Genetic Analysis of Murine Malaria

Susana Gomes Campino

Department of Medical Biosciences
Medical and Clinical Genetics
Umeå University
Umeå, Sweden, 2003

Department of Medical Biosciences
Medical and Clinical Genetics
Umeå University
SE-90187 Umeå
Sweden

New Serial Number 861
ISSN 0346-6612
ISBN 91-7305-528-X

Printed in Sweden by Solfjädern Offset AB, Umeå, 2003

a meus pais e irmã

Table of Contents

Abbreviations

Berr	Berghei resistance
CCR	Chemokine (C-C motif) Receptor
CLIS	Common Laboratory Inbred Strains
CD	Cluster of Differentiation
CM	Cerebral Malaria
cM	centiMorgans
CXCR	Chemokine (C-X-C motif) Receptor
ECM	Experimental Cerebral Malaria
G6PD	Glucose-6-phosphate-dehydrogenase
Hb	Hemoglobin
HLA	Human Leucocyte Antigen
HP	Hyperparasitaemia
ICAM	Intercellular Adhesion Molecule
IFN	Intereron
IL	Interleukine
LOD	Logarithm of the Odds
MHC	Major Histocompatibility Complex
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
OWDIS	Other Wild Derived Inbred Strains
RIS	Recombinant Inbred Strains
RCS	Recombinat Congenic Strains
SCID	Severe Combined Immunodeficience
SNP	Single Nucleotide Polymorphism
QTL	Quantitative Trait Loci
TGF	Transforming Growth Factor
TNF	Tumour Necrosis Factor
Tlr	Toll-Like receptor
Traf	Tumour necrosis factor receptor-associated factor
VDR	Vitamin D Receptor
WDIS	Wild Derived Inbred Strains
WHO	World Health Organization

Abstract

Malaria, an infectious disease caused by *Plasmodium* parasites, is one of the major world-scale health problems. Despite the efforts aimed at finding an effective way to control the disease, the success has been thwarted by the emergence of parasite drug resistance and mosquito resistance to insecticides. The understanding of the natural mechanisms of host defence against the disease could point out novel intervention targets.

This thesis focuses on the genetic analysis of resistance to murine malaria induced by the lethal *Plasmodium berghei* ANKA using a wild-derived-inbred strain (WDIS). The aim of this thesis was to exploit the genetic diversity represented among WDIS for identifying loci contributing to resistance/susceptibility to murine malaria.

The work included a genome-wide polymorphism survey using microsatellite markers performed on 10 WDIS. Comparisons of these strains to laboratory inbred strains confirmed a higher rate of polymorphism among the WDIS. We conclude that these WDIS represent repositories of unique naturally occurring genetic variability that may prove to be invaluable for the study of complex phenotypes. Next, we used the WDIS to search for novel phenotypes related to malaria pathogenesis. Whereas most laboratory strains were susceptible to experimental cerebral malaria (ECM) after infection with *P. berghei* ANKA, several WDIS were found to be resistant. To study the genetic inheritance of resistant/susceptibility to *P. berghei* ANKA infection we analysed backcross and F2 cohorts derived from crossing the WLA wild-derived strain with a laboratory mouse strain (C57BL/6). A novel phenotype represented by the cure of infection, clearance of parasitaemia and establishment of immunological memory was observed in the F2 progeny. The backcross progeny was used to genetically map one locus on chromosome 1 (*Berr*1) and one locus on chromosome 11 (*Berr*2) that mediate control of resistance to ECM induced by *P. berghei* ANKA. Genetic mapping using the F2 progeny showed that a locus on chromosome 1 (*Berr1*) and a locus on chromosome 9 (*Berr3*) were contributing to control survival time after infection with lethal *P. berghei* ANKA. Finally, we identified, a locus on chromosome 4 (*Berr*4) that appears to control time of death due to hyperparasitaemia.

This thesis underlines the value of using WDIS to reveal genetic factors involved in the aetiology of disease phenotypes. The characterisation of the genetic factors represented by the malaria resistance loci identified here are expected to provide a better understanding of the malaria pathology and may suggest new targets for therapeutic intervention.

Publications

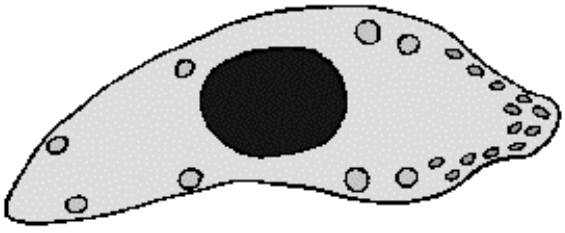
This thesis is based on the following published papers and manuscripts, which will be referred to by their roman numerals:

I. **S. Campino**, C. Behrschmidt, S. Bagot, J.-L. Guénet, P. André Cazenave, D. Holmberg, C. Penha-Gonçalves.
Unique Genetic Variation Revealed by a Microsatellite Polymorphism Survey in Ten Wild-Derived Inbred Strains. Genomics. 2002 May;79(5):618-20.

II. S. Bagot, M. Idrissa Boubou, **S. Campino**, C. Behrschmidt, O. Gorgette,J.-L. Guénet, C. Penha-Gonçalves, D. Mazier,S. Pied,and P.-A. Cazenave.
Susceptibility to Experimental Cerebral Malaria Induced by *Plasmodium berghei* ANKA in Inbred Mouse Strains Recently Derived from Wild Stock. Infect Immun. 2002 Apr; 70(4):2049-56.

III. S. Bagot **& S. Campino**, S. Pied, C. Penha-Gonçalves, P. André Cazenave,D. Holmberg.
Identification of two cerebral malaria resistance loci using an inbred wild-derived mouse strain. Proc Natl Acad Sci U S A. 2002 Jul 23; 99(15):9919-23

IV. **S. Campino**, S. Bagot, M.-L. Bergman, P. Almeida, N. Sepúlveda, S. Pied, C. Penha-Gonçalves, D. Holmberg, P-A. Cazenave
Genetic analysis of murine malaria resistance that underlies parasite clearance and immunological memory. Manuscript



Introduction

Malaria

An overview of the disease problem

Malaria is considered to be one of the major world-scale health problems. According to the World Health Organization (WHO), 300-500 million new cases of this parasitic disease occur every year causing 1.1 million deaths mainly in tropical Africa where malaria is endemic. Malaria is of higher concern in poor and underdeveloped areas and it has been estimated that 40% of the world's population is at risk of being infected. Over 90% of the disease burden occurs in sub-Saharan Africa and the remaining is distributed among South-East Asia and Oceania, the Indian sub-continent and Latin America (Fig 1)[1].

In Africa, malaria related death occurs mainly in children under 5 years old. Pregnant women, young adults and travellers are also high-risk groups. Conversely, older African individuals have a reduced risk due to the continuous exposure that leads to the development of clinical immunity against infection [2]. Outside Africa, since there is less repeated exposure to the parasite, the disease burden extends to adulthood, affecting mainly individuals who lack immunity in areas where diagnosis and treatment are not readily available [2].

After the Second World War intensive programs aimed at the prevention and control of the disease through the use of anti-malarial drugs and insecticides, lead to a reduction of the incidence and mortality. However, over the last few decades, a worsening of the situation has been observed, as malaria is reappearing in many areas where it had been reduced or eradicated. This is mainly the result of emerging parasite resistance to anti-malarial drugs and of mosquito resistance to insecticides. In addition, control strategies have deteriorated or have been halted mainly due to economic crises and civil or military disturbances[1,2]. Therefore, given the high impact of malaria as a public health problem, it is essential to develop novel control methodologies and strategies, being an effective vaccine one of the most awaited control tools.

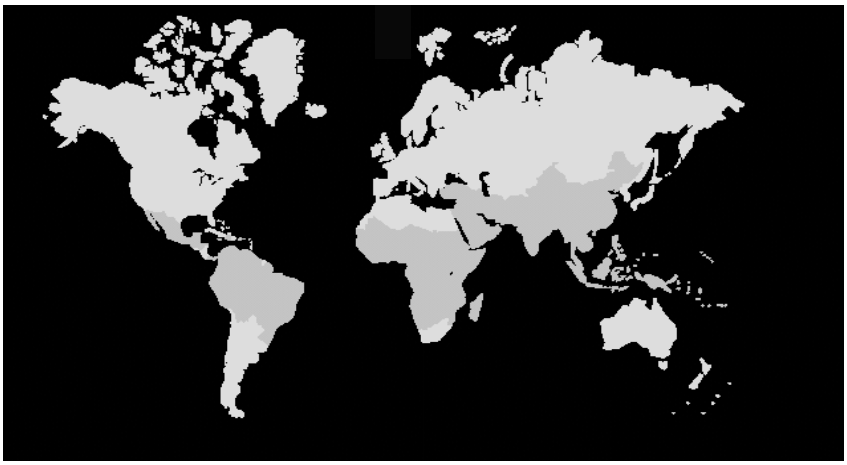


Fig.1 Geographic distribution of malaria worldwide (WHO, 2002)

Plamodium life cycle

Malaria is caused by protozoan parasites from the genus *Plasmodium*, family Plasmodiidae, order Haemosporida, class Hematozoa and phylum Apicomplexa. All the Apicomplexa members are parasites characterised by a distinctive structure localized at the apical end of the parasite - the apical complex- present at the invasive stages of the life cycle.

The genus *Plasmodium* includes around 200 known species able to infect amphibians, reptiles, birds and mammals. Most *Plasmodium* species infect birds[3].
Human malaria is caused by four species of *Plasmodium*: *P. malariae, P. ovale, P. vivax* and *P. falciparum*. These four species have different geographic distributions, relapse patterns and drug responses. *P. falciparum* causes the more severe forms of the disease and is the leading cause of morbidity and death. The other parasites-mainly *P. vivax* - cause considerable morbidity but are rarely fatal[4]. In malaria endemic regions, humans are commonly infected by a mix of different species and strains of parasites[5].

The analysis of small subunit ribosomal RNA gene sequences, have shown that *P. falciparum* is more closely related to *Plasmodium* species that infect chimpanzees and birds than to other *Plasmodium* species that infect humans and non-human primates [3]. The genome of *P. falciparum* was recently sequenced and consists of 23 million base pairs of DNA split over 14 chromosomes[6]. The recent freely available genome information is an important tool for the malaria research community.

The life cycle of *Plasmodium* is complex. It includes a sexual phase developing in an invertebrate host (definitive host) and an asexual phase occurring in a vertebrate host (intermediate host) (Fig 2). All mammalian *Plasmodium* species have similar life cycles. In human malaria the female mosquito of the genus *Anopheles* is the definitive host where

the sexual stage takes place. Only the female mosquitoes have haematophagous habits, necessary as a protein source for the development of a batch of eggs. Worldwide, more than 400 species of *Anopheles* are known, from which only around 50 are considered malaria vectors. In Africa, *Anopheles gambiae* and *Anopheles funestus* are the main vectors of malaria transmission [7,8]. Some *Anopheles* species prefer to take blood meals from animals and therefore transmit malaria to humans only very rarely or never; other species are rare in nature or do not live long enough to allow the parasites to multiply, and in some species the parasites seem to be incapable of developing[8].

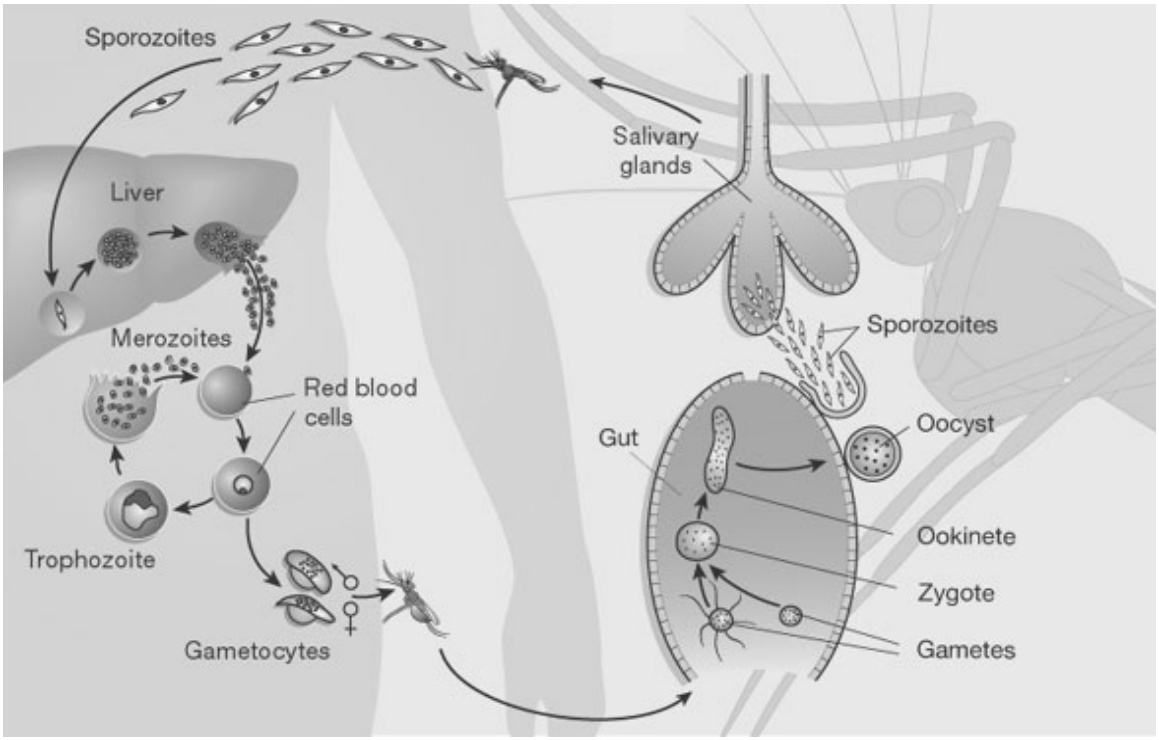


Fig. 2 Life cycle of human *Plasmodium*

Asexual phase

The infection starts with the bite of an infected female mosquito that injects the sporozoites into the subcutaneous tissue, and less-frequently directly into the bloodstream of the intermediate host. Within minutes, inoculated sporozoites reach the liver and invade the hepatocytes. In the hepatocyte the parasite undergoes differentiation and asexual reproduction (schizogony) to produce a large pre-erythrocytic schizont that contains a few thousand merozoites [9]. This stage is completely asymptomatic and in humans can last approximately 5-15 days, depending on the parasite species. In the liver stage of *P. vivax*

and *P. ovale*, some sporozoites may transform into hypnozoites, and remain latent for months or even years, being responsible for subsequent relapses [9].

With the rupture of the infected hepatocytes, the merozoites are released into the bloodstream and are able to invade erythrocytes. Each species of parasites show specific capacity to infect immature or mature erythrocytes. The merozoite, after erythrocyte invasion, differentiates into a round trophozoite that grows and develops the erythrocytic schizont, which contains more merozoites. During this process the parasite induces an increase in the permeability of the erythrocyte membrane, which enables the uptake of nutrients and discharge of waste products; the parasite also ingests and digests 70% of the haemoglobin in the erythrocyte[10]. In humans, the time of the intra-erythrocytic development can be either 48 or 72 hours and the number of produced merozoites varies from 8 to 32, depending on the *Plasmodium* species.

The erythrocyte rupture releases merozoites that are able to invade more erythrocytes, starting a new cycle. This process cycles logarithmically. The clinical manifestations of the disease are associated with the rupture of the infected red blood cells.

A small percentage of merozoites upon invading a new red blood cell differentiate into male or female gametocytes. These sexual forms cannot undergo more differentiation and will be waiting in an arrested state of development to be ingested by a female A*nopheles*.

Sexual phase

During the mosquito meal the gametocytes are taken up and after some minutes they transform into gametes in the acidic, low-temperature environment of the midgut. The male microgametes fertilize the female macrogametes to form a zygote that develops into an ookinete. This is a mobile form able to cross the ciliated wall of the stomach and situate itself in the outer surface where it develops into an oocyst that grows and matures. The oocyst undergoes repeated division (sporogony) and a large number of sporozoites are produced. Sporogony can take from 7 days to 8 weeks depending on the parasite species, nature of the vector and environmental factors[8]. The infective sporozoites, after oocyst rupture are released to the hemolymph of the mosquito and migrate to the salivary glands where they wait to be injected during a blood meal into a new host, continuing the transmission cycle.

Clinical manifestations

The clinical outcome of malaria infection can range from asymptomatic infection to severe malaria and death. Although severe malaria is uncommon when compared to the more mild malaria manifestations, it still remains an important heath problem when considering its prevalence. The typical symptoms of malaria are non-specific with headache and muscle aches, loss of appetite, nausea, chill sensation, fever and sweating[11]. These clinical manifestations may pose difficulties in the early diagnosis because they mimic many diseases such as influenza. The symptoms are associated with the blood stage parasite cycle, specifically with the rupture of the infected red blood cells and the liberation of parasites and erythrocyte substances into the circulation[12,13]. The periodicity of the clinical picture depends on the parasite species and can be tertian (every 48 hours) for *P. falciparum*, *P. vivax* and *P. ovale* or quatrain (every 72 hours) for *P. malariae*[12].

The intensity of the clinical pictures varies with previous exposure to the parasite. Immune individuals may be completely asymptomatic or can present mild anaemia. Non-immune patients can become very ill, mostly if treatment is postponed or the parasite is resistant to antimalarial drugs. The progression of the infection can lead to anaemia and enlargement of the spleen and liver [12]. Infections with *P. falciparum* can lead to more severe complications such as impaired consciousness, hypoglycemia, renal failure, pulmonary edema, severe anaemia and cerebral malaria (CM), the two latter being the major causes of death [13]. The WHO defines severe malaria as the appearance of one or more complications in addition to a positive blood smear indicative of malaria infection.

P. falciparum infected erythrocytes are capable of binding to unaffected red blood cells to form clumps called rosettes and sequester by cytoadherence in the endothelium cells of different organs (e.g., brain, heart, liver, lungs, placenta, kidneys). This phenomenon of sequestration is one of the possible explanations for some of the severe malaria clinical complications[13].

Cerebral malaria and severe anaemia

CM is the most serious clinical complication of the *P. falciparum* infection. It may account for 10% of complicated *P. falciparum* malaria and has a poor prognosis with a 20-50% mortality rate[14]. In addition, up to 10% of the individuals that recover can present neurological sequels ranging from weakness and hearing impairment to severe symptoms such as quadriplegia and cortical blindness[1,15].

CM is not a homogeneous condition. In general, this neurological syndrome is characterised by the loss of consciousness and fitting[12,14]. However, different clinical conditions have been observed which cause difficulties in defining the nature of the disease. Besides geographical variations in clinical presentation, significant differences between CM in children and in adults have also been reported [14,16].

The pathogenesis process underlying CM is still not well understood, however two hypotheses have been proposed. The obstruction hypothesis proposes that the infected erythrocytes sequester and adhere to the cerebral endothelium leading to cerebral vessel obstruction, eventually resulting in hypoxia and hemorrhagic necrosis[17,18]. The inflammatory hypothesis supports that parasitized erythrocytes, lymphocytes and macrophages sequestered in brain vessels induce an inflammatory response, eventually increasing vascular permeability and causing disturbances in brain functions [18,19]. These hypotheses are not mutually exclusive.

Another serious complication of malaria infection is severe anaemia, which is one of the major causes of death arising from malaria in Africa. Anaemia is multifactorial and is usually associated with the level of parasitaemia [12]. Anaemia results from the destruction of both infected and non-infected red blood cells and the decrease production of these cells, mainly due to an inadequate erythropoietic response of the bone marrow [13,20,21]. The spleen plays a major role in removing parasitized and non parasitized erythrocytes. In fact, this clearance was observed to be increased in patients with splenomegaly[13]. Studies on bone marrow indicated a depression and a defective erythropoiesis during malaria infection[20,22]. The removal of non-infected red blood cells has been proposed as the major mechanism leading to persistence and worsening of anaemia following parasitaemia clearance. This could explain the lack of correlation between parasitaemia level and anaemia observed in some studies that reported cases of patients with severe anaemia and low parasitaemias[22].

The clinical outcome of malaria infection depends on several factors with regards to the parasite and host as well as geographic and social factors (Table 1)[4,20]. The combination of these factors determines the outcome of the disease.

Table 1 Factors influencing the clinical outcome of malaria infection[20,4]

Parasite factors	Host factors	Geographical and social factors
Species	Immunity	Climatic changes
Strain	Health status	Transmission intensity
Multiplication capacity	Inflamatory response	Treatment access
Cytoadherence	Age	Cultural and economic factors
Rosetting	Genetics	
Drug resistance	Pregnancy	
Antigenicity		
Red cell selectivity		

Genetic control of malaria

In 1948, J.B.S. Haldane reported an extremely high gene frequency of thalassemia in certain racial groups from the Mediterranean regions, suggesting that this disease has come under intense selection because of heterozygote advantage against malaria. This was one of the first observations pointing to infectious diseases as being a major selective pressure in human evolution. It fact, Haldane's remarkable insight opened up the field of the investigation of the genetic factors underlying the pathogenesis of malaria and other infectious diseases. Over the past 50 years, mainly after J. B. S. Haldane´s hypothesis, considerable evidences had been accumulated indicating that genetic variants influence onset, progression, severity and ultimate outcome of human malaria infection. The understanding of the natural mechanisms of host defence against the disease could point out novel approaches for prophylactic and therapeutic interventions.

Genetic analysis of complex traits

The term complex trait refers to any phenotype that does not follow a classic Mendelian mode of inheritance controlled by a single locus. The complexity is due to a lack of one-to-one correspondence between genotype and phenotype. This occurs because the same genotypes can give rise to different phenotypes or different genotypes can produce the same phenotype. The complexity factors include involvement of multiple loci, environmental factors, incomplete penetrance, genetic heterogeneity, polygenic inheritance and high frequency of disease causing alleles in the population[23-25].

Since there is no recognizable Mendelian mode of inheritance, the genetic mapping of complex diseases makes use of samples with a large number of individuals and many families or extended family pedigrees, which are rarer. To genetically analyse complex traits there are a number of methods available that include linkage analysis, allele-sharing methods and association studies in human populations.

Linkage analysis is a parametric method since it requires a precise model of inheritance, gene frequencies and genotypes penetrances [24,26]. If a valid model of inheritance is possible to define for a trait, linkage analysis provides the best method of analysis. This method has been successfully used to study simple Mendelian traits and allowed the identification of several genetic loci controlling monogenic diseases. However, the majority of traits with medical relevance such as diabetes type I, heart disease, multiple sclerosis and infectious diseases, are complex genetic disorders, which are less amenable to use linkage analysis since it is harder to define a genetic model. In this case allele-sharing methods are better applicable.

Allele-sharing methods test if in a pedigree the affected relatives inherit or a given allele more often than what was expected by random Mendelian segregation. This method is non parametric, assumes no mode of inheritance and is more robust then linkage analysis for complex traits[24].

Association studies do not analyse familial inheritance patterns but compare unrelated affected and unaffected individuals in a population (case-control studies)[24]. These studies are more applied to identify variants in genes that have a possible biological relation to the trait. It usually compares if a certain allele is more frequent in the affected than in the unaffected individuals.

Genetic factors influencing malaria infection in human populations

Malaria is the infectious disease with more progresses done towards the discovery of genetic factors responsible for host resistance/susceptibility. The genetic component is complex and has been investigated using a multitude of approaches including genetic epidemiology, linkage and association studies, testing candidate genes and genetic mapping using mouse models. Almost all malaria candidate genes have been selected because of geographical overlapping of certain disorders with malaria or due to their known physiological role in the disease.

Inherited Hemoglobin disorders

The inherited hemoglobin disorders are the most common type of monogenic diseases[27]. The geographic distribution of malaria and of hemoglobinopathies largely overlap, suggesting that these disorders could confer protection against the disease.

Homozygocity of Hemoglobin S (HbS) allele is responsible for sickle cell anaemia, a disease with short life expectancy; heterozygocity confers a less severe phenotype. Individuals heterozygous for HbS, especially young children, seem to be protected against severe malaria and death. Case-control studies indicate that this variant confers more than 90% protection [28,29]. The mechanism by which HbS allele mediates protection is not clear. *In vitro* studies have proposed a low capacity of the parasite to invade and growth during the erythrocyte stage and an enhanced splenic clearance of infected erythrocytes [29-31].

For HbC and HbE the data for a selective advantage against malaria is less convincing, although some epidemiological data indicated protection against the more severe malaria manifestation [32-34].

The thalassemias are the most common hemoglobinopathies resulting from the deficient synthesis of α or β globin chains [27]. It has been observed that in some areas where malaria exists, thalassemia also exists, often with a high frequency [20,35,36]. The possible explanations for this includes a greater efficiency in clearing the infected erythrocytes, lower susceptibility of clumping and higher level of parasite antigens at the erythrocyte surface leading to a better recognition of the immune system [29-31,35].

Other erythrocytes polymorphisms

The geographical observation of remarkably uncommon *P. vivax* infections in West Africa led to the discovery that erythrocytes of Duffy blood group negative individuals are resistant to invasion by *P. vivax* but not by other malaria parasite[37,38]. The Duffy antigen encodes a chemokine receptor expressed on erythrocytes used by *P. vivax* parasites as an attached receptor to mediate entry. A polymorphism at a GATA-1 binging site in the promoter of this gene alters the expression of the receptor preventing the parasite from entering[39].

The X-linked glucose-6-phosphate-dehydrogenase (G6PD) deficiency is in general geographically correlated with malaria. It has been shown that both heterozygotic females and hemizigotic males have a significantly reduce risk (~50%) of developing severe malaria[40-42]. *In vitro* studies proposed that protection is due to impaired parasite growth and survival or more efficient clearance of infected red cells[43]. It has been shown that

deficient erythrocytes have more phagocytic removal markers at their surface than normal erythrocytes [43].

Ovalocytosis is a dominant inherited trait that occurs at high frequencies in malarious regions of the western Pacific. It is caused by a deletion in the erythrocytes membrane band 3 protein, also know as anion exchange protein 1[29]. Homozygocity is lethal[37]. Ovalocytosis is associated with increase red cell rigidity and characterised by the presence of oval red cells [29,44]. In population studies it was found that ovalocytosisis was associated with protection from CM in children, possible due to changes in the red cell membrane that interfere with the binding of infected erythrocytes to the brain vascular endothelium [38,45].

Genetic Epidemiology and Candidate-gene based approach

The study of heterogeneity in human malaria can be approached at the population level by comparing groups with different genetic backgrounds exposed to similar parasite inoculation rates - for instance different ethnic groups living together in an endemic zone. Comparisons of different ethnic groups (Fulani, Mossi, and Rimaibe) exposed to intense *P. falciparum* transmission in rural savanna areas in Burkina Faso have identified the Fulani as having lower levels of parasitaemia and malarial illness[46,47]. Other studies have also found that the Fulani have higher antibody levels against various malaria antigens[48]. Such findings call attention to the importance of the genetic background of the host to the outcome of the infection although differences in lifestyle can not be excluded from also influencing these observations.

Since long investigators have focused on the influence of the human leucocyte antigen (HLA) in malaria. HLA class I molecules, HLA-A, -B and –C, determine the specificity of the cytotoxic $CD8^+$ T cells, crucial in the defence against intracellular pathogens. HLA class II molecules, HLA-DR, -DQ and –DP determine the specificity of $CD4^+$ T cells that secrete cytokines and activate other cells such as macrophages and B cells. A large case-control study was performed with West African children infected with *P. falciparum* and found association of HLA –B53 class I antigen and HLA class II haplotype DRQB1*1302-DQB1*0501 with protection against severe malaria (40 to 50% reduced risk)[49,50]. Subsequent molecular analysis has shown that HLA-B53-restricted T cells recognized a conserved peptide from the parasite liver stage specific antigen-1 (LSA-1), suggesting that HLA-B53 antigen may facilitate cytotoxic T-cells to kill sporozoite-infected hepatocytes [51].

The tumour necrosis factor-α (TNF-α) cytokine has attracted interest because of its role in host defence and in pathogenesis of CM and other manifestations. It has been shown that TNF-α is a major mediator of malaria fever and several studies found increased TNF-α levels in the serum of children with severe malaria or CM, markedly in fatal cases[52-56]. However, some studies have failed to find differences in the level of TNF-α between severe and uncomplicated malaria [57]. Epidemiological data have described three polymorphisms in the TNF-α promoter correlated with susceptibility/ resistance to severe malaria. Gambian children homozygous for a variant at position –308 had a 7-fold increased risk of dying with CM[58]. This polymorphism increased levels of TNF *in vitro* and was associated with susceptibility to CM in other African groups and to susceptibility to leishmaniasis and lepromatous leprosy [59,60]. A variant at position –238 was associated with severe anaemia and low TNF in the plasma of Gambian children[40,61]. Another variant at position -376 associated with a 4-fold increased susceptibility to CM was found in East and West Africans[62].

The analysis of the mechanisms underlying the sequestration of infected red cells has indicated other potential candidate genes. The cell adhesion process is proposed to be one of the contributors to the pathology of *P.falciparum* malaria. The intercellular adhesion molecule type 1 (ICAM-1) acts as an endothelial ligand for infected erythrocytes[63]. Autopsy studies showed that parasitized red cells tend to aggregate in small cerebral blood vessels expressing ICAM-1[14]. An ICAM-1 variant (Kilifi allele) was associated with severe malaria in Kenya but with mild malaria in Gambian children[64,65]. In contrast, another study in Gambia showed no effect of this allele in the outcome of *P. falciparum* infection[66]. *In vitro* studies have demonstrated that ICAM-1^{kilifi} differentially binds to different *P. falciparum* strains[67,68].

CD36 is another major receptor for infected red cells. It is expressed on monocytes and macrophages and has been reported to be necessary for the phagocytosis of infected erythrocytes [69]. An exceptionally high frequency of mutations on CD36 is common in African populations and many individuals are deficient. A polymorphism causing CD36 deficiency was found to be associated with protection from severe malaria but not CM in children from Kenya[70]. Another group reported three polymorphisms encoding truncated CD36 proteins with higher frequencies in patients with severe malaria (Gambia and Kenya)[71]. Similar work was conducted in patients with severe malaria from Thailand and a variant was strongly associated with CM protection[72].

Nitric oxide synthase (NOS) type 2 is an isoform of the NOS gene, which is induced in response to both infection and cytokines, being capable of generating large quantities of nitric oxide (NO). NO may cross the blood-brain barrier and disrupt neurotransmission, inducing a state of unconsciousness [73]. In fact, increased NOS expression was found in brains of fatal CM patients and some authors have associated higher plasma NO with coma duration and fatal CM[74]. Other studies contradict these results correlating increased plasma levels of NO and NOS with accelerated clinical cure and finding lower concentration in CM patients [75-78]. In Gabon a polymorphism in the promoter (NOS2 [Lambaréne]) was associated with protection from severe malaria and decreased risk of re-infection[79]. Longer forms of CCTTT repeats in the promoter were associated with protection against severe malaria in Gambian children [80]. Another NOS2 promoter polymorphism was associated with increase NO production and severe malaria protection in Kenyan and Tanzanian children[81]. Recently, in an extensive analysis of nucleotide and haplotype of the NOS2 5´flanking region, a weak but significant association between this locus and CM susceptibility was found.[82]

Interferon (IFN)– γ and its receptor are important elements of innate and adaptive immunity and have been implicated in several infectious diseases. IFN-γ is produced in abundance during clinical episodes of malaria [83]. In a case-control study, individuals heterozygous for a promoter polymorphism IFNGR1-56 appeared to have 2-fold protection against CM and 4-fold protection against death resulting from CM[84]. Analysis of a family study by transmission disequilibrium tests revealed similar result[84].

Segregation analyses have shown that blood parasitaemia density is under complex genetic control with evidence of a major gene[85,86]. In two independent studies (sib-pair linkage analysis) blood infection levels were linked to the 5q31-33 chromosomal region [85 87,88]. This region deserves attention since it contains candidate genes that could be involved in the control of immunity to *P. falciparum*. Moreover, the importance of this region is highlighted by its linkage to plasma immunoglobulin E, atopy, asthma, bronchial hyperesponsiveness and schistosomiasis infection [87].

Host genetics in other infectious diseases

An increasing number of studies have reported the role of host genetic variation in conferring susceptibility/resistance to other infectious diseases.

Polymorphisms on the HLA gene complex have been associated with susceptibility/resistance to several infectious diseases (tuberculosis, HIV, hepatitis B and C, typhoid fever and leprosy) (Table 2) [29,38,89]. It is becoming apparent that the HLA may only account for a small proportion of the hereditability with non-HLA genes also having an important effect. A variety of polymorphisms relating to cytokines and immune effectors are associated with susceptibility/resistance to infectious agents. These associations are well exemplified in the cases of the gene for solute-carrier-family-11 member, SLC11A1 (until recently designated NRAMP1) and for the vitamin D receptor gene (VDR) (Table 2). Variants in the SLC11A1 gene have been reported to be associated with tuberculosis in different populations and also to be associated with leprosy[38,89]. This gene is expressed in macrophages and appears to influence macrophage activation[29].

The active metabolite of vitamin D is important for modulating several immune responses. A polymorphism in this gene is strongly associated with resistance to tuberculosis, modification of the course of leprosy and hepatitis B and reduces risk of HIV-1 infection[29,38].

Families with deletions in the interferon-γ receptor gene show susceptibility to opportunistic non-tuberculous mycobacterial and S*almonella* infections (Table 2). On the other hand, it appears to decrease risk of infections with tuberculosis or leprosy[38].

A variant in the promoter of the chemokine receptor 5 (CCR5) was observed to confer protection against HIV infection and other variants were shown to be associated with increase rate of transmission and accelerated progression to AIDS (Table 2) [29,38,90]. A variant in the CCR2 gene was associated with the rate of disease progression [29,38].

Table 2 Some genes implicated in resistance/susceptibility to several infectious diseases

Disease	Genes influencing susceptibility/resistance
Malaria	α-globin,β-globin, erythrocyte band 3, Duffy receptor, G6PD, HLA-B, HLA-DR, TNF, CD36, ICAM-1, NOS, IFNγR1
Tuberculosis	HLA-A,HLA-DR, SLC11A1,VDR, IFNγR1
Leprosy	HLA-B,HLA-DR, VDR, TNF
HIV	CCR5,CCR2, VDR, IL10
Hepatitis-B	HLA-DR, IL10

It is important to mention that some human epidemiology studies are based on sample sizes that are not large enough to achieve confident results and sometimes are confined to one single nucleotide polymorphism (SNP) and one microsatellite. It is necessary, therefore, to confirm these results to understand the relevance of such polymorphisms as aetiological factors in the disease. Unquestionably many more genetic determinants have yet to be discovered. Overall, the study of genetic determinants of susceptibility/resistance to malaria and other infectious diseases in humans may provide important information on proteins playing a key role in parasite pathogenesis and host immune response.

Animal models of malaria infection

Current experimental animal models of malaria infection do not reflect all the features of the human disease. However, these models can be use for particular study purposes once they exhibit specific pathological characteristics depending on the particular combinations of the animal and parasite strain used. The experimental animal models are advantageous because they allow the control of several variables not easily controlled in human malaria, such as environmental factors, parasite status, dose, degree of the host immunity, which allow precise dynamic analysis and well-defined interventions.

Primate models

The non-human primate models have been widely used to investigate several aspects of malaria because of their phylogenetic proximity to humans and also the similarity of the parasite-host interaction. Several non-human primate species are capable of sustaining the development of *P. falciparum* and *P. vivax* to various degrees. However, the pathology outcome can be influenced by unnatural hosts, and such models should be treated carefully. The host specificity of malaria parasites has limited the number of non-human primate species mainly to the chimpanzee, Saimiri and Aotus monkeys in which all or part of the parasite life cycle of human *Plasmodium* can develop[91].

Several features observed in human malaria, such as anaemia, perturbations of the circulating leukocytes and also neurological symptoms have been observed and studied in Saimiri monkeys infected with *P. falciparum.* Neurological symptoms in this model can be detected as ataxia, mobility perturbations, blindness and even coma, similar to human CM. As for humans, only few animals developed the neurological syndrome [91,92].

Malaria infections with non-human primate parasites in the natural primate host are usually mild and without neurological symptoms[93]. For example, *P. knowlesi* and *P. coatneyi* cause a mild infection in cynomolgus monkeys (*Macaca facicularis*), but *P. knowlesi*, *P. coatneyi* or *P. fragile* infections are severe in rhesus monkeys (*M. mulatta*)[92,94]. Rhesus monkey infected with *P. knowlesi* can develop coma with the presence of infected erythrocytes sequestration in the brain and if infected with *P. coatneyi* might display some features common with human cerebral pathology and also multiorgan involvement [92,94]

The non-human primate models provide information about malaria in general, mainly in respect to immune responses and pathogenic effects and have been very important in the evaluation of anti-malarial drugs and candidate vaccines. However, their use is limited due to high cost, legal requirements and ethical considerations.

Murine Models

Studies of murine models offer many advantages and have given important contributions to the understanding of the biology and pathology of human malaria.

The majority of the murine *Plasmodium* species were isolated from *Thamnomys*, the main rodent host, found mostly in localities of the Congo forest basin[95]. There are four species of murine *Plasmodium* used in experimental infection of mice: *P berghei, P. chabaudi, P yoelli* and *P. vinckei.*

The course of infection depends on factors derived both from the host and the parasite species. In laboratory mice, infections are usually acute and can be either lethal or self-curing. Some clinical features observed in humans are reproduced in certain murine models, such as splenomegaly and hepatomegaly, renal alterations, pulmonary distress, severe anaemia and CM[96]. Severe anaemia in murine malaria is similar to that observed in humans. The parasitaemias are usually higher than in humans, anaemia develops rapidly being either of short duration and reversible or acute and lethal[95]. The intensity varies with the mouse strain and the parasite used. *Plasmodium chabaudi* produces a blood stage infection in mice with the most similarities to *P. falciparum* in humans. It also includes sequestration in several organs but not in the brain[95].

To study experimental cerebral malaria (ECM) the models of choice are *P. berghei* ANKA and K173. Susceptible animals develop symptoms such as deviation of the head, convulsions, decreased body temperature, ataxia, paralysis and coma. The neurological syndrome is not reversible and leads to death[93,97,98]. The major differences between

murine and human CM are the type of cells sequestered in the brain vessels. The obstruction of the cerebral capillaries in mice seems to be due to accumulation of monocytes and leucocytes while in humans is mainly attributed to the sequestration of parasitized red blood cells. In murine models the percent of sequestered parasitized erythrocytes and platelets is much lower than what has been reported in humans [92]. Mice resistant to the neurological syndrome induced by *P. berghei* ANKA infection died with hyperparasitaemia and severe anaemia[93,98]. Survival to infection by this parasite strain has not been reported.

The effect of some natural occurring or experimental induced mutations on response to malaria has been extensively investigated. Studies in nude mice (athymic mice with no T cells) and in SCID mice (devoid of B and T lymphocytes) infected with *P. berghei* ANKA have shown that these mutations confer resistance to ECM, revealing a role for lymphocytes in malaria pathology [92,99].It has also been shown that anti-CD4 antibody treatment prevents ECM in CBA susceptible mice, while either anti-CD4 or anti-CD8 antibodies can prevent ECM in the susceptible C57BL/6 mice[100-102]. The role of certain cytokines has also been studied in the development of ECM induced by *P. berghei*[103]. The introduction of IFN- γ receptor or IFN-γ regulatory factor gene defects in susceptible mouse strains made them resistant to the development of ECM.[104] Likewise, TNF-α/β deficient mice were resistant to ECM induced by *P. berghei* ANKA[105]. ECM did not develop in transgenic mice expressing high levels of TNF-α inhibitor [106]. TNF-α seems to be an important mediator of ECM in CBA/ca mice and treatment with anti-TNF-α antibodies prevented ECM in this strain[106]. Moreover, the administration of recombinant murine TNF (daily from day 4 of infection) induced a lethal syndrome in an ECM-resistant strain [107].

It is proposed that the up-regulation of the expression of adhesion molecules in the vascular endothelium, such as ICAM-1/CD54 and CD36, is a key mechanism in the development of ECM. These molecules appear to have a role in the sequestration of infected erythrocytes and activated leucocytes to endothelial cells[108]. It was also observed that ICAM-1 knockout mice (C57BL/6 background) infected with *P. berghei* ANKA were protected against ECM[108].

Immunoregulatory cytokines such as interleukine-10 (IL-10) and transforming growth factor-beta1 (TGF-β1) have also been shown to ameliorate pathology and death in murine malaria[92]. A defect in IL-10 causes a high mortality rate in *P. chabaudi* infections and susceptibility to ECM in *P. berghei* infection [99]. TGF-β1 abrogation exacerbated pathology in both P. *chabaudi* and *P. berghei* models while treatment with recombinant

TGF-β1 increases survival time in *P. berghei* infections [109,110]. These and several other studies at the molecular level that have been conducted in the mouse illustrate the advantages of the murine models over monkey models and human studies. In fact, much of malaria cell and molecular pathology has been learnt through the use of mouse strains. Therefore, it is hoped that the identification of genetic factors controlling malaria pathology in these models may bring light to potential pathogenic mechanisms and candidate genes relevant to the human disease.

Genetic analysis of quantitative traits

The crossing of phenotypically different inbred strains enables the mapping of quantitative and qualitative trait loci. Analysis of a complex trait typically involves choosing phenotypically distinct parental strains, but these strains should also be genotypically distinct because genetic mapping depends on the polymorphic differences between parents. Genetic mapping studies can be performed in second-generation cohorts such as F2 and backcross. The phenotype observed in these progenies reflects the genetic re-assortment of the two parental strains and may reveal new genetic combinations that cause phenotypes diverse from the original observed on the parental strains.

Genetic inherited characters that can be easily classified into distinct phenotypic categories such as resistance/susceptibility to disease are considered to be qualitative traits and frequently can be studied according classical Mendelian analysis. However, a large part of the genetic inherited characters are measurable as a continuous variable, demonstrating large variation in the population and are considered to be quantitative traits showing complex patterns of inheritance. Such traits can be analysed under the assumption that the phenotype in a given individual is quantitatively contributed by several loci, called quantitative trait loci (QTL). A number of statistical methods and study designs have been developed that have made it possible to determine the locations of QTLs that control such traits. The identification of QTLs is based on methods of linear regression of the phenotypic values on the genotype that allow inferring the variance explained by a given QTL[111]. The chromosomal location of the QTLs can be estimated using the interval-mapping method that calculates association of the trait to loci located between genetic markers. The maximum likelihood odds (LOD-score analysis) for a QTL at each point can then be estimated and plotted against a framework linkage map.

The standard approach for mapping QTL contributing to variation in a quantitative trait makes use of the assumption that the variation follows a normal distribution in the

population in question[112]. However, many quantitative traits of interest are not normally distributed. Recently a number of attempts have been made to develop genetic models that allow the analysis of non-normal distributed quantitative traits as is the case of the parametric two-part model and the non-parametric model[113].

The parametric two-part model is designed for analysis of quantitative traits that show a spike in the distribution. If the proportion of individuals within the spike is appreciable and the phenotype is well separated from the rest phenotypic distribution, the normal model of QTL mapping has a tendency to produce false LOD scores peaks in regions of low genotype information. To circumvent this problem the two-part model considers two separated maximum-likelihood estimations: the analysis of the quantitative phenotype by standard interval mapping using only the individuals within the normal distribution and the analysis of the binary trait that considers the individuals in the spike against the remaining individuals[114]. The resulting LOD-score is simply the sum of the LOD scores from the two separate analyses.

The nonparametric model applicable for non-normal distributions considers a rank-based analysis of the phenotype. Each individual is represented by a rank value and the average rank for each genotype category is then compared using a Kruskal-Wallis statistic test[113]. This nonparametric statistics follows approximately a chi-square ($\chi 2$) distribution under the null hypothesis of no linkage and can be mathematically transformed into a LOD score[113].

Genetic linkage analysis of murine malaria

Inbred mouse strains differ markedly in their susceptibility to *Plasmodium* infection. Depending on the parasite used, differences can be measured by the extent of blood stage parasite replication, parasitaemia levels, overall mortality or appearance of neurological manifestations. The clear inter-strain differences have been used to genetic map host factors involved in the resistance/susceptibility to infection.

Genetic studies have predominantly been carried out for susceptibility to *P. chabaudi* infection that is characterised by high levels of parasitaemia followed by death. In contrast, resistant mice limit the parasite replication, have a robust erythropoietic response and survive infection. The strains A/J, C3H/HeJ, SJL, Balb/C, AKR, DBA/1 and 129/ICR are susceptible to *P. chabaudi*. Resistant strains include C57BL/6, CBA, B10.A and DBA/2 [96]. In general the F1 hybrids between susceptible and resistant strains are more resistant to infection than their parents. Gender differences are also observed, with

males being more susceptible than females with respect to peak parasitaemia and survival, possible due to hormonal influences[96].

Genetic linkage studies using crosses between the *P. chabaudi* susceptible A/J strain and the resistant C57BL/6 strain allowed the identification of a number of loci, that were designated *Char* loci (for *chabaudi resistance*) (Table 3). Genetic association between peak parasitaemia and a central region on chromosome 8 (*Char2*) was found in a subset of (A/JxC57BL/6)F1xA/J backcross mice and was validated in a (A/JxC57BL/6)F2 cohort [115]. Independently, similar linkage studies were conduct in (SJAxC57BL/6)F2 and (C3HxC57BL/6)F2 female mice and two loci, again on central chromosome 8 and on distal chromosome 9 (*Char*1) were found to be associated with survival of infection[116]. The *Char*1 locus was also associated with peak parasitaemia in both crosses, while *Char*2 was found to control peak parasitaemia only in the C3H derived cross. Analysis of recombinant inbred strains (RIS) containing a 50:50 ratio of A/J:C57BL/6 genomic DNA fixed as chimeric chromosomes with distinct combinations, corroborate the role of *Char*1 and *Char*2 in *P.chabaudi* infection[96]. The RIS showing A/J haplotypes in these loci were the most susceptible to infection (measured both by peak parasitaemia and survival). Additionally, a locus on chromosome 17 (*Char*3) linked to the major histocompatibility locus was mapped using (C3HxC57BL/6)F2 female mice, and was claimed to control parasitaemia at day 11 post-infection (one day after peak parasitaemia)[117].

A whole genome scan was conducted in a cohort of backcross mice derived from *P. yoelli* susceptible NC/Jic and resistant 129/Sv mice[118]. A locus close to *Char1* (designed *Pymr* for *P. yoelii* resistance) was found to control host survival and parasitaemia after infection (Table 3). Possibly, *Char*1 and *Pymr* represent the same genetic factor capable of controlling blood parasitaemia and survival to infection by different *Plasmodium* species.

Linkage studies aimed at finding loci conferring resistance to *P. berghei* ANKA were completed using F2 mice derived from crossing DBA/2 and C57BL/6[119]. In this case, C57BL/6 mice are susceptible and die with neurological syndrome designed experimental severe malaria. The DBA/2 mice are resistant to experimental severe malaria but die later due to the high level of parasitaemia. A locus on a central region of chromosome 18 was found to be associated with resistance to death before day 14 post infection (Table 3).

The identification of the genes underlying these quantitative trait loci will be important to understand the mechanism of the host response against malaria. However, this step might not be straightforward since the intervals of the loci mapped range between 15 and 30 cM. Many genes are located in these intervals and usually a good part of them can be viewed as potential candidate genes. The establishment of congenic mice

circumvents this issue. A major advance has been made towards the positional cloning of *Char*2 using a panel of congenic mouse strains each encompassing different chromosomal segments around this locus[120]. These congenic strains also enabled confirmation that *Char*2 plays a significant role in the outcome of malarial infection.

Recombinant congenic strains (RCS) also offer advantages for gene mapping experiments and to find smaller contributions to the overall phenotype[121]. Recently, a set of RCS derived from A/J and C57BL/6 was established[122]. RCS mice are derived by systematic inbreeding of double-backcrossed mice each containing an amount (12,5% representing 3 backcrosses) of genome from one of the parents fixed as congenic segments in the background of the other parent. RCS mice were genotyped and each congenic segment was localised. The relative small size of the congenic segments fixed facilitates the search and test of candidate genes. Phenotyping, by infecting with *P. chabaudi,* this set of RCS allowed the confirmation of *Char*1 and *Char*2. Mapping a F2 cross between a high resistance RCS and A/J, a novel locus (*Char*4) controlling peak parasitaemia was found in a small region (6 cM) on chromosome 3[123] (Table3).

The discovery of these malaria loci in mouse models raises the possibility that the syntenic regions in humans may be relevant in the genetic control of human malaria. The identification of the genes underlying the mapped loci will be important in trying to understand more about the malaria pathology and will possibly help in finding new intervention approaches and therapeutic targets.

Table 3 Loci associated with susceptibility/resistance to murine malaria

Chromosome region	**Locus**	**Host combination**	***Plasmodium* sp.**	**Phenotype controlled**
Distal chr. 9	*Char*1	(C3HxC57BL/6)F2	*chabaudi adami* DS	Peak parasitaemia
		(SJLxC57BL/6)F2		and mortality
	Pymr	(NC/Jicx129/SvJ)F1xNC/Jic	*yoelii* 12XL	
Central chr 8	*Char*2	(A/JxC57BL/6)F1xA/J	*chabaudi chabaudi* AS	Peak parasitaemia
		(A/JxC57BL/6)F2		and mortality
		(C3HxC57BL/6)F2	*chabaudi adami* DS	
Chr 17	*Char*3	(C3HxC57BL/6)F2	*chabaudi adami* DS	Parasitaemia day11
Chr 3	*Char*4	(RICxA/J)F2	*chabaudi chabaudi* AS	Peak parasitaemia
Central chr 18		(DBA/2xC57BL/6)F2	*berghei* ANKA	Experimental severe malaria

Wild-derived inbred mouse strains

The value of inbred mouse strains for genetic studies has long been recognised. The study of genetic inheritance in mice started around 1902 with the work of William Castle[124]. The first inbred mouse strain, DBA, was established in 1909 by a student of Castle, Clarence Little[124]. The mice used by Castle are the ancestors of many of the current laboratory inbred strains.

In 1952 the guidelines for establishing an inbred mouse strain were first published. A strain is considered inbred after 20 or more generations of brother/sister mating [124]. On average 98.6% of the loci are homozygous at generation 20. More than 450 strains have been established during the past century and some of them have been bred for more that 150 generations. Inbred strains are genetically identical, which enables the construction of a genetic profile of a strain by typing only one individual. The phenotypic hererogeneity observed among inbred strains allows them to be used for a variety of comparative studies and subsequent genetic analysis. Because the classical laboratory mouse is derived from a small pool of ancestors their natural genetic diversity is restricted. Genetic polymorphism analysis indicates that laboratory strains have a mosaic genome derived from parental components of *Mus musculus domesticus, Mus musculus musculus* and *Mus musculus castaneous,* which are the main ancestral populations of inbred strains [124,125]. Interestingly, it has been described that most of the standard inbred strains share identical mitochondrial DNA derived from *Mus musculus domesticus* and carry an undistinguished Y chromosome coming from the *Mus musculus musculus* species.[125,126].

The fact that the standard inbred strains have a limited allelic variation restricts its usefulness in genetic studies. This can be circumvented by using of inbred strains derived from wild progenitors belonging to different taxa of the genus *Mus*. These wild-derived inbred strains (WDIS) provide more genetic variability, making them valuable tools for evolution and systematic research[127,128]. The progeny from interspecific crosses is especially useful for the construction of genetic maps. Several of the WIDS naturally carry Robertsian chromosomes that are useful as tissue or cell markers for chimera and transplantation studies and FISH (fluorescence in situ hybridization) gene mapping. The natural genetic variability of the WDIS allows the discovery of novel and unique phenotypes and can provide new models to study human diseases.

Discussion

General strategy

Linkage studies aiming to find loci associated with resistance/susceptibility to murine malaria have been restricted to the use of common laboratory mouse strains. Moreover, the majority of the genetic analysis was done using *P. chabaudi*. Only one case analysed resistance/susceptibility to *P. berghei* ANKA. This is mainly because most laboratory mouse strains die from ECM when infected with this *Plasmodium* strain, with only a minority being resistant to neurological complications, which limits genetic mapping.

The overall aim of this work was to use WDIS to develop new mouse models of resistance to malaria infection and to reveal the genetic components conferring disease resistance/susceptibility.

Initially the work focused on a genome polymorphism survey for ten inbred strains recently derived from wild progenitors, creating a tool available for the genetic research community. Next we searched for novel malaria phenotypes with respect to *P. berghei* ANKA infection in the WDIS. Lastly, to identify novel loci associated with resistance/susceptibility to this *Plasmodium* we performed genome-wide genetic analysis in F2 and backcross cohorts derived from crossing a WDIS with a laboratory mouse strain.

Genome-wide polymorphism survey in ten wild-derived inbred strains
(Paper I)

Microsatellites are abundant in most eucaryotic genomes and constitute an excellent tool for genetic mapping due to their high rate of polymorphism. The genetic map of the mouse has been constructed and contains several thousand microsatellite markers evenly distributed among the entire mouse genome[129]. It is publicly available information concerning microsatellite length and polymorphisms for several laboratory mouse strains and some WDIS. The database of the Whitehead/MIT Institute presents polymorphisms among 12 inbred strains including two WDIS SPRET/Ei and CAST/ei. Several studies also reported the description of polymorphisms for other laboratory and WDIS[130-132]. Recently, a database was created (Mouse Microsatellite DataBase of Japan), which complements the MIT database and also includes data for WDIS established in Japan[133]. However, the data concerning WDIS is confined only to a few strains, which limits the use of the available WDIS for genetic studies.

We generated genetic polymorphism data for 10 WDIS (Table 4) with 254 microsatellite markers selected according to their chromosomal position on the Whitehead/MIT database. We compared our data with the allele size data available for a group of 13 common laboratory inbred strains (CLIS) and four other WDIS (OWDIS).

Table 4 Species and geographic origin of the 10 WDIS

Strain	***Mus* species**	**Origin**
WMP/Pas	*M. musculus domesticus*	Tunisia
WLA/Pas	*M. musculus domesticus*	France
BIK/g /Pas	*M. musculus domesticus*	Israel
38CH/Pas	*M. musculus domesticus*	Italy
MBT/Pas	*M. musculus musculus*	Bulgaria
PWK/Pas	*M. musculus musculus*	Former-Czechoslovakia
MAI/Pas	*M. musculus musculus*	Austria
STF/Pas	*Mus spretus*	Tunisia
SEG/Pas	*Mus spretus*	Spain
ZYD/Pas	*Mus spicilegus*	Former-Yugoslavia

This comparison revealed a high degree of polymorphism among all the WDIS and also among *Mus musculus* WDIS, reflecting the genetic heterogeneity these strains represent. As we expected the polymorphism among the CLIS was low (48.9%), however when comparing with the WDIS it increases significantly (79.8%). This confirms the advantage of using WDIS for genetic mapping studies. We found 1117 alleles among the WDIS not present in any CLIS, which represents an increase of 61% over the genetic variability described for laboratory strains. We also verified that on average 14.5% of the alleles found in the WDIS were unique, i.e., not present in any other strain included in the study (WDIS, CLIS and OWDIS). When comparing each group of WDIS with the laboratory strain, we found that *M.m. domesticus* WDIS was less polymorphic compared to CLIS, supporting the notion that CLIS are more closely related to *M.m domesticus* (Table 5)[125,126].

Table 5 Pairwise polymorphism between groups of WIDS of different *Mus* species

M.m domesticus	*M. m musculus*	*M. spretus*	*M.specilegus*	Species
74.6	82.5	85.8	85.2	Laboratory strains
	81.0	83.4	82.8	*M.m domesticus*
		81.4	81.4	*M. m musculus*
			81.8	*M. spretus*

Overall, this data confirms that WDIS exhibit a high degree of polymorphism and carry unique genetic variability that is likely be valuable in the search of novel phenotypes and construction of new mouse models.

Phenotype screening of wild-derived inbred strains with *P. berghei* ANKA
(Paper II)

As discussed above, infection with *P. berghei* ANKA is lethal due to either ECM or to severe anaemia and hyperparasitaemia, depending on the host. Mice susceptible to ECM show a decrease in body temperature, can have respiratory distress and show clear neurological manifestations such as deviation of the head, ataxia, paralysis and coma. Death usually occurs early, between days 6 to 12 after infection. Mice resistant to the neurological syndrome developed hyperparasitaemia (HP) and severe anaemia and died usually 2-4 weeks after infection. The genetic factors determining these differences in susceptibility are largely unknown.

The influence of the Major Histocompatibility Complex (MHC) on malaria susceptibility has been long debated. In Paper II we provided evidence that for *P. berghei* ANKA infection the H-2 haplotype is not to sufficient to determine the outcome of the disease, according to previous reports [92,134]. Different patters of susceptibility to ECM were observed among different mouse strains that share the same H-2 haplotype such as C57Bl/10.D2, BALB/c, DBA/2 (H-2d haplotype) and C57BL/6, 129/Sv, C3H/.B10 (H-2b haplotype). Moreover, H-2 congenics in C57BL/10 and C3H background were all susceptible to ECM, although different degrees of susceptibility were observed.

Since the WDIS carry a high degree of genetic variability, the probability of finding novel malaria resistant phenotypes increases. We screened 12 WDIS (the 10 strains mention above plus SPRETUS/Ei and CAST/Ei) for susceptibility/resistance to *P. berghei* ANKA infection. The strains from *Mus musculus musculus* subspecies developed ECM along with the C57BL/6 control strain. Among the *Mus musculus domesticus*, only the

strain WMP was susceptible to the neurological syndrome while the other three strains displayed a strong resistant phenotype but died later due to HP. All of the *Mus spretus* strains tested were also resistant to ECM, and died due to anaemia and cachexia. The ZYD strain (*Mus spicilegus*) and the CAST/Ei (*Mus musculus castaneous*) were both susceptible to the neurological manifestation (Table 6).

Table 6 Susceptibility/Resistance to ECM among WDIS

Cerebral malaria susceptible	Cerebral malaria resistant
WMP, MAI, MBT, PWK, CAST/Ei, ZID	SPRETUS/Ei, SEG, STF, WLA, BIK/g, 38CH

Segregation of malaria phenotypes

Among the WDIS strains tested, we found some strains that were resistant to ECM being ideal for genetic mapping studies. Three of these strains (WLA, 38CH and BIK/g) were bred with the susceptible C57BL/6 laboratory strain. The analysis of these crosses revealed that:

- F1 progenies derived from 38CH and BIK/g mice were susceptible to ECM (81% and 53-69% respectively) suggesting that C57BL/6 susceptibility alleles in these crosses act in a dominant fashion;
- In the backcross of the (38CH x C57BL/6)F1 susceptible mice to the parental 38CH ECM resistant, a majority of the cohort was still susceptible to ECM. The poor segregation of the malaria phenotypes suggested that many loci are involved in conferring the susceptible phenotype and discouraged the use of this cross for further genetic studies.
- All the (WLA x C57BL/6)F1 were resistant to ECM and displayed a phenotype similar to the parental WLA strain, indicating that resistance to ECM was a dominant trait in this cross;
- By backcrossing the (WLAxC57BL/6)F1 to the parental C57BL/6 we observed that 50% of the cohort was resistant to ECM while the remained died with typical neurological symptoms;
- The (WLAxC57BL/6)F2 progeny revealed a interesting novel phenotype with mice capable of surviving the lethal *P. berghei* ANKA infection. This observation was confirmed and analysed in detail in another independent (WLAxC57BL/6)F2 cohort.

10% of the F2 mice were found to reach limited parasitaemia peaks and rapidly clear parasitaemia and became cure. These animals remained asymptomatic for more than one year and were able to breed and give normal progeny. Some of these individuals were re-infected one-year after first infection and developed even lower parasitaemia levels and again survived, suggesting a memory response;

- The majority of the (WLA x C57Bl/6)F2 mice (72.68%) died due to the high levels of parasitaemia and 17.4% showed a severe form of disease, dying early with low parasitaemias. Within this group some individuals showed clear signs of ECM while no sings were detected in the remaining mice. However, we can not exclude the hypothesis that neurological syndrome in these mice developed rapidly and was not detected. It is also possible that these mice developed a severe form of disease, leading to early death, devoid of clear neurological symptoms;
- No obvious gender difference was observed in the parental strains or in any of the crosses studied.

Genetic mapping of resistance to cerebral malaria

(Paper III)

Having analysed the phenotypic differences, we aimed to identify the genetic factors responsible for the susceptibility/resistance to ECM induced by *P. berghei* ANKA infection. To facilitate the identification of those genetic factors we chose to analyse an (WLAx C57BL/6)F1xC57BL/6 backcross progeny. Two chromosomal regions located on chromosomes 1 and 11 were identified as being significantly associated with resistance to ECM. Since no genetic loci related to *P. berghei* infection have been identified before, these resistance loci were named *Berr*1 and *Berr*2 respectively *(Berghei resistance1 and 2*). The *Berr*1 locus spans a 20 cM region and reached the highest significant value at marker D1Mit221 (χ2=18.98; *P* =1.3x 10^{-5}). The *Berr*2 locus spans a region of 7 cM and the maximum value was over *D11Mit338* (χ^2=16.51; *P*=4.8x 10^{-5}). An additional locus on chromosome 14 with suggestive linkage (χ^2=10.19; *P*=1.4 x 10^{-3}) was also observed. As expected, according to the WLA parental phenotype, we observed an over-representation of the WLA alleles at *Berr*1 and *Berr*2 among the individuals that resisted to ECM.

Genetic mapping of malaria survival

(Paper IV)

In paper IV the genetic analysis was conducted using a cohort of 219 (WLAxC57BL/6)F2 mice. As described above, by analysing the course of infection (parasitaemia and survival time) the response to malaria in the F2 progeny was segregating in three different phenotypic groups: cure from infection, early death from severe malaria and later death from HP. We provided evidence of significant linkage of the three phenotypic groups to regions on distal chromosome 1 and distal chromosome 9.

We searched for QTL controlling survival time after infection using the two-part model[135]. Survival time was associated to both loci on chromosomes 1 (LOD=6.4 at D1Mit221) and 9 (LOD=4.9 at D9Mit18). The locus on chromosome 1 mapped within the previously reported *Berr*1 locus (Paper III). The locus on chromosome 9 was designated *Berr*3 once it was found not to be related previously to *P. berghei* infection. We also verified that among the animals that died due to hyperparasitaemia a different parasitaemia kinetics could be observed. We searched for QTLs controlling the survival time in these group of mice using the non-parametric QTL model[136]. We found association with a novel locus on chromosome 4, being designated *Berr*4 (LOD=3.42 at D4Mit27).

We considered that the WLA allele at the locus on chromosome 1 increased the probability of resistance to early death, as it was clearly under-represented among the mice that died early. At the *Berr*3 locus it seems that the C57BL/6 allele increased the probability to cure and survive infection as it is over–represented among the surviving mice. At the *Berr*4 locus an increase in the representation of WLA allele was observed among the group of animals that resisted longer to hyperparasitaemia before succumbing.

The *Berr* loci

*Berr*1

The *Berr*1 locus was found to be associated with resistance to ECM in a backcross progeny and the same region was associated with control of survival time in a F2 cohort. This probably represents the same locus confirmed in two different crosses. Data from papers III and IV suggests that individuals with WLA alleles, homozygous or heterozygous, show an increased probability to resist early death due to ECM or other severe manifestations. Although the resistance mechanism is unknown it is possible that this phenotype could result from lower sequestration of leukocytes, monocytes and infected and non-infected erythrocytes in certain organs, like the brain, thus preventing the development of pathology leading to early death.

It is interesting that several QTLs associated with resistance/susceptibility to intracellular pathogens (*Trypanosoma congolense, Leishmania major, Mycobacterium tuberculosis, Salmonella Typhimurium*) have been mapped in the *Berr*1 region[137-140](Fig 3). If these different QTLs represent the same genetic factor it would reveal the existence of a common genetic factor that determines the host response to different pathogens.

The *Berr*1 locus covers a substantial chromosomal segment on distal chromosome 1 (20 cM in the backcross, narrowed to 10 cM in the F2 progeny) which makes it difficult to propose potential candidate genes. Since numerous studies in murine models have demonstrated the role of several molecules of the immune system and of cell adhesion processes in preventing ECM, we looked for genes with immunological related functions in this region. Interesting candidate genes overlapping the region with highest association include the Toll-like receptor 5 (Tlr5), the Transforming growth factor beta 2 (Tgfβ-2) and the Tumour necrosis factor receptor-associated factor 5 (Traf5).

The Tlrs are pattern-recognition molecule receptors important for recognition of microbe-derived products to activate host innate immunity[141-144]. Some studies showed that protozoa could activate the Tlrs signalling to induce pathological changes in the host[145]. Flagellin from Gram-negative bacteria was shown to signal via Tlr5 and to stimulate several cells such as monocytes, fibroblats and epithelial cells to produce cytokines [146,147].

The Tgf-β superfamily members have been demonstrated to affect the development and function of immune cells. The Tgfβ-1 is known as an inflammation regulator and is described to be important for the regulation of host-parasite interactions [148-150]. It was reported to be inversely related with malaria severity in mice [110,151-153]. Interesting, Tgfβ2 was described to inhibit lymphocyte proliferation and treatment with recombinant Tgfβ-2 reduced severity of clinical symptoms after infection of rats with Borna disease virus-induced encephalomyelitis[154,155].

The Trafs are major signal transducers for the TNF receptor and the interleukin-1 receptor/Toll like receptor superfamilies[156]. They have been associated with a wide range of biological functions such as adaptive and innate immunity. Traf5 is involved in TNF-induced NF-kB and AP-1 transcription factors and in CD27 and CD4 signalling[157,158]. Traf5 deficiency leads to defects in CD40 and CD27 mediated lymphocyte activation[159].

Still in the *Berr*1 region several other genes can be mentioned due to their known function in host immune or inflammatory responses such as proteins that regulate complement activation, the Fas ligand, selectins (L, P and E), signaling lymphocyte

activation molecule, and also the F11 receptor, a component of endothelial and epithelial cells. The Nramp1 gene would be regarded as a potential candidate gene since it was associated with resistance against several intracellular infections in mice and NRAMP1 alleles have been associated with susceptibility to tuberculosis and leprosy in humans[140,160]. However it is localised in the proximal region of this chromosome very distant from *Berr*1.

***Berr*2**

This locus was found to control death due to ECM (paper I). Similar to *Berr*1, homozygocity or heterozygocity for WLA in this locus increased the probability to resist to ECM. We searched for potential candidate genes in this locus that spans a region of 7 cM in distal chromosome 11. A potential candidate gene close to the region with highest association is the CD7 antigen. This immunoglobulin is expressed in mature T and NK cells. Disruption of CD7 is associated with resistance to lipopolysaccharide (LPS) shock [161]. CD7 has been suggested to constitute a key molecule in the inflammatory response leading to LPS-induced shock and in regulating peripheral T and NK cell cytokine production[162].

Close to *Berr*2 mapped the immunoregulatory cytokine granulocyte colony-stimulating factor 3 (G-Csf-3) and the adhesion molecule Icam-2. It was observed that mice infected with the attenuated *P. berghei* XAT and treated with human recombinant G-CSF showed an increased neutrophil count and significant suppression of parasitaemia[163].

***Berr*3**

The *Berr*3 locus, mapped to the distal chromosome 9, was found to be associated with survival time after infection with *P.berghei* ANKA infection. The C57BL/6 allele at this locus conferred a high probability to survive infection. This novel phenotype of complete resistance to death was a very surprising observation. Mice rapidly cleared parasitaemia and cured similar to course of infection in mouse strains resistant to *P. chabaudi* and *P. yoelli*, non-lethal model infections. The F2 mice displaying this phenotype also showed an improved response to reinfection, suggesting that they established immunological memory.

The *Berr*3 locus overlaps with the loci *Char*1[164] and *Pymr*[118], described to be associated with resistance to *P. chabaudi* and *P. yoelli* respectively, emphasising the importance of this region for resistance to different *Plasmodium* strains (Fig 3).

The *Berr*3 locus spans a region of 10 cM, which contains several chemokine receptors (Ccr1-5, Cxcr6, Ccr9) mapping close to the highest associated region. These molecules play a wide role in immune homeostasis, and key roles in driving the maturation, homing, and activation of leukocytes[165]. It was observed that deficiency in Ccr1 increases susceptibility to *Toxoplasma gondii* and deficiency in Ccr5 decreases specific migration of *Toxoplasma gondii*-primed CD8 lymphocytes to inflammatory intestinal epithelial cells and also reduces susceptibility to ECM induced by *P. berghei* ANKA [166-168].

The adaptor protein Myd88 (myeloid differentiation), necessary to be recruited by TLRs to initiate signalling pathways, also maps in the same region[169]. Mice defective for Myd88 failed to control *Toxoplasma gondii* infection, are more susceptible to *Leishmania major* infection and do not show liver injury induced by *P. berghei* Nk65 infection although no difference in parasitaemia and mortality was found [170 171,172].

***Berr*4**

The *Berr*4 locus on chromosome 4 is involved in the control of time of death due to hyperparasitaemia. The WLA allele at this locus appears to increase the probability of resisting longer to high levels of parasitaemia. This phenomenon could be the result of an improved mechanism of host defence against parasite infection, lower parasite replication on red blood cells or improved clearance of infected erythrocytes.

This locus covers a region of 6 cM. The attractive genes in this region were: one member of the toll-like receptors (Tlr4) that regulates innate resistance to infection with *Salmonella Typhimurium* [173]; a granulocyte colony-stimulating factor receptor (G-Csf3r), and the complement component 8 beta subunit that interacts with other complement components to form a membrane attack complex (MAC) important for the killing of some pathogens [174,175].

It is important to mention that the *Berr* loci include numerous putative genes whose functions are still not known. The candidate genes mentioned are only possibilities, which association with susceptibility/resistance to malaria has yet to be formally tested. The fine mapping of these loci will help to refine the region of linkage and significantly decrease the number of candidate genes.

Although the genes predisposing to the disease in humans may not be identical to those in mouse models, the hope is that the underlying genetic basis in terms of number of genes involved and their influence on physiological disease processes may present similarities between the two species.

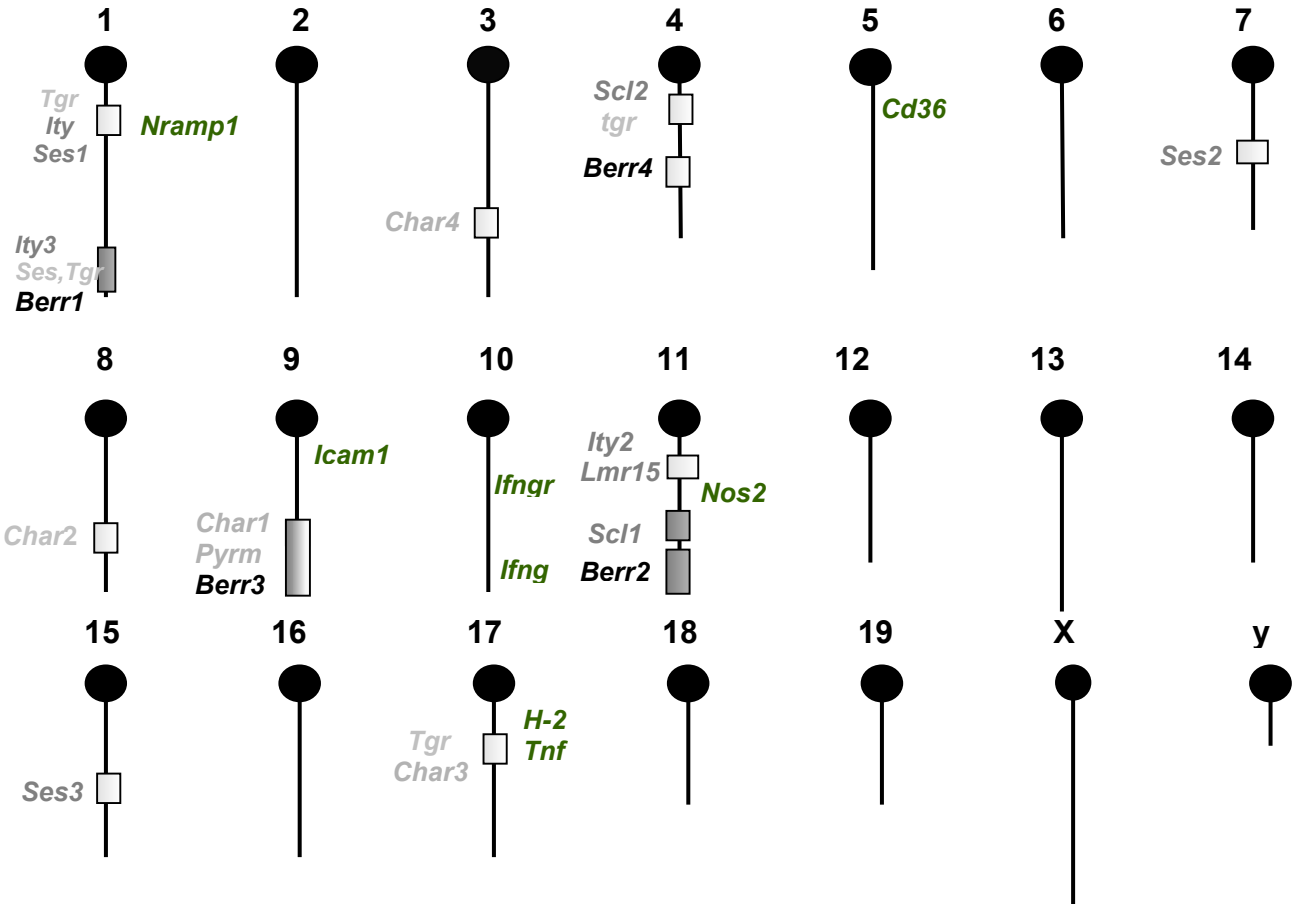


Fig 3.Host resistance/susceptibility to infectious diseases loci mapped in the mouse genome. The positions of the loci and genes were taken from the Mouse Genome Informatics database. Some genes studied in human malaria and other infectious diseases are shown on the right side. Boxes represent some loci involved in susceptibility/resistance to different infectious diseases that were mapped by QTL analysis. *Berr- P.Berghei* resistance; *Char- P.Chabaudi* resistance; *Pymr- P. yoelli* resistance; *Ses- S. enteritidis* susceptibility; *Ity-* immunity to *S. typhimurium*; *Tgr- T. gondii* resistance; *Scl-* susceptibility to cutaneous *leishmania*.

Genetic interaction among the *Berr* loci

The genes of an individual do not operate isolated from one another, but function in a common cellular or organic environment. Thus, it is expected that interactions between the genetic effects of individual loci would occur specially in cases of complex inheritance.

Since the *Berr* loci share the genetic control of malaria resistant phenotypes we analysed the possibility that resistance to disease results from joint genetic effects of the resistance loci. We performed two-loci interaction analysis starting by calculating the relative penetrance of the combined genotypes at two *Berr* loci to detect the presence of joint genotypic effects at the level of the resistant phenotypes. This was followed by fitting the observed joint effects in mathematical models of statistical interaction according to Cordell analysis[176].

We observed (Paper III) that resistance to ECM is controlled by the two major loci *Berr*1 and *Berr*2 in the backcross progeny. The relative penetrance of the combined genotypes of markers *D1MIT221* and *D11MIT338* suggests that the WLA allele at both loci have a joint effect in the penetrance of the ECM resistance phenotype (Fig 4). We mathematically modelled the joint effect of the two loci, on the linear, log odds of the penetrance and on the liability scales using several genetic models of interaction (additive, multiplicative and heterogeneity). We rejected the three genetic models, however modeling the joint effect of the loci on the penetrance scale did not rule out any of the fitted models of genetic interaction. We should mention that the use of a backcross progeny confines modeling to four genotype categories, which limits the power to discriminate between models.

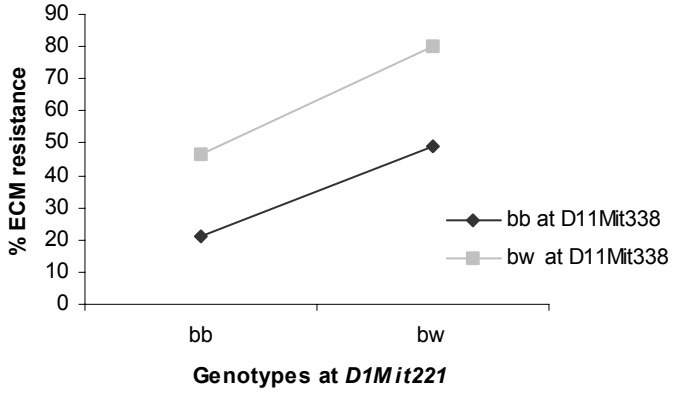


Fig 4 Penetrance of the ECM resistant phenotype given the combined genotypes at *D1MIT221* and *D11MIT338*. Penetrance was calculated as a ratio ECM resistance/total animals within each genotype category.

We also analysed the possibility of interaction between *Berr*1 and *Berr*3 in the F2 progeny at the survival time level after infection. Analysis of F2 survival identified three groups of mice: mice that die at an early stage with low parasitaemia; mice dying at a later stage with hyperparasitaemia; and mice that survive infection. Standard genetic analysis showed that both *Berr*1 and *Berr*3 are major loci in controlling survival time. To better understand the role of these loci to the survival time we analysed separately their contribution to early death and to survival of infection.

The effect of the combined genotypes at *Berr*1and *Berr*3 on the penetrance of the early death phenotype shows that the Berr1 locus has a major effect on the control of early death (Fig 5). Homozygous state of WLA allele at *Berr*1 locus confers resistance to early death and is able to override the effect of the *Berr*3 locus. When the *Berr*1 locus shows C57BL/6 homozygous configuration the *Berr* 3 locus reveals a significant effect on the resistance to early death. This situation suggests that the WLA allele of *Berr*1 conditions the phenotypic effect of the *Berr*3 locus excluding a model of genetic heterogeneity, which implies independent gene action.

To test whether these genotypic effects follow defined models of interaction we fitted the observed data to multiplicative and additive models of genetic interaction. This analysis excluded that interaction of *Berr*1 and *Berr*2 follow an additive model and suggested that the interaction may influence the penetrance of the early death phenotype in a multiplicative fashion.

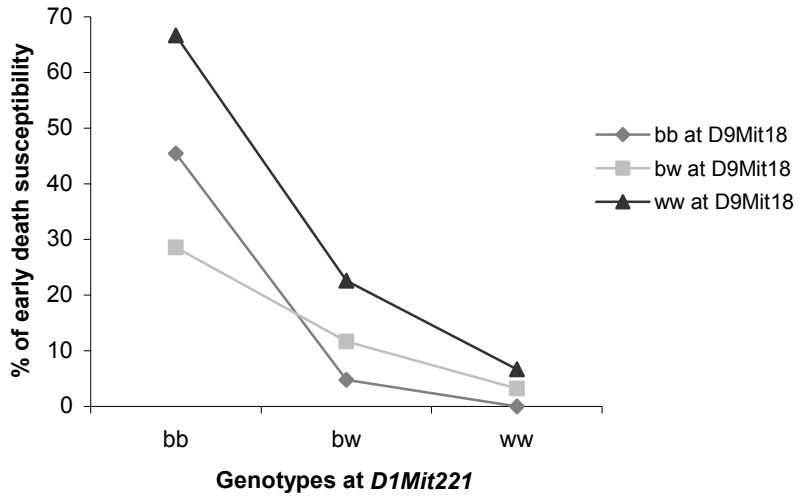


Fig 5 Penetrance of the early death susceptibility phenotype given the combined genotypes at *D1MIT221* and *D9MIT18*. Penetrance was calculated as a ratio the early death susceptibility /total animals within each genotype category.

Likewise, the combined effect of *Berr*1 and *Berr* 3 was analysed at survival to infection level. This analysis shows that the C57BL/6 homozygous configuration at *Berr* 3 is a major locus in conferring survival of infection (Fig 6). This resistance effect is increased by WLA allele at *Berr*1 locus. Statistical modelling showed that this interaction most probably follows a multiplicative mode of action.

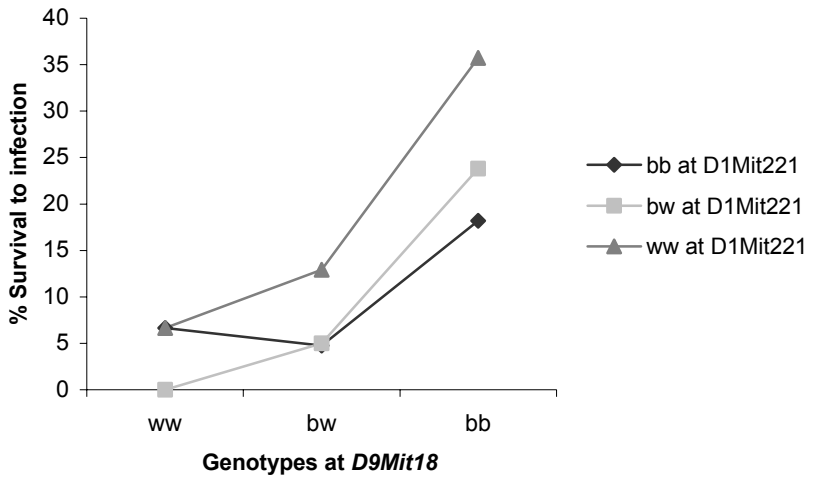


Fig 6 Penetrance of the survival to infection phenotype given the combined genotypes at *D1MIT221* and *D9MIT18*. Penetrance was calculated as a ratio the early death susceptibility /total animals within each genotype category.

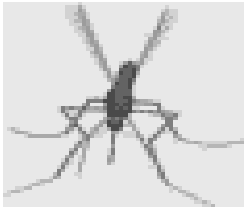
It is important to mention that the detection of a statistical interaction does not necessarily imply interaction on the biological or mechanistic level. Moreover, since the presence of a statistical interaction depends on the scale of measurement (i.e., whether we choose to model the effects in penetrance, the log odds, or the liability), it is unclear what the biological interpretation should be.

However, identification of the most parsimonious statistical model for the joint effects of alleles at several loci provides a means for improved prediction of phenotypes, compared to considering the loci in isolation. Further research concerning the interpretation and significance of statistical results will be required if statistical analyses such as those presented here are to be used to elucidate the underlying biologic mechanisms involved in complex traits.

Establishment of *Berr* congenic strains

The construction of congenic strains aims to isolate the genetic factors underlying a single QTL, permitting evaluation of the contribution of the individual QTLs to the malaria pathogenesis. We established a series of congenic strains for the loci *Berr*1 and for *Berr*2. Congenic strains are being generated by repeated backcrossing to the C57BL/6 parental strain with selection for the differential segment with WLA alleles. To establish a congenic strain we use a breeding scheme (known as 'speed congenics') involving both positive selection for the desired differential segment and selection against the rest of the donor genome amongst progeny in order to accelerate the establishment congenic strains[121].

This process leads to the generation of a congenic strain with less than 0.5% contaminating donor genome in fewer generations than the classical strategy. We have already establish the congenic strains for both *Berr*1 and *Berr*2 and we are now reducing the size of the congenic regions and setting up several series of congenic mice each containing a different congenic segment. The different congenic strains will be tested for resistance to early death after *P. berghei* ANKA infection. The congenic strains will hopefully refine the regions of linkage and evaluate the contribution of each *Berr* loci to malaria pathogenesis.



Concluding remarks

The aim of this thesis work has been to find novel loci associated with resistance to murine malaria induced by the lethal *Plasmodium berghei* ANKA by exploiting the high genetic variability provide by WDIS. This thesis provides evidence that:

By doing a genome polymorphism survey using 10 WDIS we observed that these strains encompass a high rate of polymorphism and represent a source of new and unique naturally occurring genetic variability.

While most laboratory strains were shown to be susceptible to ECM induced by *P. berghei* ANKA infection, in this thesis we demonstrated that several wild-derived strains were found to be resistant to ECM.

Resistance to ECM in a backcross (WLAxC57BL/6)F1xC57BL/6 progeny is genetically controlled by genes linked to a region on chromosome 1 (*Berr*1 locus) and on chromosome 11 (*Berr*2 locus). Resistance results from action of genetic factors derived from the WLA wild-derived inbred strain at both loci.

Unprecedented cure to lethal *P. berghei* ANKA infection was observed in a cohort of a (WLAxC57BL/6)F2 progeny. This represents the first observation of mice capable of clearing parasitaemia and establishing immunological memory providing a unique model for studying cure to *P. berghei* ANKA infection.

Genetic mapping of the F2 progeny showed that the survival time in this cohort is controlled by a locus on chromosome 1 (*Berr*1) and at a locus on chromosome 9 (*Berr*3). Survival to infection was showed to be a combinatorial effect of genetic factors derived from the two parental strains.

The phenotype of delayed death due to HP observed in the F2 cohort is controlled by a locus on chromosome 4 (*Berr*4). Resistance was conferred by genetic factors derived from the WLA strain.

Acknowledgement

To Dan for always trusting me and giving me all the freedom to perform my project. For all your enthusiasm about work; for all the secret experiments and for being so Portuguese.

To Carlos, for teaching me almost everything I know about science; for all the remarkable help and support, for being a friend always there even when we were in different countries.

To Prof. Cazenave and Sylviane Pied, thank you for collaboration and support during the entire project. Merci pour m´avoir si bien reçut à l´Institut Pasteur.

To all others that collaborate in the performance of this thesis work.

To Prof. Coutinho for guiding me to Dan, and for all the enthusiasm with my work.

To all the people at the Gulbenkian Institute that made each day a better day and the Institute a fantastic place to work. A special thank to Margarida V., Moisés and Maria M., for reading and correcting my thesis.

To all the people at Cazenave and Sylviane´s Lab at Pasteur Institute for the great help and support. Merci à tout!

To all the people of Dan´s group at Umeå University for all the fun and support mainly at end of my thesis! Jag skulle vilja tacka alla för den trevliga arbetsmiljön och för många mysiga fika tillfällen. A special thank to Kurt for correcting the language of my thesis.

To all my friends, life is much easy with you! Martinha you were the first, a long time ago! We gave so many steps in our lives together. Lisa, my baby, such a team we make at work and such a team we make in friendship. You are good! Patricia, Sofia, Ana and Susana, for your nice friendship. To my new but great friends, Margarida C. for all the gorgeous moments, I miss our afternoons at Cascais, Margarida V. for all our secret conversations in your office, Moisés for being such a nice person.

Nádia, whenever I am sad or happy, I know you are with me. For all the funny conversation, for all the great moments we spent together and for all your patience!!

A special thank to my family that are so precious to me. My mother for being more than a mother, for being my best friend, for the 100% trust and love. My father, for being an amazing father and friend, and for the 100% concern and love. My lovely sister, for being the 100% best sister in the world, “carne da mesma carne, sangue do mesmo sangue”. Eu sei que posso sempre contar com vocês! Os 3 amores da minha vida!

To Mário and Lídia, for all the support and for being like parents to me.

To the others “brothers and sister”, and closest family, thank you!

And to Luky, my little baby, thank you for understanding us so much!

Obrigado!

References

1. Greenwood, B. & Mutabingwa, T. Malaria in 2002. *Nature* 415, 670-2. (2002).
2. Trigg, P. & Kondrachine, A. The current global malaria situation. in *Malaria: parasite biology, pathogenesis and protection* (ed. Sherman, I.) 11-22 (ASM Press, Washington, 1998).
3. Ayala, F., Escalante, A., Lal, A. & Rich, S. Evolutionary relationships of human malaria parasites. in *Malaria: parasite biology, pathogenesis and protection* (ed. Sherman, I.) 285-300 (ASm Press, Washington, 1998).
4. Miller, L.H., Baruch, D.I., Marsh, K. & Doumbo, O.K. The pathogenic basis of malaria. *Nature* 415, 673-9. (2002).
5. Bruce, M.C. et al. Cross-species interactions between malaria parasites in humans. *Science* 287, 845-8. (2000).
6. Gardner, M.J. et al. Genome sequence of the human malaria parasite Plasmodium falciparum. *Nature* 419, 498-511 (2002).
7. Miller, L.H. & Greenwood, B. Malaria--a shadow over Africa. *Science* 298, 121-2. (2002).
8. Beier, J. & Vanderberg, P. Sporogony development in the mosquito. in *Malaria: parasite biology, pathogenesis and protection* (ed. Sherman, I.) 49-61 (Washington, 1998).
9. Frevert, U. & Crisanti, A. Invasion of vertebrate cells:hepatocytes. in *Malaria: parasite biology, pathogenesis and protection* (ed. Sherman, I.) 73-91 (ASM Press, Washington, 1998).
10. Barnwell, J.W. & Galinski, M.R. Invasion of vertebrate cells:erythrocytes. in *Malaria: parasite biology, pathogenesis and protection* (ed. Sherman, I.) 93-120 (ASM Press, Washington, 1998).
11. Clark, I.A. & Schofield, L. Pathogenesis of malaria. *Parasitol Today* 16, 451-4 (2000).
12. White, N. Malaria pathophysiology. in *Malaria: parasite biology, pathogenesis and protection* (ed. Sherman, I.) 371-385 (ASM Press, Washington, 1998).
13. Miller, L.H., Baruch, D.I., Marsh, K. & Doumbo, O.K. The pathogenic basis of malaria. *Nature* 415, 673-9 (2002).
14. Turner, G. Cerebral malaria. *Brain Pathol* 7, 569-82 (1997).
15. van Hensbroek, M.B., Palmer, A., Jaffar, S., Schneider, G. & Kwiatkowski, D. Residual neurologic sequelae after childhood cerebral malaria. *J Pediatr* 131, 125-9 (1997).
16. Warrell, D.A. Cerebral malaria: clinical features, pathophysiology and treatment. *Ann Trop Med Parasitol* 91, 875-84 (1997).
17. Berendt, A.R., Turner, G. & Newbold, C. Cerebral Malaria: the sequestration hypothesis. *Parasitol Today* 10, 412-414 (1994).
18. Grau, G. & de Kossodo, S. CErebral Malaria:Mediators,Mechanical Obstruction or more? *Parasitol Today* 10, 408-409 (1994).
19. Clark, I. & Rockett, K. The cytokine theory of human cerebral malaria. *Parasitol Today* 10, 410-411 (1994).
20. Weatherall, D.J. et al. Malaria and the red cell. *Hematology (Am Soc Hematol Educ Program)*, 35-57 (2002).
21. Menendez, C., Fleming, A.F. & Alonso, P.L. Malaria-related anaemia. *Parasitol Today* 16, 469-76 (2000).
22. Miller, L.H., Good, M.F. & Milon, G. Malaria pathogenesis. *Science* 264, 1878-83 (1994).
23. Lander, E.S. & Schork, N.J. Genetic dissection of complex traits. *Science* 265, 2037-48 (1994).
24. Lander, E. & Kruglyak, L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11, 241-7 (1995).
25. Schork, N.J. Genetics of complex disease: approaches, problems, and solutions. *Am J Respir Crit Care Med* 156, S103-9 (1997).
26. Balding, D. *Handbook of Statistical Genetics*, (John wiley& Sons, 2001).
27. Weatherall, D.J. Phenotype-genotype relationships in monogenic disease: lessons from the thalassaemias. *Nat Rev Genet* 2, 245-55. (2001).
28. Aidoo, M. et al. Protective effects of the sickle cell gene against malaria morbidity and mortality. *Lancet* 359, 1311-2 (2002).
29. Hill, A.V. The immunogenetics of human infectious diseases. *Annual Review of Immunology* 16, 593-617 (1998).
30. Weatherall, D.J. Host genetics and infectious disease. *Parasitology* 112 Suppl, S23-9 (1996).
31. Carlson, J., Nash , G., Gabutti, V., al-Yaman, F. & Wahlgren, M. Natural protection against severe Plasmodium falciparum malaria due to impaired rosette formation. *Blood* 84(11), 3909-14. (1994).
32. Hutagalung, R. et al. Influence of hemoglobin E trait on the severity of Falciparum malaria. *J Infect Dis* 179, 283-6. (1999).
33. Modiano, D. et al. Haemoglobin C protects against clinical Plasmodium falciparum malaria. *Nature* 414, 305-8 (2001).

34. Agarwal, A. et al. Hemoglobin C associated with protection from severe malaria in the Dogon of Mali, a West African population with a low prevalence of hemoglobin S. *Blood* 96, 2358-63 (2000).
35. Allen, S.J. et al. alpha+-Thalassemia protects children against disease caused by other infections as well as malaria. *Proc Natl Acad Sci U S A* 94, 14736-41. (1997).
36. Weatherall, D.J. Phenotype-genotype relationships in monogenic disease: lessons from the thalassaemias. *Nat Rev Genet* 2, 245-55 (2001).
37. Miller, L.H. Impact of malaria on genetic polymorphism and genetic diseases in Africans and African Americans. *Proc Natl Acad Sci U S A* 91, 2415-9 (1994).
38. Weatherall, D.J. & Clegg, J.B. Genetic variability in response to infection: malaria and after. *Genes Immun* 3, 331-7 (2002).
39. Tournamille, C., Colin, Y., Cartron, J. & Le Van Kim, C. Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in Duffy-negative individuals*.: Nat Genet.* 2, 224-8. (1995).
40. Migot-Nabias, F. et al. Human genetic factors related to susceptibility to mild malaria in Gabon. *Genes Immun* 1, 435-41 (2000).
41. Saunders, M.A., Hammer, M.F. & Nachman, M.W. Nucleotide variability at G6pd and the signature of malarial selection in humans. *Genetics* 162, 1849-61 (2002).
42. Tishkoff, S.A. et al. Haplotype diversity and linkage disequilibrium at human G6PD: recent origin of alleles that confer malarial resistance. *Science* 293, 455-62 (2001).
43. Cappadoro, M. et al. Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)- deficient erythrocytes parasitized by Plasmodium falciparum may explain malaria protection in G6PD deficiency. *Blood* 92, 2527-34. (1998).
44. Hill, A.V. & Weatherall, D. Host genetic factors in resistance to malaria. in *Malaria:Parasite, biology, pathogenesis and protection* (ed. Sherman, I.) 445-455 (ASM, Press, Washington, 1998).
45. Genton, B. et al. Ovalocytosis and cerebral malaria. *Nature* 378(6557), 564-5. (1995).
46. Modiano, D. et al. Different response to Plasmodium falciparum malaria in west African sympatric ethnic groups. *Proc Natl Acad Sci U S A* 93, 13206-11 (1996).
47. Modiano, D. et al. The lower susceptibility to Plasmodium falciparum malaria of Fulani of Burkina Faso (west Africa) is associated with low frequencies of classic malaria-resistance genes. *Trans R Soc Trop Med Hyg* 95, 149-52 (2001).
48. Luoni, G. et al. Antimalarial antibody levels and IL4 polymorphism in the Fulani of West Africa. *Genes Immun* 2, 411-4 (2001).
49. Hill, A.V. et al. Extensive genetic diversity in the HLA class II region of Africans, with a focally predominant allele, DRB1*1304. *Proc Natl Acad Sci U S A* 89, 2277-81 (1992).
50. Hill, A.V. et al. Common west African HLA antigens are associated with protection from severe malaria. *Nature* 352, 595-600 (1991).
51. Hill, A.V. et al. Molecular analysis of the association of HLA-B53 and resistance to severe malaria. *Nature* 360, 434-9 (1992).
52. Odeh, M. The role of tumour necrosis factor-alpha in the pathogenesis of complicated falciparum malaria. *Cytokine* 14, 11-8 (2001).
53. Kwiatkowski, D. et al. TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated Plasmodium falciparum malaria. *Lancet* 336, 1201-4 (1990).
54. Kwiatkowski, D. Tumour necrosis factor, fever and fatality in falciparum malaria. *Immunol Lett* 25, 213-6 (1990).
55. Kern, P. et al. Soluble tumor necrosis factor receptors correlate with parasitemia and disease severity in human malaria. *J Infect Dis* 166, 930-4 (1992).
56. Deloron, P. et al. Plasma levels of TNF-alpha soluble receptors correlate with outcome in human falciparum malaria. *Eur Cytokine Netw* 5, 331-6 (1994).
57. Jakobsen, P., CA, B., J, T. & JH., P. Malaria: toxins, cytokines and disease. *Parasite Immunol* 17(5), 223-31. (1995).
58. McGuire, W., Hill, A.V., Allsopp, C.E., Greenwood, B.M. & Kwiatkowski, D. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. *Nature* 371, 508-10 (1994).
59. Roy, S. et al. TNF promotor polymorphism and susceptibility to lepramatous leprosy. *J Infect Dis* 176 (2), 530-2 (1997).
60. Wilson, A.G., Symons, J.A., McDowell, T.L., McDevitt, H.O. & Duff, G.W. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci U S A* 94, 3195-9 (1997).
61. McGuire, W. et al. Severe malarial anemia and cerebral malaria are associated with different tumor necrosis factor promoter alleles. *J Infect Dis* 179, 287-90 (1999).
62. Knight, J.C. et al. A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria. *Nat Genet* 22, 145-50 (1999).

63. Smith, J.D. et al. Identification of a Plasmodium falciparum intercellular adhesion molecule-1 binding domain: a parasite adhesion trait implicated in cerebral malaria. *Proc Natl Acad Sci U S A* 97, 1766-71 (2000).
64. Kun JF & Klabunde J, L.B., Luckner D, Alpers M, May J, Meyer C, Kremsner PG. Association of the ICAM-1Kilifi mutation with protection against severe malaria in Lambarene, Gabon. *Am J Trop Med Hyg.* 61(5), 776-9. (1999).
65. Fernandez-Reyes, D. et al. A high frequency African coding polymorphism in the N-terminal domain of ICAM-1 predisposing to cerebral malaria in Kenya. *Hum Mol Genet* 6, 1357-60 (1997).
66. Bellamy, R., Kwiatkowski D & AV., H. Absence of an association between intercellular adhesion molecule 1, complement receptor 1 and interleukin 1 receptor antagonist gene polymorphisms and severe malaria in a West African population. *Trans R Soc Trop Med Hyg.* 92(3), 312-6 (1998).
67. Craig, A. et al. A functional analysis of a natural variant of intercellular adhesion molecule-1 (ICAM-1Kilifi). *Hum Mol Genet* 9, 525-30 (2000).
68. Adams, S. et al. Differential binding of clonal variants of Plasmodium falciparum to allelic forms of intracellular adhesion molecule 1 determined by flow adhesion assay. *Infect Immun* 68, 264-9 (2000).
69. Febbraio, M., Hajjar, D.P. & Silverstein, R.L. CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. *J Clin Invest* 108, 785-91 (2001).
70. Pain, A. et al. A non-sense mutation in Cd36 gene is associated with protection from severe malaria. *Lancet* 357, 1502-3 (2001).
71. Aitman, T.J. et al. Malaria susceptibility and CD36 mutation. *Nature* 405, 1015-6 (2000).
72. Omi, K. et al. CD36 polymorphism is associated with protection from cerebral malaria. *Am J Hum Genet* 72, 364-74 (2003).
73. Clark, A.G., Rockett, K. & Cowden, W.B. Posible role of nitric oxide in conditions clinically similat to cerebral malaria. *The Lancet* 340, 894-896 (1992).
74. Perkins, D.J. et al. Blood mononuclear cell nitric oxide production and plasma cytokine levels in healthy gabonese children with prior mild or severe malaria. *Infect Immun* 67, 4977-81 (1999).
75. Anstey, N.M. et al. Nitric oxide in Tanzanian children with malaria: inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. *J Exp Med* 184, 557-67 (1996).
76. Chiwakata, C.B., Hemmer, C.J. & Dietrich, M. High levels of inducible nitric oxide synthase mRNA are associated with increased monocyte counts in blood and have a beneficial role in Plasmodium falciparum malaria. *Infect Immun* 68, 394-9 (2000).
77. Lopansri, B.K. et al. Low plasma arginine concentrations in children with cerebral malaria and decreased nitric oxide production. *Lancet* 361, 676-8 (2003).
78. Boutlis, C.S. et al. Nitric oxide production and mononuclear cell nitric oxide synthase activity in malaria-tolerant Papuan adults. *Infect Immun* 71, 3682-9 (2003).
79. Kun, J.F. et al. Nitric oxide synthase 2(Lambarene) (G-954C), increased nitric oxide production, and protection against malaria. *J Infect Dis* 184, 330-6 (2001).
80. Burgner, D. et al. Inducible nitric oxide synthase polymorphism and fatal cerebral malaria. *The Lancet* 352, 193-194 (1998).
81. Hobbs, M.R. et al. A new NOS2 promoter polymorphism associated with increased nitric oxide production and protection from severe malaria in Tanzanian and Kenyan children. *Lancet* 360, 1468-75 (2002).
82. Burgner, D. et al. Nucleotide and haplotypic diversity of the NOS2A promoter region and its relationship to cerebral malaria. *Hum Genet* 112, 379-86 (2003).
83. Hunt, N.H. & Grau, G.E. Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. *Trends Immunol* 24, 491-9 (2003).
84. Koch, O. et al. IFNGR1 gene promoter polymorphisms and susceptibility to cerebral malaria. *J Infect Dis* 185, 1684-7 (2002).
85. Rihet, P. et al. Human malaria: segregation analysis of blood infection levels in a suburban area and a rural area in Burkina Faso. *Genet Epidemiol* 15, 435-50 (1998).
86. Abel, L., Cot, M., Mulder, L., Carnevale, P. & Feingold, J. Segregation analysis detects a major gene controlling blood infection levels in human malaria. *Am J Hum Genet* 50, 1308-17 (1992).
87. Rihet, P. et al. Malaria in humans: Plasmodium falciparum blood infection levels are linked to chromosome 5q31-q33. *Am J Hum Genet* 63, 498-505. (1998).
88. Flori, L. et al. Linkage and association between Plasmodium falciparum blood infection levels and chromosome 5q31-q33. *Genes Immun* 4, 265-8 (2003).
89. McLeod, R., Buschman, E., Arbuckle, L. & Skamene, E. Immonogenetics in the analysis of resistance to intracellular pathogens. *Curr Opin Immunol* 7, 539-552 (1995).
90. Martin, M., M, D. & Smith, M. Genetic acceleration of AIDS progression by a promotor variant of CCR5. *Science* 282 (6497), 1907-1911 (1998).
91. Gysin, J. Animal models:primates. in *Malaria: parasite biology, pathogenesis and protection* (ed. Sherman, I.) 419-441 (ASM Press, Washington, 1998).

92. de Souza, J.B. & Riley, E.M. Cerebral malaria: the contribution of studies in animal models to our understanding of immunopathogenesis. *Microbes Infect* 4, 291-300 (2002).
93. Lou, J., Lucas, R. & Grau, G.E. Pathogenesis of cerebral malaria: recent experimental data and possible applications for humans. *Clin Microbiol Rev* 14, 810-20, table of contents (2001).
94. Chitnis, C. & Blackman, M. Host Cell invasion by malaria parasites. *Parasitol Today* 16, 411-15 (2000).
95. Laudau, I. & Gautret, P. Animal models:rodents. in *Malaria: parasite biology, pathogenesis and protection* 401-417 (ASM Press, Washington, 1998).
96. Fortin, A., Stevenson, M.M. & Gros, P. Complex genetic control of susceptibility to malaria in mice. *Genes Immun* 3, 177-86 (2002).
97. Tylor-Robinson, A. Murine Models of Cerebral Malaria: Aqualified Defense. *Parasitology Today* 11, 407-408 (1995).
98. Neill, A.L. & Hunt, N.H. Pathology of fatal and resolving Plasmodium berghei cerebral malaria in mice. *Parasitology* 105 (Pt 2), 165-75 (1992).
99. Yanez, D., Manning, D., Cooley, A., Weidanz, W. & van der Heyde, H.C. Participation of lymphocyte subpopulations in the pathogenesis of experimental murine cerebral malaria. *Journal Immunology* 157, 1620-1624 (1996).
100. Chang, W.L. et al. CD8(+)-T-cell depletion ameliorates circulatory shock in Plasmodium berghei-infected mice. *Infect Immun* 69, 7341-8 (2001).
101. Xu, H. et al. The mechanism and significance of deletion of parasite-specific CD4(+) T cells in malaria infection. *J Exp Med* 195, 881-92 (2002).
102. Hermsen, C., van de Wiel, T., Mommers, E., Sauerwein, R. & Eling, W. Depletion of CD4+ or CD8+ T-cells prevents Plasmodium berghei induced cerebral malaria in end-stage disease. *Parasitology* 114 (Pt 1), 7-12 (1997).
103. Clark, I.A. & Cowden, W.B. The pathophysiology of falciparum malaria. *Pharmacol Ther* 99, 221-60 (2003).
104. Senaldi, G. et al. Protection against the mortality associated with disease models mediated by TNF and IFN-gamma in mice lacking IFN regulatory factor-1. *J Immunol* 163, 6820-6 (1999).
105. Rudin, W. et al. Resistance to cerebral malaria in tumor necrosis factor-alpha/beta-deficient mice is associated with a reduction of intercellular adhesion molecule-1 up-regulation and T helper type 1 response. *Am J Pathol* 150, 257-66 (1997).
106. Grau, G.E., Piguet, P.F., Vassalli, P. & Lambert, P.H. TNF and other cytokines in cerebral malaria:experimental and clinical data. *Immunological Reviews* 112, 49-70 (1989).
107. Clark, I.A., Ilschner, S., MacMicking, J.D. & Cowden, W.B. TNF and Plasmodium berghei ANKA-induced cerebral malaria. *Immunol Lett* 25, 195-8 (1990).
108. Favre, N. et al. Role of ICAM-1 (CD54) in the development of murine cerebral malaria. *Microbes Infect* 1, 961-8 (1999).
109. de Kossodo, S. & Grau, G.E. Profiles of cytokine production in relation with susceptibility to cerebral malaria. *J Immunol* 151, 4811-20 (1993).
110. Omer, F.M. & Riley, E.M. Transforming growth factor beta production is inversely correlated with severity of murine malaria infection. *J Exp Med* 188, 39-48 (1998).
111. Camp, N.j. & Cox, A. *Quantitative Trait loci: Methods and Protocols*, (Humana press, Totowa, New Jersey, 2002).
112. Lander, E. & Kruglyak, L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11, 241-7. (1995).
113. Broman, K.W., Wu, H., Sen, S. & Churchill, G.A. R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 19, 889-90. (2003).
114. Broman, K.W. Mapping quantitative trait Loci in the case of a spike in the phenotype distribution. *Genetics* 163, 1169-75. (2003).
115. Fortin, A. et al. Genetic control of blood parasitaemia in mouse malaria maps to chromosome 8. *Nat Genet* 17, 382-3 (1997).
116. Foote, S.J. et al. Mouse loci for malaria-induced mortality and the control of parasitaemia. *Nat Genet* 17, 380-1. (1997).
117. Burt, R.A., Baldwin, T.M., Marshall, V.M. & Foote, S.J. Temporal expression of an H2-linked locus in host response to mouse malaria. *Immunogenetics* 50, 278-85. (1999).
118. Ohno, T. et al. Chromosomal mapping of the host resistance locus to rodent malaria (Plasmodium yoelii) infection in mice. *Immunogenetics* 53, 736-40. (2001).
119. Nagayasu, E. et al. Association of a determinant on mouse chromosome 18 with experimental severe Plasmodium berghei malaria. *Infect Immun* 70, 512-6 (2002).
120. Burt, R.A. et al. Mice that are congenic for the char2 locus are susceptible to malaria. *Infect Immun* 70, 4750-3. (2002).
121. Rogner, U.C. & Avner, P. Congenic mice: cutting tools for complex immune disorders. *Nat Rev Immunol* 3, 243-52 (2003).

122. Fortin, A. et al. Recombinant congenic strains derived from A/J and C57BL/6J: a tool for genetic dissection of complex traits. *Genomics* 74, 21-35 (2001).
123. Fortin, A. et al. Identification of a new malaria susceptibility locus (Char4) in recombinant congenic strains of mice. *Proc Natl Acad Sci U S A* 98, 10793-8 (2001).
124. Beck, J.A. et al. Genealogies of mouse inbred strains. *Nat Genet* 24, 23-5 (2000).
125. Wade, C.M. et al. The mosaic structure of variation in the laboratory mouse genome. *Nature* 420, 574-8 (2002).
126. Silver, M.L. *Mouse Genetics Concepts and Applications*, (Oxford University Press, New York, Oxford., 1995).
127. Guenet, J.L. Wild mice as a source of genetic polymorphism. *Pathol Biol (Paris)* 46, 685-8. (1998).
128. Guenet, J.L. & Bonhomme, F. Wild mice: an ever-increasing contribution to a popular mammalian model. *Trends Genet* 19, 24-31. (2003).
129. Waterston, R.H. et al. Initial sequencing and comparative analysis of the mouse genome. *Nature* 420, 520-62 (2002).
130. Montagutelli, X., Serikawa, T. & Guenet, J.L. PCR-analyzed microsatellites: data concerning laboratory and wild-derived mouse inbred strains. *Mamm Genome* 1, 255-9 (1991).
131. Routman, E.J. & Cheverud, J.M. Polymorphism for PCR-analyzed microsatellites between the inbred mouse strains LG and SM. *Mamm Genome* 6, 401-4 (1995).
132. Fowlis, G.A., Adelman, S., Knight, A.M. & Simpson, E. PCR-analyzed microsatellites of the mouse genome--additional polymorphisms among ten inbred mouse strains. *Mamm Genome* 3, 192-6 (1992).
133. Kikkawa, Y. et al. Microsatellite database for MSM/Ms and JF1/Ms, molossinus-derived inbred strains. *Mamm Genome* 12, 750-2 (2001).
134. Grau, G.E. et al. Significance of cytokine production and adhesion molecules in malarial immunopathology. *Immunol Lett* 25, 189-94 (1990).
135. Broman, K.W. Mapping quantitative trait Loci in the case of a spike in the phenotype distribution. *Genetics* 163, 1169-75 (2003).
136. Broman, K.W., Wu, H., Sen, S. & Churchill, G.A. R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 19, 889-90 (2003).
137. Mitsos, L.M. et al. Genetic control of susceptibility to infection with Mycobacterium tuberculosis in mice. *Genes Immun* 1, 467-77 (2000).
138. Iraqi, F. et al. Fine mapping of trypanosomiasis resistance loci in murine advanced intercross lines. *Mamm Genome* 11, 645-8 (2000).
139. Badalova, J. et al. Separation and mapping of multiple genes that control IgE level in Leishmania major infected mice. *Genes Immun* 3, 187-95 (2002).
140. Roy, M.F. & Malo, D. Genetic regulation of host responses to Salmonella infection in mice. *Genes Immun* 3, 381-93 (2002).
141. Kopp, E. & Medzhitov, R. Recognition of microbial infection by Toll-like receptors. *Curr Opin Immunol* 15, 396-401 (2003).
142. Kopp, E.B. & Medzhitov, R. The Toll-receptor family and control of innate immunity. *Curr Opin Immunol* 11, 13-8 (1999).
143. Underhill, D.M. Toll-like receptors: networking for success. *Eur J Immunol* 33, 1767-75 (2003).
144. Sabroe, I. et al. Toll-like receptors in health and disease: complex questions remain. *J Immunol* 171, 1630-5 (2003).
145. Qureshi, S.T. & Medzhitov, R. Toll-like receptors and their role in experimental models of microbial infection. *Genes Immun* 4, 87-94 (2003).
146. Mizel, S.B., Honko, A.N., Moors, M.A., Smith, P.S. & West, A.P. Induction of macrophage nitric oxide production by Gram-negative flagellin involves signaling via heteromeric Toll-like receptor 5/Toll-like receptor 4 complexes. *J Immunol* 170, 6217-23 (2003).
147. Sebastiani, G. et al. Cloning and characterization of the murine toll-like receptor 5 (Tlr5) gene: sequence and mRNA expression studies in Salmonella-susceptible MOLF/Ei mice. *Genomics* 64, 230-40 (2000).
148. Fitzpatrick, D.R. & Bielefeldt-Ohmann, H. Transforming growth factor beta in infectious disease: always there for the host and the pathogen. *Trends Microbiol* 7, 232-6 (1999).
149. Letterio, J.J. Murine models define the role of TGF-beta as a master regulator of immune cell function. *Cytokine Growth Factor Rev* 11, 81-7 (2000).
150. Omer, F.M., Kurtzhals, J.A. & Riley, E.M. Maintaining the immunological balance in parasitic infections: a role for TGF-beta? *Parasitol Today* 16, 18-23 (2000).
151. Li, C., Sanni, L.A., Omer, F., Riley, E. & Langhorne, J. Pathology of Plasmodium chabaudi chabaudi infection and mortality in interleukin-10-deficient mice are ameliorated by anti-tumor necrosis factor alpha and exacerbated by anti-transforming growth factor beta antibodies. *Infect Immun* 71, 4850-6 (2003).

152. Tsutsui, N. & Kamiyama, T. Transforming growth factor beta-induced failure of resistance to infection with blood-stage Plasmodium chabaudi in mice. *Infect Immun* 67, 2306-11 (1999).
153. Deininger, M.H., Kremsner, P.G., Meyermann, R. & Schluesener, H.J. Differential cellular accumulation of transforming growth factor-beta1, -beta2, and -beta3 in brains of patients who died with cerebral malaria. *J Infect Dis* 181, 2111-5 (2000).
154. Kezuka, T. & Streilein, J.W. Analysis of in vivo regulatory properties of T cells activated in vitro by TGFbeta2-treated antigen presenting cells. *Invest Ophthalmol Vis Sci* 41, 1410-21 (2000).
155. Stitz, L., Planz, O., Bilzer, T., Frei, K. & Fontana, A. Transforming growth factor-beta modulates T cell-mediated encephalitis caused by Borna disease virus. Pathogenic importance of CD8+ cells and suppression of antibody formation. *J Immunol* 147(10), 3581-6. (1991).
156. Chung, J.Y., Park, Y.C., Ye, H. & Wu, H. All TRAFs are not created equal: common and distinct molecular mechanisms of TRAF-mediated signal transduction. *J Cell Sci* 115, 679-88 (2002).
157. Tada, K. et al. Critical roles of TRAF2 and TRAF5 in tumor necrosis factor-induced NF-kappa B activation and protection from cell death. *J Biol Chem* 276, 36530-4 (2001).
158. Aizawa, S. et al. Tumor necrosis factor receptor-associated factor (TRAF) 5 and TRAF2 are involved in CD30-mediated NFkappaB activation. *J Biol Chem* 272, 2042-5 (1997).
159. Nakano, H. et al. Targeted disruption of Traf5 gene causes defects in CD40- and CD27-mediated lymphocyte activation. *Proc Natl Acad Sci U S A* 96, 9803-8 (1999).
160. Bellamy, R. The natural resistance-associated macrophage protein and susceptibility to intracellular pathogens. *Microbes Infect* 1, 23-7 (1999).
161. Sempowski, G., Lee, D., Scearce, R., Patel, D. & Haynes, B. Resistance of CD7-deficient mice to LPS-induced shock syndrome. *J Exp Med* 189(6), 1011-6 (1999).
162. Heinly, C. et al. Comparision of thymocyte development and cytokine production in Cd7 deficient, Cd28-deficient and CD7/Cd28 double deficient mice. *Int Immunol* 13(2), 157-66 (2001).
163. Wak, i.S., Kurihara, R., Nemoto, H. & Suzuki, M. Effect of recombinant human colony-stimulating factor on the course of parasitaemia in non-lethal rodent malaria. *Parasitol Res* 79(8), 703-5. (1993).
164. Foote, S.J. et al. Mouse loci for malaria-induced mortality and the control of parasitaemia. *Nat Genet* 17, 380-1 (1997).
165. Campbell, D., Kim, C. & Butcher, E. Chemokines in the systemic organization of immunity. *mmunol Rev.* 195, 58-71 (2003).
166. Khan, I. et al. Mice lacking the chemokine receptor CCR1 show increased susceptibility to Toxoplasma gondii infection. *J Immunol* 166(3), 1930-7 (2001).
167. Nitcheu, J. et al. Perforin-dependent brain-infiltrating cytotoxic CD8+ T lymphocytes mediate experimental cerebral malaria pathogenesis. *J Immunol* 170, 2221-8 (2003).
168. Belnoue, E. et al. CCR5 deficiency decreases susceptibility to experimental cerebral malaria. *Blood* 101, 4253-9 (2003).
169. Barton, G. & Medshitov, R. Toll-like receptor signalling pathways. *Science* 300 (5625), 1524-5 (2003).
170. Adachi, K. et al. Plasmodium berghei infection in mice induces liver injury by an IL-12- and toll-like receptor/myeloid differentiation factor 88-dependent mechanism. *J Immunol* 167, 5928-34 (2001).
171. Scanga, C. et al. Cutting edge:MyD88 ir required for resistance to T. gondii infection and regulates parasite-induced IL-12 production by dendritic cells. *J Immunol* 168(12), 5997-6001 (2002).
172. Muraille, E. et al. Genetically resistant mice lacking MyD88-adapter protein display a high susceptibility to Leishmania major infection associated with apolarized Th2 response. *J Immunol* 170(8), 4237-41 (2003).
173. Poltorak, A. et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282, 2085-8 (1998).
174. Janeway, C., Travers, P., Walport, M. & Shlomchik, M. Innate immunity. in *Immunobiology* (ed. Gibbs, S.) 35-92 (Garland Publishing, New York, 2001).
175. Parker, C. & Sodetz, J. Role of the human C8 subunits in complement-mediated bacterial killing: evidence that C8 gamma is not essential. *Mol Immunol* 39(7-8), 453-8 (2002).
176. Cordell, H.J. et al. Statistical modeling of interlocus interactions in a complex disease: rejection of the multiplicative model of epistasis in type 1 diabetes. *Genetics* 158, 357-67 (2001).