in the IEDB, reflects the intense immunological investigation of the field decades, starting mostly in the late-1980s and early 1990s.

To date, all reported plasmodial epitopes are peptidic in nature. While some epitopes have been derived from well-known lipoproteins, the defined epitope itself is strictly peptidic. Surprisingly, no carbohydrate epitopes have been reported to date. While genomic analysis suggests that *Plasmodium* species generally lack certain machinery necessary for complex post-translation glycosylation (N- and O-linked) (24), other studies have confirmed the existence of protein glycosylation, albeit at low levels (25). Thus the lack of carbohydrate epitopes is likely a reflection of the difficulty in identifying epitopes of complex biochemical nature, and not necessarily a reflection of lack of a functional role in parasite pathogenesis. Therefore, the role of these moieties has yet to be fully elucidated. To date, the only well-documented plasmodial carbohydrate modification pathway is that of the glycosyl-phosphatidylinositol (GPI) anchors. These modifications are essential for parasite survival and affect such functionally important proteins as CSP, MSP-1, MSP-2, p71 and the 55 kDa merozoite rhoptry antigens (25). Determinants derived from these molecules maybe of interest for future epitope analysis. A vaccine targeting the malaria glycosylphosphatidylinositol toxin has indeed been proposed to prevent severe malaria (26).

Phenotype of Defined T Cell Epitopes

A total of 892 T cell epitopes have been reported for all species within the *Plasmodium* genus. The $CD4^+$ vs. $CD8^+$ phenotype of most of these (more than 500) was undefined by the authors. However, the effector cell phenotype can in many cases be inferred from the assay used. Accordingly we enumerated $CD4^+$/class II vs. $CD8^+$/class I epitopes as 686 and 157, respectively. The remaining T cell epitopes remained as unassigned. Thus, reported $CD4^+$/class II epitopes out-numbered $CD8^+$/class I epitopes by a ratio of more than 4 to 1. While it is possible to conclude that this apparent disparity may have biologic significance (27), it is more likely that the higher number of $CD4^+$ T cell epitopes may simply reflect the technical ease of certain assays (for example, lymphoproliferation vs. cytotoxicity), or reflect the focus of the scientific investigations performed to date. It is our assessment that the over abundance of $CD4^+$/class II vs. $CD8^+$/class I data is a result of experimental bias, and that this tells us that more work defining $CD8^+$ epitopes is warranted. Indeed, evidence from the literature suggests that both $CD4^+$ and $CD8^+$ T cells contribute to malaria immunity, and $CD4^+$ and $CD8^+$ T cell epitopes often map to similar regions and in many cases overlap (28–30).

MHC Restriction of Epitopes

Epitope-specific T cell responses were further characterized in terms of their MHC class restriction (Table S1 in Supporting Information). However, the vast majority of studies focused on only a few haplotypes. In humans, both class I (HLA-A, B) and class II (HLA-DR, DP, DQ) data were present, including certain MHC alleles (HLA-B35, DRB1*1302 and DQB1*1501) that have been associated with protection from severe disease in certain human populations (31,32). However, the majority of defined restrictions were mediated by HLA-A2 or other frequent alleles. This distribution mirrors a general bias in definition of HLA epitopes present in all epitope-related literature. These results suggest that epitopes recognized in the context of a more diverse set of HLA molecules need to be defined, especially for those alleles expressed by populations in malaria-endemic regions. This will be required to support evaluation of vaccines in field studies targeting populations of diverse ethnicities. In mice there a slightly greater array of MHC was tested; however, most responses were either restricted by the $H\text{-}2^d$-restricted alleles for class I and $H\text{-}2^d$ or $H\text{-}2^b$-

restricted alleles for class II. This bias is reflective of the MHC alleles expressed in the murine strains most frequently utilized in experimental studies.

The Nature of B Cell Epitopes

While it is generally considered that antibody responses against conformational epitopes on *Plasmodium* antigens are important in anti-malaria immunity, the vast majority of epitopes defined in the literature are actually linear. Of the 896 antibody epitopes captured within the IEDB, only 20 are conformational in nature. This is likely a reflection of the difficulty in defining these epitope types for complex pathogens. Indeed, a large fraction of conformational epitopes defined to date are derived from simpler pathogens, such as viruses, using escape mutants selected by specific antibodies, and this approach is not readily feasible in the case of *Plasmodium*. It is also possible that the nature of the parasite's genome may contribute to the difficulty in defining non-linear epitopes. Indeed, approximately 40% of the plasmodial genome is enriched in intrinsically unstructured proteins, which are either entirely disordered or disordered in large segments (33). We believe that defining *Plasmodium* derived discontinuous epitopes will by an important area for future investigation, which will also benefit from high throughput definition of antibody antigen 3D structures, data which to date represents only a tint fraction of the B cell epitope data.

Another important point to consider is that the vast majority of B cell epitope identification comes from the analysis of linear, overlapping peptides using the ELISA technique. This aspect of B cell epitope identification is universal, and not unique to *Plasmodium* species; indeed, we have found this phenomenon in all meta-analyses performed to date. While efficient and standardized, ELISAs cannot discriminate between low and high affinity binding, and cannot therefore, fully characterize the structural relationship of overlapping peptides, preventing the identification of residues potentially involved in larger conformational determinants. Therefore the B cell epitope data from the literature reflects a bias towards linear epitopes. The IEDB can, and does capture NMR and X-ray structures when these data are reported. However, these data are seldom reported.

The isotype of antibodies recognizing the various epitopes has also been captured in the IEDB, whenever this information was available in the published reports. This issue is relevant in the case of blood stage immunity, since IgG1 and IgG3 in humans (34), as well as IgG2a and IgG2b in mice (35) have been associated with protective immunity. Not surprisingly, we found that the majority of epitope reactivity was defined for total IgG (364), however, IgG1(75), IgG2 (3), IgG2a (6), IgG2b (18), IgG3 (12), IgG4 (1), and IgM (50) isotypes were also described. It was somewhat surprising, though, that IgE epitopes were not reported, given the putative role of this isotype in the control of parasitic infections. A role for this isotype has been implicated in malarial infection (36–38).

Interestingly, we also found that more than 200 plasmodial epitopes (*c.* 14%) are recognized by both B and T cells. And indeed, a survey of the literature shows that certain antigenic determinants have been reported as being recognized by both arms of the immune system in the course of plasmodial infection (39–45), including the tandem repeat regions of certain surface proteins (NANP of CSP and EENV of RESA). However, it is also true that many epitopes are identified as 15–20-mer peptides, and that these same peptides are often found to be reactive in both ELISA and proliferation assays (two different epitopes nested within the same peptide). Moreover, many of the well-known plasmodial antigens were first identified by the characterization of humoral immunity; these antigens were then subsequently studied for the identification of T cell epitopes. Thus the phenomenon is likely to result from a combination of biological and experimental factors.

Plasmodium Species and Strain Distribution

Epitope data has been captured from a total of 12 different species within the *Plasmodium* genus. This includes data from *Plasmodium* species that represent three of the four known human pathogens (*P. falciparum*; Pf, *P. vivax*; Pv and *P. malariae*, those used in rodent models (*P. berghei, P. yoelii* and *P. chabaudi*), as well as those specific to non-human primate models (*P. cynomolgi, P. simiovale, P. knowlesi, P. simium, P. brasilianum, P. fragile* and *P. reichenowi*). Table 1 enumerates epitope distribution among all reported *Plasmodium* species and strains (a separation according to effector phenotype is also provided). The vast majority of epitopes were described for *P. falciparum* (1373 epitopes records representing some 15 different strains), followed distantly by *P. vivax* (152 records representing the 2 major strains). For non-human primates 14 epitopes are derived from *P. knowlesi* and 8 epitopes derived from the six remaining species for which epitope data was reported. For rodent species, the greatest number (89) of epitopes is derived from *P. yoelii*; followed by *P. berghei* (53) and then *P. chabaudi* (44). No epitopes have been reported to date for *P. ovale*, which has been identified as a fourth species involved in human malaria, and *P. vinckei*, which is common rodent malaria parasite. Overall, the relative ratio of defined epitopes (nearly 10 : 1 for Pv compared to Pv) reflects, as expected the priority in which the different *Plasmodium* species have been targeted by immunological investigations.

Genomic Distribution of Defined Epitopes

To date, epitopes have been reported from antigens expressed in one or more of all major life cycle stages of the plasmodial parasite (150 total unique proteins). Table 2a shows the distribution of antigens associated with each life stage of *P. falciparum*: the pre-erythrocytic (sporozoite/liver), erythrocytic/asexual (blood) and transmission/sexual (mosquito) stage. Also shown in this table is the number of epitopes reported for each antigen. Not surprisingly, the majority of reported epitopes come from surface antigens expressed during the pre-erythrocytic and erythrocytic stages. Thus far, the majority of epitopes have been defined from circumsporozoite protein (CSP), liver stage antigen 1 (LSA-1), merozoite surface proteins (MSP-1 and MSP-2), sporozoite surface protein 2 (SSP2/TRAP), ring-infected erythrocyte surface antigen (RESA), rhoptry associated protein 1 (RAP-1), apical membrane antigen (AMA-1) and the erythrocyte binding antigen (EBA-175). Epitopes from proteins expressed during the sexual stage have also been reported: ookinete surface protein (P25), gametocyte-specific surface protein (Pfs230), antigen Pfg27/25, 11-1 polyprotein, chitinase, multidrug resistance protein (MRP), sexual stage and sporozoite surface antigen and antigen QF122. Table 2b shows results for *P. vivax*; a significantly smaller distribution of epitopes and antigens currently exist for this species which reflects the fact that most research efforts to date have focused on *P. falciparum* rather than *P. vivax*.

It is important to note that the prominence of a given antigen in the epitope data can be attributed to historical and/or experimental factors rather than a true reflection of immunodominance. For example, a large number of epitopes have been defined for CSP and MSP-1, but this does not mean that these are necessarily the only or the most immunodominant proteins. Recent reports in the literature have supported an immunodominant and protective role of CSP (46). However, CSP was the first of the malarial proteins to be cloned, and as a result, significant efforts have been devoted to analysis and reagent development specific for this protein. This point is particularly relevant in the context of past difficulties in generating recombinant *Plasmodium* antigens for a broader characterization of reactivity of malarial proteins (47–49) (Dr Lee, personal communication).

Genome-wide searches for correlates of immunity for vaccine development and sequence comparisons for identifying diagnostic candidates may be of significant interest to those in the malaria community. However, thus far epitopes have been described for only a small fraction of the more than 5000 predicted open reading frames (ORFs) for *Plasmodium* species, and many investigators are therefore cautious due to the low yield of actionable data from genomic analyses. Controversy currently exists in the malaria community as to the relative value of expending resource and time on the identification of new antigens using this data vs. focusing on the optimization of delivery systems for existing promising candidates (i.e. CSP, MSP-1, and AMA-1). Nevertheless, because it is likely that protective immunity results from the summation of immune responses against multiple antigens/epitopes, genomic data will no doubt play a critical future role in understanding pathogenesis and immunobiology of malaria.

The data reported in the IEDB and analysed herein was also interpreted in a broader context, taking advantage of other database applications, synergistic in scope with the IEDB. To this end, we can coalesce genomic, proteomic and gene expression data (transcriptome), data available in the *Plasmodium* Genome Resource (PlasmoDB) (50) with the output from our epitope analysis (data not shown). All reported epitopes can thus be aligned to specific expression time points (life cycle stages) and assigned function to the derivative antigens (both at the gene and protein level). Here we can assign both terms associated with gene function (so-called GO terms), as well as terms relevant for identifying roles in pathogenesis. Currently, PlasmoDB houses the complete genome of *P. falciparum* 3D7, including complete gene expression data. In this way, individual epitopes can be correlated with genes as they are expressed during the process of infection and potentially extrapolate for related species/strains. Ultimately, it is our goal to provide links between the IEDB and PlasmoDB. In the future it may be possible to chart the number of new epitopes discovered per year and determine whether the rate of epitope discovery has increased with the volume of genomic data.

Epitope Reactivity Associated With Different Parasite Life Cycle Stages

We evaluated the relationship between epitope reactivity and life cycle stage. For this, we considered all records in which the immunogen, was the *epitope* and the antigen used to test reactivity was the *whole organism* of a specified stage (i.e. sporozoite, schizont, or merozoite) (Table 3a). We also considered records in which the immunogen was the whole organism of a specified stage and the antigen was the epitope (Table 3b). This sort of analysis has implications for identifying epitopes and/or antigens for use in vaccines, as well as diagnostics.

A total of 35 epitopes (*c.* 70% B cell and 30% T cell) were found to generate reactivity specific to sporozoites, 21 to schizonts (all B cell), 18 to merozoites (all B cell), 2 to gametocytes, 2 to schizonts and trophozoites (all B cell), 1 to schizonts and gametocytes (all B cell) and 1 to schizonts and merozoites (all B cell). This includes all *Plasmodium* species (Table 3a). Some epitopes (shown in **bold**) were found to generate responses to more than one life cycle stage. As expected, no common epitope reactivity was observed when the whole organism was used as immunogen (Table 3b) for any host species, consistent with the known genetic restriction of protective immunity against malaria. This data may help identify potential targets for incorporation into candidate vaccines or for evaluation of the immunogenicity of candidate whole organism vaccines. Currently, only one epitope (CSP NANP) has been reported that, when used to immunize humans, generated immunity against the whole organism (sporozoites). However, more than 700 different epitopes are recognized by humans following natural infection/exposure to plasmodia (data not shown). These

epitopes are derived from more than 40 different sporozoite, liver and/or blood stage (asexual and sexual forms) antigens.

Host Distribution of The Epitope Reactivities

Epitopes were most frequently defined using human hosts (940), followed by murine hosts (689). Smaller numbers of epitopes were defined in non-human primates, as well as in rabbits, rats, guinea pigs and goats (Table 4; a separation according to effector phenotype is also provided). While the majority of epitope identification in humans focused on T cell reactivity, the reverse was true in mice.

For human epitopes we found a broad distribution of populations from which the data was derived, consistent with the universal nature of the problem of malaria (Table 5; a separation according to effector phenotype is also provided). To date, epitope data has been reported from many countries in which malaria has been defined as problematic including Africa, Central and South America, Asia, as well as N. America and Europe (where many experimental vaccine studies are conducted); from areas of high, medium and low malaria endemicity (hyper-endemic, holoendemic, mesoendemic, etc.) and from subjects of all age groups lsqb;adults, children, infants and neonatesrsqb;. The greatest number of epitopes has been reported from populations within the African continent (633), followed distantly by Indonesia (150), and North America (135), South America (101), Australia (57), Europe (41), Asia (30) and Central America (3) reflecting the relative focus of research efforts. In only a few instances was the geographical assignment of epitopes more generally ascribed to ‘non-endemic region’ or ‘endemic region’ (16).

As shown in Table 4, in non-human primate models, epitopes were most frequently defined in *Aotus* monkeys (mostly B cell epitopes in this species) (60), followed by chimpanzees (18), squirrel monkeys (12) and rhesus macaques (7), reflecting that *Aotus* monkeys are considered good models of *P. falciparum* and *P. vivax* blood stage immunity/vaccination (51) and rhesus macaques are considered good models for T cell-mediated immunity. Increased availability of epitope data for NHP species would facilitate the evaluation of candidate vaccines in these models and provide greater understanding of malarial and immunobiology. Only a few epitopes were defined in other NHP species (*M. fascicularis*, Saki monkeys, Howler monkeys and Red-handed tamarins).

Prominent among rodent models of malaria were those utilizing standard inbred mouse strains, such as BALB/c (365) and C57BL (211) (> 80% of total; data not shown), which are regarded as established models of malaria infection/immunity (*P. chabaudi* and *P. yoelii*) or cerebral malaria (*P. berghei* ANKA) (52–54). Outbred mice were more rarely used to define malarial epitopes. Epitopes defined in BALB/c and C57BL mice include those from *P. falciparum*, *P. vivax*, *P. berghei*, *P. yoelii*, and *P. chabaudi*. Epitopes have also been defined in rats (*P. falciparum* and *P. berghei*), and New Zealand White rabbits (*P. falciparum*, *P. berghei*, *P. yoelii* and *P. knowlesi*).

Epitope Data Associated With Different Clinical Stages of Malarial Disease

Disease severity and outcome are influenced by a combination of host and parasite-specific factors. Severe clinical disease is more frequently seen in children, the elderly and in those whose immune system has been compromised either by co-infection, pregnancy or malnutrition. Similarly, length of exposure/endemicity of the affected population is associated with disease susceptibility or resistance. To this end, fields within the IEDB have been specifically designed for the capture of patient histories, including age, gender, MHC type, ethnicity/geographical location, disease state/stage, the manner in which the immunogen was encountered (natural exposure/immunization), timing of sample collection

relative to episode and parasitaemia level (if provided). This information can then be used to probe for different patterns of epitope recognition between individuals with active disease and those who recovered uneventfully from infection.

Table 6a shows the distribution of reported epitope reactivities according to five defined malarial disease states (see Table S2 in Supporting Information for definitions): healthy-exposed individuals from endemic areas [exposed, no symptoms at time of sampling] (474), uncomplicated *falciparum* malaria [423], subjects from endemic areas with no clinical histories [exposure-unknown] (192; Pf and Pv combined), uncomplicated *vivax* malaria (77), healthy-exposed individuals from non-endemic areas [experimentally immunized volunteers/travellers] (34) and severe/complicated *falciparum* malaria (5). When possible, disease states were further categorized by the stage of disease: acute (parasitaemia; symptomatic at sampling), post (past malaria; clinically immune; convalescent), or unknown (parasitaemic, but no clinical signs/asymptomatic; includes infants (chord blood) of mothers with placental malaria). Table 6b lists all epitopes distinctly identifiable by disease *stage* (acute or post). Here, only epitopes that were empirically tested for reactivity in both stages are presented.

The distribution of epitopes by age of disease shows, not surprisingly, that the vast majority of epitopes were defined for uncomplicated *falciparum* malaria in adults (273), followed by children and adults combined (120), children (20), pregnant women (13), infants (11) and neonates (4). For uncomplicated *vivax* malaria 60 epitopes were defined in adults and 33 were described in groups containing adults and children. Of the five epitopes defined for severe/complicated *falciparum* malaria, four were defined in adults and one in a child. Given the prevalence of severe disease in children, additional epitope identification would be desirable in this group. Finally, the largest number of epitopes was defined in exposed-healthy (asymptomatic) adults (348), followed by adults and children combined (65) and pregnant women (7) from endemic regions.

Recognition profiles were different in non/low-exposure individuals (children < 10 years and naïve travellers), and these were distinguishable from individuals characterized by high-exposure. This finding may be consistent with the reports indicating that rates of malaria in adults are lower in areas of high endemicity, whereas in areas of low exposure/endemicity, there are higher rates of malarial disease (55,56).

Lacking is a better definition of whether recognition of certain epitopes/antigens is associated with different malarial disease states: severe/complicated malaria, cerebral malaria, pregnancy-associated malaria (PAM)/placental malaria and co-infection (HIV/HBV). Epitopes defined during *Plasmodium* species co-infection is also lacking. Due to the importance of these data for our overall understanding of immunological distinction between mild and severe malaria, further investigation is warranted in this area.

Protective Epitopes and Epitopes Associated With Protective Responses

The IEDB information details natural and experimental exposure/infection and identifies epitopes putatively associated with protective responses. We first searched for protective epitopes, defined as those utilized as isolated molecular structures to immunize and confer protection. Protection was most often defined *in vivo* as reduction in parasitaemia or as sterile protection (pre-erythrocytic stage). According to this definition and available data, no epitopes defined in humans have been associated with protection from infection and/or disease. This is not surprising as human studies utilizing direct immunization with defined epitopes have been limited.

In rodents, however, more than 30 epitopes (B cell and T cell) have been associated with protection following direct immunization with the epitope itself (Tables 7 and 8). These epitopes were mainly derived from *P. berghei* and *P. yoelii* (lethal and non-lethal strains), and come from a narrow range of antigenic proteins (CSP, MSP-1, HEP17, EXP-1 and ribosomal phosphoprotein). Interestingly, one protective epitope was defined for *P. falciparum 3D7* using an outbred mouse strain. Protective epitopes were also defined for Norway brown rats and New Zealand White rabbits. While slightly more B cell epitopes were reported than T cell epitopes, we found that both B cell and T cell epitopes were associated with protection from disease in these models, as expected based on current knowledge of mechanisms of protective immunity against malaria (28–30). The majority of reported protective epitopes were derived from CSP reflecting the fact that this is an extensively studied model protein.

We have also considered two additional classes of epitopes. First, epitopes that are associated with assays linked to protection or neutralization *in vitro*, such as neutralization, growth inhibition assays (GIA) or antibody dependent cellular inhibition (ADCI), inhibition of sporozoite invasion assay (ISI) or inhibition of liver stage development assay (ILSDA) or CTL. As a further category, epitopes associated with protection in the context of multiple epitope specificities directed against a whole malaria antigen or the whole organism (for example irradiated sporozoites) were also considered (Tables 9 and 10). More than 60 B and T cell epitopes that have been identified using humans immunized with sporozoites, and these were derived from CSP, LSA-1, CRA, EXP-1 and TRAP/SSP2. Of those with defined HLA restriction, the majority were DR, followed distantly by A2 and B8.

It is of interest to determine if any epitopes identified as protective in non-human models (*in vivo*) were common to those found to be associated with protective responses (*in vitro* functional assays) in humans, and then ascertain what type of immune responses were measured. Of the protective epitopes identified in non-human models, 10 were common to those identified as being associated with protective responses *in vitro* (Table 9; in bold) determined most frequently *in vitro* using neutralization or inhibition assays (such as, ADCI) for antibody-mediated protection, as well as cytotoxicity and inhibition of growth assays for T cell-mediated protection.

Summary of Key Findings

Herein we present a meta-analysis of epitope data related to the *Plasmodium* genus. We anticipate that this analysis will help establish a broader picture of malaria-related epitope data with relevance for the identification of epitopes and antigens associated with protective immunity, assisting in vaccine design and development, and for the characterization of immune response to *Plasmodium* variant strains. The results of this analysis are also relevant for the identification of knowledge gaps and areas of further investigation in the field.

More than 1500 different epitope structures are described in the literature. About half are T cell epitopes, and CD4 epitopes out-number CD8 epitopes 4 : 1. This may be a reflection of predominance of $CD4^+$ T helper cell activity in both the blood and liver stages. However, more work defining $CD8^+$ epitopes is warranted particularly since $CD8^+$ T cells are primary effectors of pre-erythrocytic (sporozoite/liver) stage immunity. At the level of MHC restriction, we found low HLA diversity. Most defined epitopes are associated with alleles abundant in Caucasians populations, underscoring the need for the identification of epitopes recognized by a more diverse set of HLA molecules. Likewise for B cell epitopes, a striking experimental bias exists in the favour of definition of linear vs. discontinuous epitopes. We believe that defining *Plasmodium*-specific discontinuous epitopes will be an important area

for future investigation, which will also benefit from high throughput definition of antibody antigen 3D structures.

Looking at the *Plasmodium* species distribution for human pathogens, the vast majority of epitopes were described for *P. falciparum*, followed distantly by *P. vivax*, while in rodents as expected *P. yoelii* and *P berghei* constituted the majority of entries. Epitope identification studies may be desirable in several additional *Plasmodium* species, and in those associated with non-human primates in particular to better correlate mechanisms of immunity and pathogenesis between animal models and human disease. In addition, further epitope identification in *P. knowlesi* may be of significant interest, given the recent association of this species with human fatality (57–59).

Despite the fairly broad distribution of epitopes among malarial proteins, we note that only a small fraction of the more than 5000 predicted ORFs for *Plasmodium* species have been associated with defined epitopes. It is likely that these results reflect experimental bias rather that an extreme form of immunodominance in responses, as it is well-known that *Plasmodium* specific responses are broad and multi-specific. Thus, genome-wide searches for correlates of immunity for vaccine development and sequence comparisons for identifying diagnostic candidates may be of significant interest to those in the malaria research community.

Assessing how many epitopes are associated with reactivity to different life cycle stages has implications for identifying target epitopes and/or antigens for use in vaccines. Epitopes have been reported from antigens expressed in all three life cycle stages of the plasmodial parasite (150 total unique proteins), consistent with the long recognized fact that different life stages can be targeted by immune responses. In terms of function of the recognized proteins, the preponderance of reported epitopes was derived from antigens that are expressed at the parasite surface, during the sporozoite stage and/or the asexual erythrocytic stage of the infection. This seems to be true regardless of effector cell (antibody or T cell), and highlights the need for epitope identification in a more diverse set of plasmodial antigens.

The distribution of the hosts from which the epitopes are derived is relevant for malaria vaccine and diagnostic development. Epitopes were most frequently defined using human hosts, followed by murine hosts. In humans to date, epitope data has been reported for populations from most, if not all, countries in which malaria has been defined as problematic and from subjects of all age groups [adults, children, infants and neonates]. However lacking from the human epitope data is a better definition of epitopes associated with different malarial disease states. Further investigation is highly desirable and strongly recommended.

Because fields within the IEDB have been specifically designed for the capture of patient histories, it should be possible in the future to use this information to probe for different patterns of epitope recognition. However, the current data for malaria is not yet comprehensive enough to allow for this analysis, even though interesting trends were noted, and might be confirmed as additional reports are published and included in the IEDB.

While identification of protective epitopes (those that when used as immunogen provide protection against infection/disease) was fairly broad in animal models, no protective epitopes have been defined for humans, However, epitopes recognized in the course of natural infection represent targets for inclusion into epitope-based vaccines. Likewise, analysis of epitopic responses associated with differential activities in *in vitro* assays, taken as surrogates of protection, will be of significant interest.

One of the most promising applications of the IEDB is for human data relating to clinical trials evaluating the immunogenicity, safety and protective efficacy of different antigens and vaccine formulations. In this application, the IEDB could thus assist the process of vaccine development and testing. The data currently available in the epitope literature is somewhat limited and therefore does not reflect the large number of vaccine trials conducted to date. However, in the last few years a resurgence of interest and activity in malaria research has brought together several different scientific groups, basic scientists, clinical investigators, health organizations and funding agencies. It is possible to speculate that in the future, more data would become available relating to the immunogenicity and efficacy of different vaccine candidates. The paradigm, database structure, curation and analysis strategies developed for the purpose of the present analysis could be easily applied to hosting and curation of detailed immunological data that could be queried at any level of granularity.

Ultimately, it will be interesting to repeat this analysis in 3–5 years to evaluate the growth of epitope data for *Plasmodium* species, to assess to what extent knowledge gaps have been addressed, and further, to access how such growth correlates with the growth of genomic data. Moreover, tools available on the IEDB webpage, such as ‘Homology Mapping’ and ‘Epitope Conservancy Analysis,’ could be employed for further analysis to assess such things as the potential impact of variants (polymorphism) on *Plasmodium* immunity and/or to perform cluster analysis to further refine epitope analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Plasmodium species and strain distribution. Immune epitope distribution among *Plasmodium* species and strains is given according to three categories: human pathogens (4), species associated with non-human primate models (6) and species associated with rodent models (3). In each category, plasmodia are listed in order of epitope abundance. For *P. falciparum*, 15 different strains were reported: 3D7, 7G8, FC27/PNG, Palo Alto/Uganda, WELLCOME, CDC/Honduras, FCR-3/Gambia, K1/Thailand, LE5, Mad20/PNG, NF7/Ghana, NF54, T4/Thailand, CAMP/Malaysia and RO-33/Ghana. ***Plasmodium knowlesi* has also been associated with human infection and disease. Epitopes are also further categorized according to the phenotype of the defining effector wherever possible. Note: the total number of epitopes may differ from individual B and T cell reports due to shared B and T cell epitopes.

Human	
P. falciparum	**1373**
CD4	549
CD8	133
B cell	796
P. vivax	**152**
CD4	109
CD8	10
B cell	66
P. malariae	**1**
T cell	0
B cell	1
Non-Human Primate	
*P. knowlesi***	**14**
CD4	6
CD8	0
B cell	9
P. cynomolgi (B cell only)	**1**
P. simiovale (B cell only)	**1**
P. simium (B cell only)	**2**
P. brasilianum (B cell only)	**2**
P. reichenowi	**3**
CD4	2
CD8	1
B cell	0
Rodent	
P. yoelii	**89**
CD4	30
CD8	33
B cell	37
P. berghei	**53**
CD4	32
CD8	9
B cell	18

P. chabaudi	**44**
CD4	40
CD8	0
B cell	5

Bold, the total number of epitopes. For the species without B and T epitopes considered.

Table 2

Epitope mapping by plasmodial life cycle stage. Epitopes have been reported from antigens expressed in all three life cycle stages of the plasmodial parasite (150 total unique proteins): 2a) shows the distribution of antigens associated with each life stage of *P. falciparum*: the pre-erythrocytic (liver), erythrocytic/asexual (blood) and transmission/sexual (mosquito) stage. 2b) shows the distribution of antigens associated with each life stage of *P. vivax*. Epitopes are listed according to their abundance per antigen. **(a)** Epitope mapping by life cycle stage of *P. falciparum*

Protein name	Life cycle stage	Total epitopes
Circumsporozoite protein (CSP)	Liver (Sporozoite)	266
Merozoite surface protein (MSP-1)	Blood (Merozoite)	215
TRAP or sporozoite surface protein 2 (SSP2)	Liver	114
Merozoite surface protein 2 (MSP-2)	Blood (Merozoite)	108
Ring-infected erythrocyte surface antigen (RESA)	Blood (Merozoite)	63
Rhoptry associated protein 1 (RAP-1)	Blood (Merozoite)	55
Liver stage antigen (LSA)	Liver	35
Apical membrane antigen 1 (AMA-1)	Blood (Merozoite)	26
Erythrocyte binding antigen (EBA-175)	Blood	25
Erythrocyte membrane-associated giant protein or Antigen 332 (Ag332)	Blood	22
dnaK-type molecular chaperone	Blood	18
Glutamate-rich protein (GLURP)	Blood	15
Erythrocyte membrane protein 1 (EMP-1)	Blood	14
Serine repeat antigen (SERA)	Blood (Merozoite)	14
Liver stage antigen 3 (LSA-3)	Liver and Blood	13
Clustered-asparagine-rich protein (CARP)	Blood	12
Sexual stage and sporozoite surface antigen	Sexual (Mosquito)	12
Circumsporozoite protein-related antigen precursor (CRA)	Blood	11
Cytoadherence-linked asexual protein (CLAG)	Blood (schizont)	11
Antigen Pfg27/25	Sexual (Mosquito)	11
Acid basic repeat antigen (ABRA) or 101 kDa malaria antigen	Blood (Merozoite)	10
Rhoptry antigen protein (RAP-2)	Blood (Merozoite)	10
Antigen QF122	Sexual	8
Knob-associated histidine-rich protein (KHRP)	Blood	8
Merozoite surface protein 3 (MSP-3)	Blood (Merozoite)	5
Merozoite surface protein 4 (MSP-4)	Blood (Merozoite)	5
Merozoite surface protein 6 (MSP-6)	Blood (Merozoite)	5
Rhoptry antigen protein (RAP)	Blood (Merozoite)	5
11-1 polypeptide	Sexual (Gametocyte)	3
Exported protein 1 (EXP-1)	Liver and Blood	3
Gametocyte-specific surface protein (Pfs230)	Sexual (Mosquito)	2
Cysteine protease	Blood (schizont)	2
Hypothetical protein PFE1325w	Blood	2
Ookinete surface protein (P25)	Sexual (Mosquito)	2
Sporozoite and liver stage antigen (SALSA)	Pre-erythrocytic	2

Protein name	Life cycle stage	Total epitopes
Sporozoite threonine and asparagine-rich (STARP)	Pre-erythrocytic	2
Protective antigen (MAg-1)	Blood	1
Fructose-bisphosphate aldolase	Blood	1
Ribosomal phosphoprotein P0	Blood	1
Chitinase	Sexual (Mosquito)	1
Multidrug resistance protein (MRP)	Sexual (Gametocyte)	1
P-type ATPase	Blood	1
Glucose-regulated protein (GRP78)	Blood	1
Asparagine and aspartate-rich protein (AARP1)	Blood	1
Interspersed repeat antigen or PFE0070w	Blood	1

Table 2(b) Epitope mapping by life cycle stage of *P. vivax*

Protein name	Life cycle stage	Total epitopes
Circumsporozoite protein (CSP)	Liver (Sporozoite)	80
Merozoite surface protein 1 (MSP-1)	Blood (Merozoite)	11
Duffy binding protein (DBP)	Blood	10
Gametocyte antigen 1 (GAM1)	Sexual (Gametocyte)	1
Ookinete surface protein Pvs25	Sexual (Gametocyte)	1

Table 3

Epitope reactivity according to life cycle stage. The relationship between identified epitopes and each of the three plasmodial life cycle stages in terms of demonstrated immune reactivity is presented. (a) All records in which the immunogen, or immunizing agent, was the epitope and the antigen used to test reactivity was the *whole* organism of a specified stage. Epitope sequences shown in bold text are reactive in more than one life cycle stage. (b) All records in which the immunogen was the whole organism of a specified stage and the antigen was the epitope. Data includes epitopes derived from all *Plasmodium* species, the stage of the organism (sporozoite, merozoite, schizont, trophozoite, gamete or combination thereof), the host in which the epitope was defined and the total number of epitopes per stage. **(a)** Epitope reactivity according to life cycle stage: epitope as immunogen

Immunogen Epitope	Reactivity (Antigen) Whole organism	Host	
CSP NANP		Human, Rhesus, Mouse	**25**
CSP NAGG		Mouse	
CSP PPPPNPND		Mouse	
CSP DPPPPNPN		Mouse, Rat	
CSP DRADGQPAG		Mouse	
CSP QGPGAP		Mouse	
CSP SYIPSAEKI		Mouse	
CSP SYVPSAEQI		Mouse	
CSP ANGAGNQPG		Mouse	
CSP GDRAAGQPAGDRAAGQPA		Mouse	
CSP GDRADGQPAGDRAAGQPA		Mouse	
CSP KIYNRNIVNRLLGD		Mouse	
CSP KIYNRNTVNRLLAD	Sporozoites	Mouse	
CSP KQJRDSITEEWS		Mouse	
CSP AKKPAGKGSPSTLQTPG		Rabbit	
CSP PKKPNENKLKQPNE		Rabbit, Mouse	
CSP EWTPCSVTCGVGVRVRSRVNAAN		Mouse	
CSP RRKAHAGNKKAEDLTMDDLE		Rabbit, Mouse	
CSP YNRNIVNRLLGDALNGKPEEK		Mouse	
CSP IEQYLKKIKNSISTEWSPCSVTCGNGIQVRIK		Mouse	
CSP QAQGDGANAGQP/GQPQAQGDGANA		Rabbit	
SSP2 NEPSNPN		Mouse	
LSA-3 LEESQVNDDIFNSLVKSVQQEQQHNV		Chimp	
SSP2 CHPSDGKCN		Mouse	
SSP2 DRYIPYSP		Mouse	
RESA EENVEENV		Rabbit	**15**
RESA **EENVEHDA**		Rabbit, Mouse	
TRAP EWSPCSVTCGKGTRSRKR		Rabbit	
RESA **DDEHVEEPTVADDEHVEEPTVA**		Mouse	
TRAP WSPCSVTCG		Rabbit	
MSP-1 VTHESYQELVKKLEALEDA		Rabbit	

Immunogen Epitope	Reactivity (Antigen) Whole organism	Host	
101 kDa ENDVLNQETEEEMEK		Rabbit	
101 kDa LKNKIFPKKKEDNQAVDT	Merozoites	Rabbit	
101 kDa YKAYVSYKKRKAQEK		Rabbit	
EBA-175 NEREDERTLTKEYEDIVLK		Rabbit	
101 kDa NIISCNKNDKNQ		Rabbit	
101 kDa VPPTQSKKKNKNET		Rabbit	
EBA 175 SNNEYKVNEREDERTLTKEYEDIVLKSHMNRESDDGELYDEN		Rabbit, Mouse	
HSP70 DEIDRMVNDAEKYKAEDEENRKRIEA		Rabbit	
Surface protein GGMPGGMPGGMPG		Rabbit	
SERA **ASQPGSSEPSNPVSSGHSVSTVSVSQTSTSSEKQDTIQ**		Mouse	**22**
MSP-1 DELEAETQNVYAA		Aotus	
CRA DNNLVSGP		Rabbit, Mouse	
EXP-1 DNNLVSGP		Mouse	
MSP-2 DTIASGSQRSTNSAS		Mouse	
RESA **EENVEHDA**		Rabbit	
MSP-1 IEESKKTIDKNKNATKEEEKKKLYQA		Mouse	
MSP-2 KNESKYSNTFINNAY		Mouse	
Ag QF122 KNNNSTNSGI		Mouse	
RAP QGEDKTTDNTYKEMEE		Mouse	
RAP LEEAEGTSNLKKGLEFYKSSLKLDQLDKEKPKKKKSKRKKKRD	Schizonts	Mouse	
MSP-2 NESKYSNTFINNAYNMSIR		Mouse	
MSP-2 NSVGANAPNADTIAS		Mouse	
MSP-1 YSLFQKEKMVL		Aotus, Mouse	
MSP-1 YGLFQKEKMVL		Mouse	
MSP-1 YGLFHKEKMLL		Mouse	
MSP-1 YGLFHKEKMIL		Mouse	
MSP-1 YGGPANKKNAG		Aotus	
MSP-1 AVLTGYSLFQKEKMVLNEGTS		Aotus	
MSP-2 TAADTPTATESISPSPP		Mouse	
MSP-1 THESYQELVKKLEALEDAVLTGYGLFHKEKMIL		Mouse	
MSP-1 THESYQELVKKLEALEDAVLTGYSLFQKEKMVL		Mouse	
Pf230 EGGEGDDVYK	Gametes or gametocytes	Mouse	**2**
Pf230 SKKHTARDGE		Mouse	
SERA **ASQPGSSEPSNPVSSGHSVSTVSVSQTSTSSEKQDTIQ**	Schizonts and merozoites	Mouse	**1**
RESA **DDEHVEEPTVADDEHVEEPTVA**	Schizonts and trophozoites	Mouse	**2**
RESA **EENVEHDA**EENVEHDA		Mouse	
P-type ATPase **LSSSKANSFNSYHT**	Schizonts and gametocytes	Rabbit	**1**

Table (b) Epitope reactivity according to life cycle: epitope as antigen

Whole organism	Epitope	Host	Total
	CSP DPPPPNPNDPPPPNPN	Rabbit	37
	CSP GEKPKEGADKEKKKEKGKEKEEEPK	Rabbit	

Table (b) Epitope reactivity according to life cycle: epitope as antigen

Whole organism	Epitope	Host	Total
	CSP PKKPNENKLKQPNE	Rabbit	
	CSP QAQGDGANAGQPQAQGDGANAGQP	Rabbit	
	CSP RRKAHAGNKKAEDLTMDDLE	Rabbit	
	CSP DGQPAGDRADGQPAGDRA	Mouse	
	CSP DPAPPNANDPAPPNANDPAPPNAN	Mouse	
	CSP DPNANP	Mouse	
	CSP DPPPPNPNDPPPPNPN	Mouse	
	CSP DRAAGQPAG	Mouse	
	CSP DRADGQPAG	Mouse	
	CSP EFVKQISSQLTEEWSQCSVT	Mouse	
	CSP FVKQIRDSITEEWSQ	Mouse	
	SSP2 HLGNVKYLV	Mouse	
	SSP2 LYADSAWENVKNVIGPFMKA	Mouse	
	CSP NAAG	Mouse	
	CSP NANP	Mouse	
	CSP NANPNVDPNANPNANPNANPNANP	Mouse	
Sporozoites	CSP NDDSYIPSAEKI	Mouse	
	CSP NEDSYVPSAEQI	Mouse	
	CSP NNNNGNNNEDSYVPSAEQIL	Mouse	
	CSP NPNANP	Mouse	
	CSP NPNVDP	Mouse	
	CSP PNANPN	Mouse	
	CSP PNVDPN	Mouse	
	CSP QAQGDGANAGQP	Mouse	
	CSP QGPGAP	Mouse	
	CSP QQPP	Mouse	
	CSP RNTVNRLLADAPEGK	Mouse	
	CSP SYIPSAEKI	Mouse	
	CSP SYVPSAEQI	Mouse	
	CSP TEICKMDKCSSIFNIVS	Mouse	
	CSP VRKRKGSNKKAEDLTLRDID	Mouse	
	CSP YNRNIVNRLLGDALNGKPEEK	Mouse	
	CSP EDLTLNDLETDVCTMDKCAG	Aotus	
	CSP RRVNAANKKPEDLTLNDLET	Aotus	
	CSP EYLDKVRATVGTEWTPCSVT	Chimp	
	RAP-2 ELETILNNSPFSEEQTMK	Mouse	11
	Ag QF122 KNNNSTNS	Mouse	
	RAP LEEAEGTSNLKKGLEFYKSSLKLDQLDKEKPKKKKSKRKKKRD	Mouse	
	Ag QF122 NNNSTNSG	Mouse	
	Ag QF122 NNSTNSGI	Mouse	
Merozoites	Ag QF122 NSTNSGIN	Mouse	
	RAP QSKSTSAASTSDELSGSEGP	Mouse	

Table (b) Epitope reactivity according to life cycle: epitope as antigen

Whole organism	Epitope	Host	Total
	RAP SSLKLDQLDKEKPKKKKSKRKKKRDSSSDRILLEESKTFTSENEL	Mouse	
	Ag QF122 STNSGINN	Mouse	
	RAP TDNTYK	Mouse	
	Ag QF122 TNSGINNS	Mouse	
Schizonts	MSP-1 CFRHLDER	Mouse	30
	MSP-1 CPENSGCF	Mouse	
	MSP-1 CVKKQCPE	Mouse	
	MSP-1 DEREECKC	Mouse	
	MSP-1 DMLNISQH	Mouse	
	CRA DNNLVSGP	Mouse	
	MSP-1 ENSGCFRH	Mouse	
	MSP-1 EREECKCL	Mouse	
	MSP-1 FQDMLNIS	Mouse	
	MSP-1 FRHLDERE	Mouse	
	MSP-1 GCFRHLDE	Mouse	
	MSP-1 HLDEREEC	Mouse	
	MSP-1 ISQHQCVK	Mouse	
	MSP-1 KFQDMLNI	Mouse	
	MSP-1 KKQCPENS	Mouse	
	MSP-1 LDEREECK	Mouse	
	MSP-1 LLNYKQEG	Mouse	
	MSP-1 NSGCFRHL	Mouse	
	MSP-1 PENSGCFR	Mouse	
	MSP-1 QCVKKQCP	Mouse	
	MSP-1 QDMLNISQ	Mouse	
	MSP-1 RHLDEREE	Mouse	
	MSP-1 SGCFRHLD	Mouse	
	MSP-1 KSCDPLDL	Aotus	
	MSP-1 LEYYLREK	Aotus	
	MSP-1 LREKNKKV	Aotus	
	MSP-1 PHNVLQNF	Aotus	
	MSP-1 SATHSNSQ	Aotus	
	MSP-1 SFDLYNKY	Aotus	
	MSP-1 YNVEKQRY	Aotus	
	RESA EENVEENVEENVEENV	Mouse	3
Gametes	Protein Ag KDKDKDNTDE	Mouse	
	Ag Pfg27/25 KPLDKFGNIYDYHYEH	Mouse	
	Rhoptry protein AVNDT	Mouse	4
Schizonts and merozoites	MSP-1 NISQHQCVKKQCPENSGCFRHLDEREECKCLLNYKQEGDKCVENPNPT	Mouse	
	MSP-1 NISQHQCVKKQCPQNSGCFRHLDEREECKCLLNYKQEGDKCVENPNPT	Mouse	
	Rhoptry protein NLIESEHSNNNN	Mouse	
	Ag332 VTEEI	Mouse	6

Table (b) Epitope reactivity according to life cycle: epitope as antigen

Whole organism	Epitope	Host	Total
Asexual stage	Hypothetical Protein ESYKNSKDKELLIYLNNGELKKKNS	Rabbit	
	Hypothetical Protein GAQILQTTLCARLLTARCGCVNLTADRMKRSFTLTC	Rabbit	
	Hypothetical Protein GIDKKKKKKNI	Rabbit	
	Hypothetical Protein MNDIQIKTITIIYI	Rabbit	
	Hypothetical Protein YINNNNIPFQQKHNLPFPTDIDFDDHYIYVN	Rabbit	

Table 4

Host species distribution. The distribution of epitopes (all *Plasmodium* species) according to the host species in which they were defined is reported. Epitope data are presented for human hosts, murine hosts, including 22 different inbred, transgenic and out-bred strains, as well as 8 species of non-human primates, rabbits, rats, guinea pigs and goats. Epitopes are also further categorized according to the phenotype of the defining effector wherever possible. Note: the total number of epitopes may differ from individual B and T cell reports due to shared B and T cell epitopes

Human	940
CD4	519
CD8	91
B cell	396
Mouse	689
CD4	251
CD8	61
B cell	469
Rabbit (B cell only)	152
Non-human primate	109
Aotus money	60
CD4	11
CD8	0
B cell	58
Chimpanzee	18
CD4	13
CD8	8
B cell	11
Rhesus monkey	7
CD4	5
CD8	0
B cell	3
Squirrel monkey	12
CD4	1
CD8	0
B cell	11
Macaca fascicularis (B cell only)	1
Saki monkeys (B cell only)	1
Howler monkeys (B cell only)	5
Red-handed tamarin (B cell only)	2
Rat (B cell only)	6
Guinea pig	2
CD4	1
B cell	2
Goat (B cell only)	2

Table 5

Summary of human epitope data by geographic region. Epitope data are reported for populations from most, if not all, countries in which malaria has been defined as problematic. These include: all parts of Africa, Central and South America, numerous parts of Asia, as well as N. America and Europe; and represent areas of high, medium and low malaria endemicity. These data include epitopes from *P. falciparum* (majority) as well as *P. vivax*. A complete list of epitopes according to geographical location is provided in Table S3 (in Supporting Information). The data presented define the general region/continent, the total number of epitopes defined therein and the endemicity of that region as ascribed in the literature. Epitopes are also further categorized according to the phenotype of the defining effector wherever possible. Note: the total number of epitopes may differ from individual B and T cell reports due to shared B and T cell epitopes

Geographic location	Total epitopes	Endemicity
Africa	633	Endemic (Holo)
CD4 (258)		
CD8 (57)		
B cell (303)		
Indonesia	150	Endemic
CD4 (106)		
CD8 (4)		
B cell (49)		
North America	135	Non-endemic
CD4 (52)		
CD8 (58)		
B cell (19)		
South America	101	Endemic
CD4 (50)		
CD8 (5)		
B cell (60)		
Australia	57	Non-endemic
CD4 (54)		
CD8 (0)		
B cell (3)		
Europe	41	Non-endemic
CD4 (32)		
CD8 (7)		
B cell (0)		
Asia	30	Endemic
CD4 (22)		
CD8 (0)		
B cell (25)		
Endemic Region	9	Endemic
CD4 (9)		
CD8 (0)		
B cell (0)		

Geographic location	Total epitopes	Endemicity
Non-Endemic Region	7	Non-endemic
CD4 (6)		
CD8 (0)		
B cell (1)		
Central America	3	Endemic
CD4 (0)		
CD8 (0)		
B cell (3)		

Table 6

Epitope distribution in malaria disease states. Epitope defined in human populations can be further enumerated according to reported disease states. (a) shows the distribution of reported epitope reactivities according to 5 defined malarial disease states (DS): uncomplicated malaria (both *P. falciparum* and *P. vivax*), severe/complicated *falciparum* malaria, healthy-exposed individuals from endemic areas (exposed, no symptoms at time of sampling), healthy-exposed individuals from non-endemic areas (travellers) and subjects from endemic areas with no clinical histories (exposure-unknown). The total number of epitopes is further broken down into T cell vs. B cell reactivities. Note: the total number of epitopes may differ from individual B and T cell reports due to shared B and T cell epitopes. (b) lists all epitopes identified by disease stage: acute (symptomatic at time of sampling) or post (recovered/previous history of malaria). The data presented are strictly defined as those epitopes empirically tested in *both* disease stages and found to have differential reactivities. A complete description of disease states assignments is provided in Table S2 (in Supporting Information). **(a)** Epitope distribution in malaria disease states

Malaria disease states	**Total**
Uncomplicated malaria	
P. falciparum	423
CD4	228
CD8	20
B cell	210
P. vivax	**77**
CD4	45
CD8	5
B cell	43
Complicated/severe *falciparum* malaria	5
CD4	1
CD8	1
B cell	3
Exposed-healthy-endemic region	
P. falciparum	474
CD4	273
CD8	77
B cell	81
P. vivax (CD4 only)	**4**
P. berghei (CD8 only)	**1**
Exposed-non-endemic (sub-category of uncomplicated malaria)	
P. falciparum (CD4 only)	**34**
Exposed-endemic-unknown status	
P. falciparum	**176**
CD4	75
CD8	5
B cell	97
P. vivax	**16**
CD4	9
CD8	0

Malaria disease states	Total
B cell	7

Table 6(b) Epitopes Identified by Stage of Disease

Uncomplicated *falciparum* malaria		
Epitope Sequence	Acute	Post
KKICKMEKCSSVFNVVNSSI	NEG	POS
LEMNYYGKQENWYSLKKNSR	NEG	POS
DELDYENDIEKKICKMEKCS	POS	POS
DNEKLRKPKHKKLKQPGDGN	POS	POS
EENVEENVEENVEENV	POS	POS
EENVEHDAEENVEHDAEENVEENV	POS	POS
ENDIEKKICKMEKCSSVFNV	POS	POS
ERRAKEKLQEQQRDLEQRKADTKK	POS	POS
IKPGSANKPKDELDYENDIE	POS	POS
KPIVQYDNF	POS	POS
MPLETQLAI	POS	POS
NAKNVNDMYRDGEMS	POS	POS
NELNYDNAGTNLYNELEMNY	POS	POS
NNFMNRNMKNKNMNN	POS	POS
PSDKHIEQYLKKIKNSISTE	POS	POS
SLRWIFKHVAKTHLK	POS	POS
VTCGNGIQVRIKPGSANKPK	POS	POS
EENVEENVEENVEENV	POS	POS
LNDITKEYEKLLNEI	POS	POS
NANPNANPNANP	POS	POS
NTSDSQKE	POS	POS
SNTFINNA	POS	POS
Uncomplicated *vivax* malaria		
Epitope Sequence	Acute	Post
AANKKAEDAGGNAGGNAGGG	NEG	POS
AGGGQGQNNEGANAPNEKSV	NEG	POS
AINLNGVNFNNVDASSLGAA	NEG	POS
ANGAGNQPGANGAGGQAA	NEG	POS
ANGAGNQPGEDGAGNQPG	NEG	POS
ASRGRGLDENPDDEEGDAKK	NEG	POS
DLTLNDLETDVCTMDKCAGI	NEG	POS
DRAAGQPAGNGAGGQAAGGN	NEG	POS
DRADGQPAGDRAAGQPAGDR	NEG	POS
DRADGQPAGDRADGQPAGDR	NEG	POS
EDGAGNQPGANGAGNQPG	NEG	POS
FNVVSNSLGLVILLVLALFN	NEG	POS
GAGGQAAGGNAANKKAEDAG	NEG	POS
HCGHNVDLSKAINLNGVNFN	NEG	POS

Table 6(b) Epitopes Identified by Stage of Disease

KEYLDKVRATVGTEWTPCSV	NEG	POS
NPRENKLKQPGDRADGQPAG	NEG	POS
PDDEEGDAKKKKDGKKAEPK	NEG	POS
SLGAAHVGQSASRGRGLDEN	NEG	POS
ANGAGNQPGANGAGNQPG	POS	POS
DRAAGQPAGDRADGQPAGDR	POS	POS
GANAPNEKSVKEYLDKVRAT	POS	POS
KKDGKKAEPKNPRENKLKQP	POS	POS
MKNFILLAVSSILLVDLFPT	POS	POS
NVDASSLGAAHVGQSASRGR	POS	POS
RVNAANKKPEDLTLNDLETD	POS	POS
SILLVDLFPTHCGHNVDLSK	POS	POS
TCGVGVRVRRRVNAANKKPE	POS	POS
VCTMDKCAGIFNVVSNSLGL	POS	POS
VGTEWTPCSVTCGVGVRVRR	POS	POS

Table 7
Protective T cell epitopes defined in Non-human models (*in vivo*)

Tables 7 and 8 Protective T cell epitopes defined in non-human models. Protective epitopes are enumerated for non-human models of malaria infection (all Plasmodia) by epitope name, epitope sequence, source antigen (species/strain) and the host in which they were defined. Here, protective epitopes are defined as those that are utilized as isolated molecular structures to immunize and confer protection. Host species include mice, rats and rabbits. Table 10 represents defined protective T cell epitopes, whereas Table 11 represents defined protective B cell epitopes. Protection from disease *in vivo* was most often determined by reduced parasitaemia, or by reduced parasite counts in the liver.

Epitope name(s)	Epitope sequence	Source protein	Host(s)
T1 (57–70)	KIYNRNTVNRLLAD	CSP (Pb)	BALB/c
		SSP2/TRAP (Pyy17XNL)	A/J
$(NPNEPS)_4$			C57BL/6
PySSP2 (484–501)	NPNEPSNPNEPSNPNEPS		A/J
MSP-1 (1157–1171)	ISVLKSRLLKRKKYI	MSP-1 (Pc)	BALB/c
PyCSP (280–297)	SYVPSAEQILEFVKQISS		
PyCSP (280–295)	SYVPSAEQILEFVKQI		
PyCSP (280–288)	SYVPSAEQI	CSP (Pyy)	BALB/c
PyCSP20	SYVPSAEQILEFVKQISSQL		BALB/cByJ
T	KQJRDSITEEWS	CSP (Pb)	A/J
CS (252–260)		CSP (Pb)	BALB/c
pb9		CSP (Pb ANKA)	
CSP (245–253)	SYIPSAEKI	CSP (Pb ANKA)	
		CSP (Pyy)	BALB/c
PyCSP (57–70)	KIYNRNIVNRLLGD		BALB/cByJ
Cm21	AEFEILTKNLEKYIQIDEKL	MSP-1 (Py YM)	BALB/c
SYVPSAEQI	SYVPSAEQI		
CS epitope	SYVPSAEQI	CSP (Pyy17XNL)	BALB/c
CS (280–289)	SYVPSAEQIL		
PYCTL1	SYVPSAEQILEFVKQI		
		CSP (Py 265 BY)	BALB/c
Py1	YNRNIVNRLLGDALNGKPEEK		C57BL/6
P32 (Pf)	IEQYLKKIKNSISTEWSPCSVTCGNGIQVRIK	CSP (Pb)	C57BL/6

Table 8
Protective B cell epitopes defined in Non-human models (*in vivo*)

Tables 7 and 8 Protective T cell epitopes defined in non-human models. Protective epitopes are enumerated for non-human models of malaria infection (all Plasmodia) by epitope name, epitope sequence, source antigen (species/strain) and the host in which they were defined. Here, protective epitopes are defined as those that are utilized as isolated molecular structures to immunize and confer protection. Host species include mice, rats and rabbits. Table 10 represents defined protective T cell epitopes, whereas Table 11 represents defined protective B cell epitopes. Protection from disease *in vivo* was most often determined by reduced parasitaemia, or by reduced parasite counts in the liver.

Epitope name(s)	Epitope sequence	Source protein	Host(s)
$(PPPPNPND)_2$	PPPPNPNDPPPPNPND	CSP (Pb)	A/J
$(QGPGAP)_3QG$	QGPGAP	CSP (Pyy)	A/J
$(QGPGAP)_2$	QGPGAP	CSP (Py17XNL)	BALB/c
D-16-N	DPAPPNANDPAPPNAN	CSP (Pb)	*Mus musculus*
			BALB/cByJ
			CD1
$(QGPGAP)_4$		CSP (Pyy)	C57BL/6
PyB	QGPGAP		A/J
R3			C57BL/6
Epitope 17.1		CSP (Pb)	A/J
CS	DPPPPNPNDPPPPNPN		A/J
Epitope 17.1	DPPPPNPNDPPPPNPN	CSP (Pb)	Norway Brown rats
NYLS2 (126–140)	SFPMNEESPLGFSPE	HEP17 (Py)	A/J
NYLS3 (136–150)	GFSPEEMEAVASKFR	HEP17 (Py)	A/J
$(EENVEHDA)_4$	EENVEHDA	RESA (Pf)	New Zealand white rabbits
$(DPPPPNPN)_2$	DPPPPNPN	CSP (Pb)	BALB/c
MoAb 8E7/55 epitope	DNNLVSGP	EXP-1 (Pf)	BALB/c
CS (93–108)	PPPPNPNDPPPPNPND	CSP (Pb)	A/J
CS (265–276)	KQIRDSITEEWS	CSP (Pb)	A/J
Pf-PO-P	EEEEEEDGFMGFGMFD	ribosomal phosphoprotein P0 (Pf 3D7)	Swiss
P8	SNTFINNA	MSP-2 (Pf)	BALB/c
N2	PKKPNENKLKQPNE	CSP (Pk H)	New Zealand white rabbits

Table 9

Epitopes associated with protective responses as defined *in vitro.* Epitopes associated with assays linked to protection *in vitro* are listed according to sequence, assay type and host species. Assays that demonstrate correlates of protection *in vitro* broadly include neutralization, inhibition of antibody activity, growth inhibition, and cytotoxicity assays. Assays typically used to define protective responses against plasmodial infection are represented: antibody-dependent cellular inhibition assays (growth inhibition), inhibition of sporozoite invasion assay (neutralization), inhibition of liver stage development assay (inhibition of invasion), as well as chromium release assays (cytotoxicity).

	Sequence	Antigen	Assay type	Host
	ALYTDEDLLFDLEKQK	RESA	Neutralization	Rabbit
	CHPSDGKCN	TRAP/SSP2	Neutralization	Mouse
	DDEHVEEPTVA	RESA	Inhibition of Ab activity	Rabbit
	DDEHVEEPTVADDEHVEEPTVA	RESA	Neutralization	Mouse
	DNNLVSGP	CRA	Neutralization	Rabbit
	DRYIPYSP	TRAP/SSP2	Neutralization	Mouse
	EENVEENV	RESA	Neutralization	Rabbit
	EENVEENVEENV	RESA	Neutralization	Rabbit
	EENVEENVEENVEENV	RESA	Neutralization	Rabbit
	EENVEENVEENVEENVEENVEENVEENV	RESA	Neutralization	Rabbit
	EENVEHDA	RESA	Neutralization	Mouse
			Inhibition of Ab activity	Rabbit
	EENVEHDAEENVEHDA	RESA	Neutralization	Mouse
	EENVEHDAEENVEHDAEENVEHDAEENVEHDA	RESA	Neutralization	Rabbit
	ENDVLNQETEEEMEK	101 kDa antigen	Neutralization	Rabbit
	EWSPCSVTCGKGTRSRKR	TRAP	Inhibition of Ab activity	Rabbit NW
	GFSPEEMEAVASKFR	HEP17	Neutralization	Mouse
B cell	IEQYLKKIKNSISTEWSPCSVTCGNGIQVRIK	CSP	Neutralization	Mouse
	KEEKEEKEEKEEKEKEKE	Non-natural	Neutralization	Rabbit
	LKNKIFPKKKEDNQAVDT	101 kDa antigen	Neutralization	Rabbit
	NAGGNAGGNAGGNAGGNAGG	CSP	Neutralization	Mouse
	NANP	CSP	Neutralization	Mouse, Human
	NEREDERTLTKEYEDIVLK	EBA175	Neutralization	Rabbit
	NIISCNKNDKNQ	101 kDa antigen	Neutralization	Rabbit
	NPNANPNA	CSP	Neutralization	Rabbit
	QAQGDGANAGQPQAQGDGANAGQP	CSP	Neutralization	Rabbit
	QGPGAP	CSP	Neutralization	Mouse
	QGPGAPQGPGAPQGPGAP	CSP	Neutralization	Mouse
	SFPMNEESPLGFSPE	HEP17	Neutralization	Mouse
	SNNEYKVNEREDERTLTKEYEDIVLKSHMNRESDDGELYDEN	EBA175	Neutralization	Rabbit, Mouse
	SVTEEIAEEDKSVIEEAV	Ag332	Neutralization	Rabbit
	VPPTQSKKKNKNET	101 kDa antigen	Neutralization	Rabbit

	Sequence	Antigen	Assay type	Host
	WSPCSVTCG	TRAP	Inhibition of Ab activity	Rabbit NW
	YKAYVSYKKRKAQEK	101kDa antigen	Neutralization	Rabbit
	EVLYLKPLAGVYRSLKKQLE	MSP-1	Neutralization	Mouse
T cell	DSYIPSAEKI	CSP	Cytotoxicity (^{51}Cr)	Mouse
	ERRAKEKLQEQQSDLEQRKADTKK	LSA-1	Cytotoxicity (^{51}Cr)	Chimp
	GANAPNEKSVKEYLDKVRAT	CSP	Cytotoxicity (^{51}Cr)	Mouse
	IEKYLKTIKNSLSTEWSPCS	CSP	Cytotoxicity (^{51}Cr)	Mouse
	KIYNRNIVNRLLGD	CSP	Cytotoxicity (^{51}Cr)	Mouse
			Inhibition of growth	
	KNNNNDDSY	CSP	Cytotoxicity (^{51}Cr)	Mouse
	KPKDELDY	CSP	Cytotoxicity (^{51}Cr)	Human
	KSKDELDY	CSP	Cytotoxicity (^{51}Cr)	Human
	KPKDELDYENDIEKKICKMEKCS	CSP	Cytotoxicity (^{51}Cr)	Mouse
	LACAGLAYK	TRAP	Cytotoxicity (^{51}Cr)	Mouse
	LEESQVNDDIFNSLVKSVQQEQQHNV	LSA-3	Cytotoxicity (^{51}Cr)	Chimp
	LLSNIEEPKENIIDNLLNNI	LSA-3	Cytotoxicity (^{51}Cr)	Chimp
	LSTNLPYGK	TRAP	Cytotoxicity (^{51}Cr)	Mouse
	MKNFILLAVSSILLVDLFPT	CSP	Cytotoxicity (^{51}Cr)	Mouse
	MMRKLAILSV	CSP	Cytotoxicity (^{51}Cr)	Mouse
	NVDASSLGAAHVGQSASRGR	CSP	Cytotoxicity (^{51}Cr)	Mouse
	SAEKKDEKEASEQGEESHKKENSQESA	SALSA	Cytotoxicity (^{51}Cr)	Chimp
	SILLVDLFPTHCGHNVDLSK	CSP	Cytotoxicity (^{51}Cr)	Mouse
	SYIPSAEKI	CSP	Cytotoxicity (^{51}Cr)	Mouse
	SYVPSAEQI	CSP	Cytotoxicity (^{51}Cr)	Mouse
	VCTMDKCAGIFNVVSNSLGL	CSP	Cytotoxicity (^{51}Cr)	Mouse
	VGTEWTPCSVTCGVGVRVRR	CSP	Cytotoxicity (^{51}Cr)	Mouse
	YIPSAEKI	CSP	Cytotoxicity (^{51}Cr)	Mouse
	YNRNIVNRLLGDALNGKPEEK	CSP	Inhibition of growth	Mouse
	YPHFMPTNL	hypothetical protein	Cytotoxicity (^{51}Cr)	Mouse
	YVPSAEQI	CSP	Cytotoxicity (^{51}Cr)	Mouse
	DELFNELLNSVDVNGEVKENILEESQ	LSA-3	Cytotoxicity (^{51}Cr)	Chimp
	NGKDDVKEEKKTNEKKDDGKTDKVQEKVLEKSPK	SALSA	Cytotoxicity (^{51}Cr)	Chimp
	VESVAPSVEESVAPSVEESVAENVEESV	LSA-3	Cytotoxicity (^{51}Cr)	Chimp

Table 10

Reactivity of humans immunized with irradiate sporozoites to epitopes. Reactivities against multiple epitope specificities defined in humans following immunization with whole malarial antigen (irradiated sporozoites) are presented. The data include both B and T cell epitopes, the source antigen (species/strain) and the country of origin of immunized subjects. For those subjects identified as Caucasian, these data come from papers in which the study population was known to be Caucasian, but the country of original was not known

	Antigen	Subjects
B cell epitope		
NANPNANPNANP	CSP (Pf)	Gambia
DPNANPNVDPNANPNV	CSP (PF T4/Thailand)	USA
IKPGSANKPKDELDYENDIE	CSP (PF T4/Thailand)	USA
T cell epitope		
IKEYLNKIQNSLSTEWSPCSWSPCS	CSP (Pf NF54)	USA
PSDKHIKEYLNKIQNSLSTE	CSP (Pf NF54)	USA
EPSDKHIKEY	CSP (Pf 3D7)	USA
MNHLGNVKYLVIVFL	TRAP (Pf)	USA
EVDLYLLMDCSGSIR	TRAP (Pf)	USA
LPYGKTNLTDALLQV	TRAP (Pf)	USA
ALLQVRKHLNDRINR	TRAP (Pf)	USA
APFDETLGEEDKDLD	TRAP (Pf)	USA
LLSTNLPYGKTNLTD	TRAP (Pf)	USA
TLGEEDKDLDEPEQF	TRAP (Pf)	USA
TNLTDALLQVRKHLN	TRAP (Pf)	USA
ENVKNVIGPFMKAVC	TRAP (Pf)	USA
CEEERCLPKREPLDV	TRAP (Pf)	USA
CLPKREPLDVPDEPE	TRAP (Pf)	USA
ALLACAGLAYKFVVP	TRAP (Pf)	USA
NANPNVDPNANPNVDPNANPNVDPNANP	CSP (Pf)	USA
KPKDELDYENDIEKKICKMEKCS	CSP (Pf)	USA
KPKDELDYANDIEKKICKMEKCS	CSP (Pf 3D7)	USA
TPYAGEPAPF	CSP (Pf 3D7)	Caucasian
GLLGNVSTV	CRA (*Plasmodium* sp.)	Caucasian
ILSVSSFLFV	CSP (Pf)	Caucasian
KILSVFFLA	EXP-1 (Pf)	Caucasian
VLAGLLGNV	CRA (*Plasmodium* sp.)	Caucasian
VLLGGVGLVL	EXP-1 (Pf)	Caucasian
FILVNLLIFH	LSA-1 (Pf)	Caucasian
GVSENIFLK	LSA-1 (Pf)	Caucasian
HVLSHNSYEK	LSA-1 (Pf)	Caucasian
LACAGLAYK	SSP2 (Pf 3D7)	Caucasian
LLACAGLAY	SSP2 (Pf 3D7)	Caucasian
LLACAGLAYK	SSP2 (Pf 3D7)	Caucasian
QTNFKSLLR	LSA-1 (Pf)	Caucasian

	Antigen	Subjects
VTCGNGIQVR	CSP (Pf)	Caucasian
MPLETQLAI	Sexual-stage-specific protein (Pf NF54)	Caucasian
AGLLGVVSTVLLGGV	EXP-1 (Pf)	Caucasian
ASKNKEKAL	SSP2 (Pf)	USA
DASKNKEKALIIIKS	SSP2 (Pf)	USA
DPNANPNV	CSP (Pf WELLCOME)	USA
EYLNKIQNSLSTEWSPCSVT	CSP (Pf)	USA
GLAYKFVVPGAATPY	TRAP (Pf)	Caucasian
HNWVNHAVPLAMKLI	TRAP (Pf)	Caucasian
IRLHSDASKNKEKAL	SSP2 (Pf)	USA
KNKEKALI	SSP2 (Pf)	USA
KNKEKALII	SSP2 (Pf)	USA
KSKYKLATSVLAGLL	EXP-1 (Pf)	Caucasian
KYKIAGGIAGGLALL	SSP2 (Pf 3D7)	Caucasian
KYLKKIKNSLSTEWSPCSVT	CSP (Pf)	USA
KYLKKIQNSLSTEWSPCSVT	CSP (Pf)	USA
KYLKRIQNSLSTEWSPCSVT	CSP (Pf)	USA
KYLKTIQNSLSTEWSPCSVT	CSP (Pf)	USA
KYLQKIKNSLSTEWSPCSVT	CSP (Pf)	USA
KYLQKIQNSLSTEWSPCSVT	CSP (Pf)	USA
KYLQKIRNSLSTEWSPCSVT	CSP (Pf)	USA
LVNLLIFHINGKIIKNS	LSA-1 (Pf)	Caucasian
MNYYGKQENWYSLKK	CSP (Pf)	Caucasian
MRKLAILSVSSFLFV	CSP (Pf)	Caucasian
QYLKKIKNSISTEWSPCSVT	CSP (Pf)	USA
QYLKKIQNSLSTEWSPCSVT	CSP (Pf)	USA
RHNWVNHAVPLAMKLI	TRAP (Pf)	Caucasian
SSVFNVVNSSIGLIM	CSP (Pf NF54)	Caucasian
VKNVIGPFMKAVCVE	TRAP (Pf)	Caucasian