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Development of Humanized Mice to Study Asexual Blood Stage *Plasmodium falciparum* Infection

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1. Introduction

Infectious diseases continue to heavily burden our global society. Endemic and epidemic malaria results in severe disease in an estimated half-a-billion people each year, and causes over 1.5 million deaths annually. Although progress has been made in the prevention and treatment of *falciparum* malaria infections, more effective, tolerable and affordable therapies are urgently needed. This deadly parasite displays unique human tropism, and the development of novel intervention strategies have been hampered by the lack of robust, cost effective, and predictive animal models that accurately reproduce the hallmark of human infections. While rodents and non-human primates have been employed in biomedical research and drug/vaccine development, they often do not yield reliable preclinical results that translate into effective human treatments. "Humanized" mice have recently emerged as powerful tools in the investigation of human diseases (Legrand et al., 2006; Manz, 2007; Shultz et al., 2007). These are amenable animal models transplanted with various kinds of human cells and tissues (and/or equipped with human transgenes) that may be ideally suited for direct investigation of human infectious agents such as malaria. Despite the challenges, humanized mouse technology has made rapid progress over the last few years, and it is now possible to achieve significant levels of human chimerism in various hosts, organ/tissues, particularly the immune systems, liver and muscles. Such humanized mice provide a new opportunity to perform preclinical studies of intractable human malaria

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parasite. However, the relevance of *Plasmodium falciparum*-infected humanized mice for malaria studies has been questioned because of the low percentage of mice in which the parasite develops. Malaria caused by *Plasmodium falciparum* is difficult to model in the laboratory because of the specificity of this parasite for its human host, the complexity of its life cycle and the substantial diversity of parasite strains. Consequently, most experimental in vivo studies on malaria have heavily relied on different combinations of various murine strains and Plasmodium spp. of rodent (Carlton et al., 2001; Hernandez-Valladares et al., 2005) but biological differences between parasite species remain a major limitation. For example, there are many indications that in human cerebral malaria, the preferential sequestration of parasitized erythrocytes in the brain capillaries is the central precipitating step (Medana and Turner, 2006), whereas this phenomenon is much less evident in rodent models (Lou et al., 2001).

Limitations of experimental models have also hampered the evaluation of the impact of new drugs or vaccines prior to clinical trials. During the preclinical screening of a new drug, its activity has to be evaluated first against the development of *P. falciparum* in vitro, then against the infection of a rodent Plasmodium in a mouse model and, finally, against *P. falciparum* infection in a monkey model (Fidock et al., 2004) or in a humanized mouse model.

1.1 Perspectives

The stability, reproducibility and long-standing tendency of parasitaemia in the developed humanized model based on NOD/SCID/IL2Rγ-null mouse (NSG), a mouse strain with profoundly deficient adaptive and innate immunity (NSG-IV model) might be validated by selecting artesunate resistant mutants of *P. falciparum* through prolonged exposure of the parasite to increasing levels of the drug.

2. The development of humanized mice

Advances in the ability to generate humanized mice have depended on a systematic progression of genetic modifications to develop immunodeficient host mice. Three main breakthroughs have occurred in this field (TIMELINE). First, the discovery of the *Prkdcscid* (protein kinase, DNA activated, catalytic polypeptide; severe combined immunodeficiency, abbreviated *scid*) mutation in CB17 mice (Bosma et al., 1983) was soon followed by the observation that human PBMCs (Mosier et al., 1988), fetal haematopoietic tissues (McCune et al., 1988) and HSCs (Lapidot et al., 1992) could engraft in these mice. However, engraftment occurred at only a very low level, and the engrafted human cells failed to generate a functional human immune system. The limitations impeding human-cell engraftment in CB17-*scid* mice include the spontaneous generation of mouse T and B cells during aging (known as leakiness) and high levels of host natural killer (NK)-cell and other innate immune activity, which limit the engraftment of the human hematopoietic compartment (Greiner et al., 1998). The *scid* mutation also results in defective DNA repair and, consequently, an increase in radio sensitivity. Targeted mutations at the recombination-activating gene 1 (*Rag1*) and *Rag2* loci prevent mature T- and B-cell development in the mice but do not cause leakiness or radio sensitivity. However, these mice retained high levels of NK-cell activity and had limited engraftment of human HSCs (Greiner et al., 1998; Mombaerts et al., 1992; Shinkai et al., 1992).

The second breakthrough was the development of immunodeficient non-obese diabetic (NOD)-*scid* mice (Shultz et al., 1995). Crossing the *scid* mutation onto different strain backgrounds led to the observation that NOD-*scid* mice supported higher levels of engraftment with human PBMCs than did any of the other strains that were tested, including C3H/HeJ-*scid* and C57BL/6-*scid* mice (Hesselton et al., 1995). Furthermore, it was observed that NK-cell activity, which is one of the main impediments to the engraftment of human haematopoietic cells (Christianson et al., 1996), was lower in NOD-*scid* mice than in CB17-*scid* mice (Shultz et al., 1995). NOD-*scid* mice also have additional defects in innate immunity that allow higher levels of human PBMC (Hesselton et al., 1995) and HSC (Lowry et al., 1996; Pflumio et al., 1996) engraftment. Incremental improvements in the extent of human-cell engraftment as a result of the development of new genetic variations of NOD-*scid* mice occurred over the following 10 years (TIMELINE), but the use of humanized NOD-*scid* mice as a model for human immunity remains limited by their relatively short life span, and the residual activity of NK cells and other components of innate immunity, which impedes the engraftment of the human lymphoid compartment.

The third breakthrough was the humanization of immunodeficient mice homozygous for targeted mutations at the interleukin-2 receptor (IL-2R) γ-chain locus (*Il2rg*; also known as the common cytokine-receptor γ-chain, γc) (Ishikawa et al., 2005; Ito et al., 2002; Shultz et al., 2005;Traggiai et al., 2004). These mice support greatly increased engraftment of human tissues, HSCs and PBMCs compared with all previously developed immunodeficient humanized mouse models. The IL-2R γ-chain is a crucial component of the high-affinity receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, and it is required for signaling through these receptors (Sugamura et al., 1996). The absence of the IL-2R γ-chain leads to severe impairments in T- and B-cell development and function, and completely prevents NK-cell development (Cao et al., 1995; DiSanto et al., 1995; Ohbo et al., 1996).

3. Optimization of immunosuppression protocols: Engraftment of *P. falciparum* in humanized mice

After an obtainment of proliferation of *Theileria sergenti* in scid mouse grafted with bovine-RBC (Tsuji et al., 1992), several studies raised the possibility of obtaining *P. falciparum* in immune deficient mice grafted with huRBCs (Badell et al., 2000; Moore et al., 1995; Tsuji et al., 1995). The researchers used different strategies to improve parasite survival considering the fast clearance of the parasite in few hours after being inoculated. Moore *et al* tried to adapt parasite *in vitro* partially in the serum of scid mice in place of human serum before being inoculated into the mouse (Moore et al., 1995). A concentration of mouse serum higher than 5% was found toxic for the parasite in culture. In this study, the NOD/scid mice were intraperitoneally infected with 1% adapted parasite followed by 1 ml huRBCs injection everyday. The parasite survival lasting up to 10 days was achieved with this protocol. Although splenectomized mice had shown an improvement of parasite growth (>15 days), variations in the parasitaemia from one mouse to another was detected. Another research group (Badell et al., 1995) developed another strategy of *P. falciparum* survival in the immunodeficient mice. They also noted that parasite inoculated through i.p. route into the scid mouse was eliminated in few hours from the circulation. Moreover, mouse serum added in *in vitro* cultures did not show any toxicity to parasite growth and parasite survival was observed even at 10% serum. This experiment further confirmed the important role of

residual innate immune effectors of mice in the clearance of parasite. The efforts have been made to control innate immune effectors by various means such as irradiation and cyclophosphamide treatment however with limited success. This group was able to establish an immunomodulatory protocol for BXN mouse to deplete innate immune regulatory cells and could achieve an average of 0.1% parasitaemia. This immunomodulatory protocol comprised of an i.p. injection of 0.2 ml of dichloro-methylene-diphosphonate (CL₂MDP) loaded liposome once per week to destroy the murine macrophages in conjunction with an administration of an anti-PMN monoclonal antibody at every 5th day to block the polymorphonuclear cells and also the injection of 1 ml of huRBCs & AB+ human serum in 1 ml of 10%-RPMI every day. Tsuji *et al*, used splenectomized scid mice and administered huRBCs by intravenous route from day 1 to 5. As from day 1 to 4 mice received an intraperitoneal injection of 500µl of human serum as well as a subcutaneous injection of an anti-mouse RBC antibody to replace murine RBC by huRBCs. Afterwards, the mouse was reconstituted with the huRBCs followed by the irradiation, and was also injected with 1.6-4% parasitized huRBCs through an intravenous route. By employing this protocol almost complete substitution of murine RBC by huRBCs was achieved (Tsuji et al., 1995). Until recently, immunomodulatory protocol (destroying macrophages with CL2MDP-lip and blocking PMN with an anti-PMN monoclonal antibody) was applied to the NOD/scid mice (Moreno Sabater et al., 2005). Moreover, a comparative study was carried out to show differences in the capabilities of *P. falciparum* survival in BXN and NOD/scid mice using the protocol with two intraperitoneal infections (i.e. primary infection on 3rd day and secondary infection on 18th day) and with the injection of immunosuppressors along with fresh huRBCs at every three days interval. In brief NOD/scid mouse allowed a better development of the parasite for 35 days with 75% infectivity compared to only 8% with BXN mice. In the BXN mouse strain the parasite could grow until day 7 post second infections followed by a gradual decrease in parasitaemia. On the contrary NOD/scid mice showed 0.25% average parasitaemia until day 17 post second infection. Therefore this protocol with NOD/scid mice showed an improvement of parasite survival (Moore et al., 1995). Interestingly, this protocol does not imply either the need of splenectomy or *in vitro* adaptation of the parasite prior to infection. Recently Inigo *et al* (Angulo-Barturen et al., 2008), developed a murine model (NOD/scidβ2m-/-) to study *falciparum*-malaria in non-myelodepleted mice grafted with human erythrocytes with considerable success in terms of parasitaemia and with 100% infectivity. They claimed to generate *in vivo* strains of *P. falciparum* able to grow reproducibly in peripheral blood of humanized mouse with out using immunosuppressors to deplete murine phagocytes. Despite the significant success with this model cumbersome administration of 1ml RBC pellet i.p. everyday and *in vitro* adaptation of *falciparum* strains prior to infection raise questions over its ideal nature. The same group (Jimenez-Diaz et al., 2009) came up with the same protocol applied to NOD/SCIDIL-2Rγ-/- strain with slight modifications for the survival of the parasite.

Therefore to complement the descriptive analysis in humans by an experimental approach in a model, we decided to perform in *P. falciparum* NOD/SCID model a systematic and stepwise analysis of innate cell responses and inflammation mediators produced in response to the grafting of HuRBC, of *P. falciparum*, as well as to agents employed to control innate defences. The results brought new insights about the role and potency of innate defences against human xenografts, such as HuRBC, and human pathogens, such as *P. falciparum* (Arnold et al.).
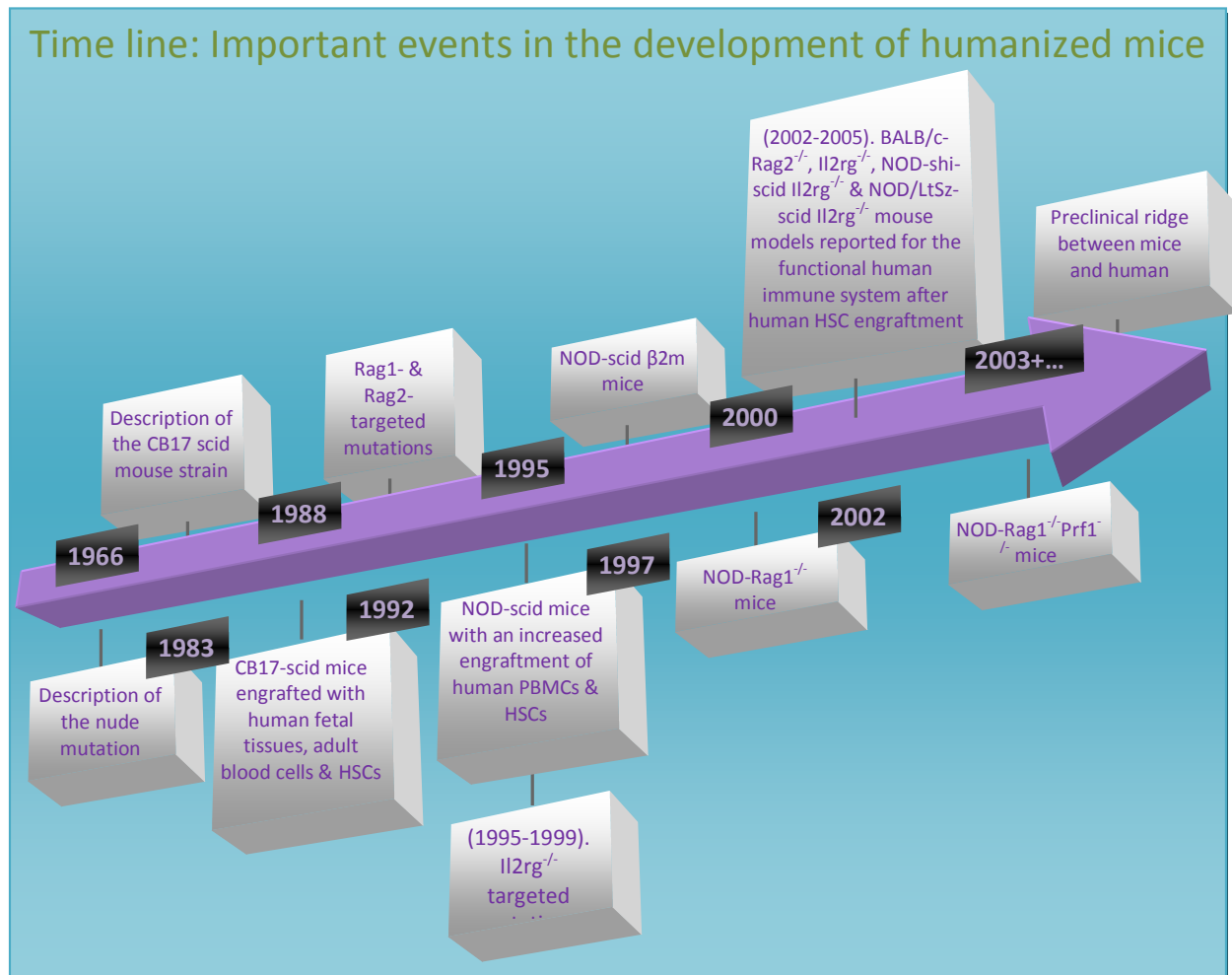
Fig. 1. Chronological events in the development of humanized mouse model

A main barrier to achieving an improved, workable *P. falciparum* mouse model is the strong pro-inflammatory effect of the parasite itself. In humans the asexual erythrocytic stages of *P. falciparum* are known to result in a systemic inflammatory process that is responsible for many of the symptoms of the disease. A second significant practical problem with all existing models developed to date is that huRBC are injected by the intra-peritoneal (IP) route, which relies on the successful migration of huRBC into the blood stream across the peritoneum. This is a process that is not properly understood, and, therefore, prevents any rational analysis and further improvement of the model. With these limitations of current models in mind, we thought to improve the *P. falciparum* humanized mouse model, particularly in terms of control of inflammatory reactions, and reproducibility of parasitemia. We decided to address these issues by using the IV route for huRBC and parasite administration, and by investigating other means to increase control over the mouse innate immune response. The use of this IV model led us to identify, among several factors investigated, the effect of aging and that of inosine as significant in reducing inflammatory reactions, and therefore improving *P. falciparum* growth. Moreover, after using various strains of immunodeficient mice, we investigated, as others [14], the value of

the NOD/SCID/IL-2Rc-null mouse (NSG mouse) which, due to the knock out of the γ-chain of the IL-2 receptor, has been shown to better tolerate a variety of transplanted human cells (Ito et al., 2002; King et al., 2007; Watanabe et al., 2007). The resulting new IV model based on NSG mice presents several advantages over previously available models. It offers greater reproducibility, with 100% of mice successfully grafted without the need for mouse-adapted parasites, consistent curves of parasitemia, and high levels of infection with up to 40–50% of total erythrocytes infected (Arnold et al., 2010).

4. Strategic planning for the development of humanized mouse model

4.1 Materials and methods

4.1.1 Mice

BALB/c, NOD/SCID and NSG mice are purchased from Charles River. Immunodeficient mice were kept in sterile isolators. They are housed in sterilized cages equipped with filter tops during the experimentation, and are provided with autoclaved tap water and a γ-irradiated pelleted diet ad libitum. They are manipulated under pathogen free conditions using laminar flux hoods.

4.1.2 Human red blood cells

Human whole blood is provided by the blood bank. Blood donors had no history of malaria and all the blood groups are used without observing any difference on parasite survival. Whole blood is washed three times by centrifugation at 900 ×g, 5 minutes at room temperature and buffy coat was separated in order to eliminate white blood cells and platelets. Packed huRBCs are suspended in SAGM (Adenine, Glucose and mannitol solution) and kept at 4°C for a maximum of 2 weeks. Before use huRBCs are washed three times in RPMI-1640 medium (Gibco/BRL, Grand Island, N.Y.) supplemented with 1 mg hypoxanthine per liter (Sigma, St Louis, MO) and warmed 10 minutes at 37°C.

4.1.3 Parasites

P. falciparum lines 3D7, UPA, and K1 are employed in the study, along with clinical isolates taken from a patient at Bichat Hospital, Paris (which was used the day after being sampled). The Uganda Palo Alto (UPA) strain employed is the Palo Alto Marburg line, used for all the experiments conducted. Parasite cultures are not synchronized and therefore a mix of various developmental stages was injected to infect mice. Parasites are maintained under *in vitro* conditions at 5% hematocrit at 37°C in a candle jar in complete culture medium (RPMI-1640 medium (Gibco/BRL), 35mM HEPES (Sigma), 24mM NaHCO$_3$, 10% albumax (Gibco/BRL) and 1mg of hypoxanthine (Sigma) per liter. Parasite samples are cryopreserved using the glycerol/sorbitol method (Rowe et al., 1968). The cultures are controlled for mycoplasma contamination by using polymerase chain reaction (PCR) technique. A non-lethal rodent parasite strain *Plasmodium yoelii* XNL1.1 is preserved in 500μl aliquot of cryo-preserving buffer at -80°C at 22% parasitaemia. The strain is thawed at room temperature, diluted twice in RPMI-1640 medium followed by the injection of 50×10^6 parasite directly into the mice.

5. Protocols to control residual innate immunity

5.1 (I.P. Protocol)

5.1.1 Immunomodulatory agents to suppress innate immunity

Numerous attempts have been made to increase the success rate of the grafting of infected RBC. Un-sized dichloromethylene diphosphonate (Cl$_2$-MDP) encapsulated in liposome (clo-lip) (provided by N. Van Rooijen, Amsterdam, The Netherlands) is injected through intraperitoneal (i.p.) route in order to reduce the number of tissue MP, as described previously (van Rooijen and van Kesteren-Hendrikx, 2003). The anti-PMN monoclonal antibody NIMP-R14 (Lopez et al., 1984) is purified from a hybridoma. Its activity is compared to that of two other anti-PMN monoclonal antibodies: RB6-8C5 (purified from the hybridoma) and 1A8 (BioXcell, Lebanon). The NIMP-R14 monoclonal antibody is used in all the studies, unless specified. Various agents (from Sigma) (Table 1) are used to further reduce innate immunity such as dexamethasone (1-5 mg/kg/day), TGF-β (100 ng - 1 µg/day) (PeproTech, Rocky Hill, NJ), cyclophosphamide (75 mg/kg/day), cisplatinium (1-10 mg/kg/day), and TMβ-1 monoclonal antibody that targets NK cells (1 mg/kg/day).

Expertiments Performed Using I.P. Protocol With Vaious Immunomodulatory Reagent					
Protocol tested	Dose	Nb of mouse	% of sucrose(more than 2 days	Paracitemia length average (days)	Best parasitemia (days)
DSMO	5 %	18	88.8	7.18	12
TGFβ	100 ng/day	17	23.5	7.25	8
	100 µg/day	3	66.6	10	13
Splenctomy		5	60	7	10
Cyclophopshamide	75 mg/Kg	7	100	7.6	9
	50 mg/Kg	12	41.6	6.8	9
Coinfection *P.Chabaudi* P.Falciparum		7	71.42	11	24
Coinfection *P.Yoellii P.Falciparum*		52	90.24	11.09	34
NAC	100 mg/Kg	25	56	11.07	19
Vitamine E	200 mg/kg	13	77	8.25	34
Trolox	4 mg/Kg	5	60	3.85	6
Anti-NK(TMBβ-1)	1 mg	15	53.3	4.12	8
Futhan	200 µg/day	4	50	2.75	4
Bleeding		20	35	7.42	14
P.Falciparum with various amount	0.3	2	100	2	2
	1%	2	100	4	4
	5%	2	100	5.5	6
	7%	2	100	4	4
	10%	2	100	5.5	6
pABA	400 mg/Kg	4	100	4.5	5
Folinic acid	mg/Kg	4	100	4.5	5
Coinfection *P.Chabaudi* and *P.Yoellii*, NAC and Vitamine E seem to have a beneficial effect in *P.Falciparum* survival; however results are very heterogeneous form one mouse to other and from one experiment to other one					

Table 1. Various immune suppressants to control innate immunity

The effect of splenectomy and of irradiation (100 - 300 cGy) is also tested. Other experiments that evaluates the addition of metabolic agents such as pABA (400 mg/kg/day), and folinic acid (400 mg/kg/day), or of antioxidants, such as vitamin E (20 mg/kg/day; Nepalm, Cenexi, Fontenay-sous, France), N-acetyl cysteine (100 mg/kg/day), trolox (4-100 mg/kg/day), 8-aminoguanidine (100 mg/kg/day).

5.1.2 Chemical immunomodulation protocol and mouse infection

A previously described immunomodulation protocol (Badell et al., 2000), modified as described in (Moreno et al., 2006) is employed. On day -13, each mouse receives a dose of 10 mg/kg of mAb NIMP-R14 by i.p. injection. On day -12, each mouse receives 0.2 ml of the suspension of clo-lip by the same route. On days -9, -6, -3 each mouse receives 0.5 ml of huRBCs i.p. mixed with a dose of 10 mg/kg of mAb NIMP-R14 and 0.2 ml of clo-lip. On day 0 mice are infected with 50μl huRBCs parasitized by *P. falciparum* at a parasitaemia of 1% (all the developmental forms, i.e. trophozoite, schizont and rings, were present) mixed with a dose of 10 mg/kg of NIMP-R14 antibody. Afterwards, a dose of 10 mg/kg of antibody NIMP-R14, 0.2 ml of clo-lip and 0.5 ml of huRBCs is injected i.p. at 3 day intervals, until the end of the study. The infection is followed-up by daily Giemsa stained thin blood films drawn from the tail vein.
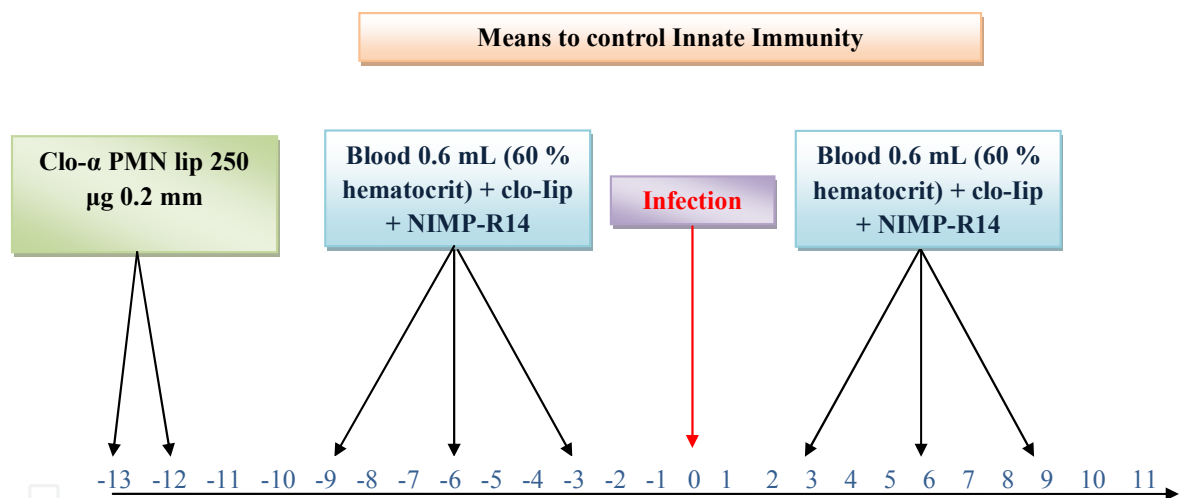

Fig. 2. Schematics of intraperitoneal immunomodulation protocol

5.2 Protocol for intravenous injection

5.2.1 Mouse infection and immunomodulation protocol

NOD/SCID mice are retro-orbitally injected with 400 μl huRBCs every 3 days to ensure a satisfactory proportion of huRBCs (i.e. chimerism), at the time of infection (≈ 60%). Simultaneously, 0.1 ml of un-sized dichloromethylene diphosphonate (Cl₂MDP) encapsulated in liposome (clo-lip) (provided by Nico Van Rooijen) diluted in 0.4 ml RPMI is intraperitoneally injected. Four injections at 2-3 day intervals are given before parasite infection. At the time of the fifth injection, mice are retro-orbitally injected with 300 μl of a *P. falciparum* infected huRBCs suspension in RPMI at a parasitaemia of 1% (all the developmental forms, i.e. rings, trophozoites and schizonts were present). After infection huRBCs and clo-lip are supplied every 3 days as described for the pre-infection step. In

some experiments, 250 mg/kg of inosine (Sigma) is injected intraperitoneally every day as the half-life of inosine is very short (Mabley et al., 2009). In experiments using NSG mice the protocol has been adopted in order to achieve varying levels of adequate huRBCs chimerism and to avoid overloading the mice. As such, different amounts of blood are employed, and either 200, 400, 550 or 750 µl huRBCs are injected 3 times per week (i.e. Monday, Wednesday and Friday) mixed with 250 µl human AB serum, as it has previously been described that human serum improves huRBCs survival in immunocompromised mice (Angulo-Barturen et al., 2008); 4 injections are done prior to infection, and clo-lip is injected as described above. The infection is followed-up by daily Giemsa stained thin blood films drawn from the tail vein.

5.2.2 Haematological parameters and grafting of *P. falciparum*-huRBCs

The study blood samples are collected from mice retro-orbital sinus on heparin. Various haematological parameters such as haematocrit, leukocyte number and phenotype (Ly-6C APC, Ly-6G APC (Miltenyi Biotec, Germany), CD115 PE, CD43 FITC, CD62L FITC, CD11b FITC, DX5 FITC, CD122 PE (BD Biosciences, UK) in peripheral blood samples were monitored, as well as the phenotype characterization of monocytes (CD11b+,CD115+), inflammatory monocytes (CD43-, CD62L+, Ly-6C+), PMN (CD11b+, ly6G+), and natural killer cells (DX5+, CD122+). Total leukocyte number (leukocytes/µl blood) is evaluated by lysing 20µl of total blood with BD FACS™ Lysing solution, and counting on Malassez haematocytometer. Since successfully grafted mice have a significant, but variable, percentage of huRBCs in their peripheral blood, parasitaemia in mice is expressed as the overall percentage of *P. falciparum* infected RBCs among total RBCs, i.e. both human and mouse RBCs observed on thin blood smears. In addition, the peritoneal blood parasitaemia is measured on the smears drawn from the peritoneum.

Blood samples drawn from mice are used to determine the percentage of huRBCs in mouse peripheral blood at regular intervals by flow cytometry on a FACScalibur (BD biosciences) using FITC labeled anti-human glycophorin monoclonal antibody (Dako, Denmark).

5.2.3 Mouse cell isolation

In NOD/SCID mice inflammation is induced by IP injection of 1ml 3% thioglycolate (Sigma) diluted in sterile PBS. 4-5 days after, the peritoneal cavity is washed with HBSS without Ca^{++} and Mg^{++}. The collected cells are washed twice in RPMI supplemented with L-glutamine, Penicillin (100U/ml), Streptomycin (100ug/ml), and 10% Fetal Calf Serum (FCS) and seeded at $3x10^5$ per well in a 96-well culture plate. Cell suspensions of splenocytes are prepared in cold RPMI 1640 medium supplemented with 10% FCS and filtered on a 100 µm cell strainer to remove debris. Erythrocytes are lysed with ACK lysis buffer, and the splenocytes are washed 2 times with RPMI supplemented with 10% FCS and seeded at $3x10^5$ per well of a 96 well culture plate.

5.2.4 Cytokines/chemokine/chemiluminesence assay

100 µl blood samples are collected from the retro-orbital plexus with a Pasteur pipette, and sera are stored at -80°C. Conditioned media obtained after 16h stimulation of peritoneal cells and splenocytes with lipopolysaccharide (LPS, 1 µg/ml) (Sigma) are stored at -80°C. Cytokines and chemokines (IL-6, MCP-1, IFNγ, TNFα, IL-12p70 and IL-10) are quantified

using the BD™ Cytometric Bead Array mouse inflammatory kit (BD biosciences) following the manufacturer's recommendations on a FACScalibur (BD biosciences).
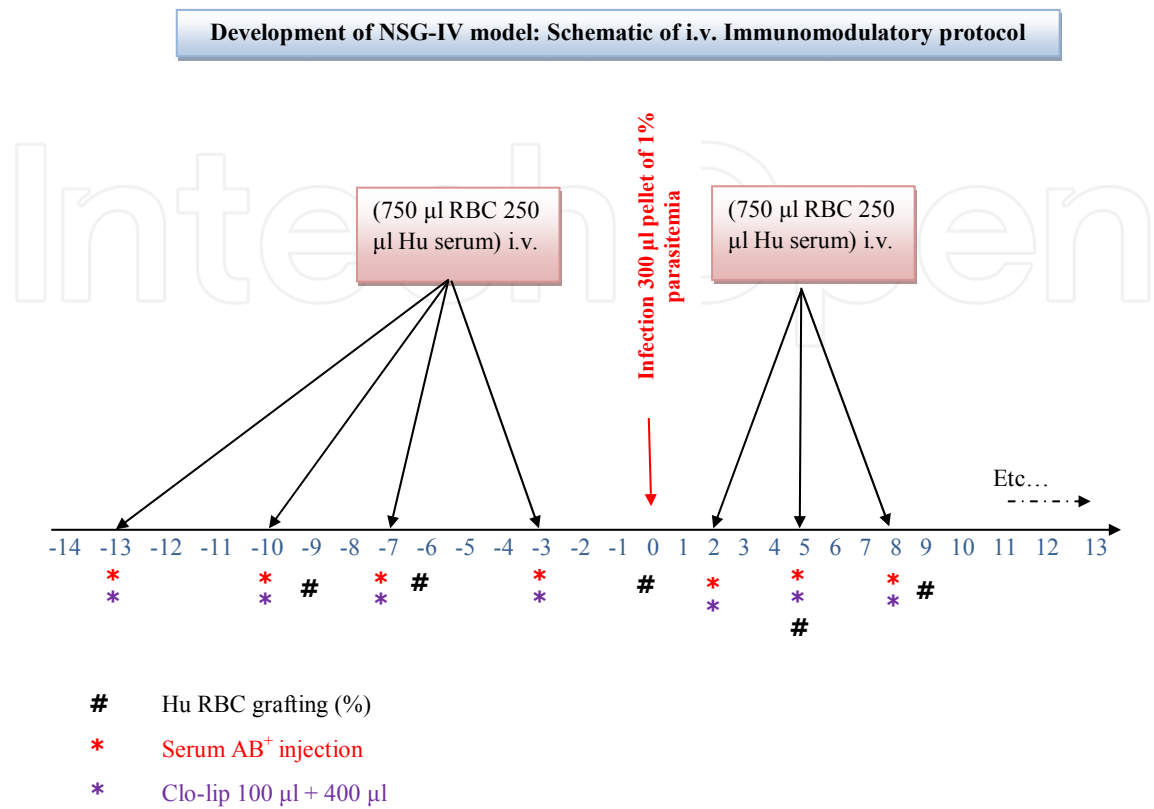

Fig. 3. Development of NSG-IV (NOD/SCIDIL2Rγ-/-) model: schematic of immunomodulatory protocol

Since production of reactive oxygen intermediates (ROI) closely mirrors the state of activation of macrophages and polymorphonuclear cells, luminol dependent photometric assay is used to measure Reactive Oxygen Intermediates (ROI). Blood samples are collected from NOD/SCID mice, washed with HBSS with freshly added Ca++ and Mg++. Washed blood is diluted 1/10 in HBSS and 90 µl blood were added to each well in a 96 well plate (Nunc, Denmark) and incubated for 30 minutes at 37⁰C after adding 10 µl PMA (final concentration 1 µg/ml) to stimulate the cells. 50µl of luminol (final concentration 200 µg/ml) solution are added immediately before measuring emissions.

5.2.5 Analysis of deep-seated organs for parasite differential count

NSG mice are used for the comparison of parasite differential counts in the peripheral blood, with that in deep-seated organs. Four mice are infected with UPA strain, and when a parasitaemia of >10% is reached a thin smear from peripheral blood is drawn before killing the mouse, and harvesting its organs. Kidney, Liver, Spleen, Lung, and Brain are removed from each mouse. Parasite content is assessed from blots made by repeatedly spotting sections from each organ. These slides are then stained with Giemsa. The last blots taken, are considered to be the most representative of the parasite content in the organ's vascular bed, and are examined at 1000x magnification to perform differential counts of each stage (> 200 parasites from each organ counted).

6. Anticipated results

The present protocols illustrate an improvement of the mice with genetic deficiencies in adaptive immunity for successful grafting of human cells or pathogens to study human biological processes *in vivo*. Since non-adaptive immunity received little attention, we have deployed our efforts to study innate defences, responsible for the substantial control of *P. falciparum* and highlighted some of the remaining limitations on the development of the optimal humanized mouse. In addition, numerous attempts were made to enhance the success rate of infected huRBC by employing various immunomodulatory agents intraperitoneally to further suppress the residual innate immunity. This study came up with some conclusions such as, 1) stable parasitaemia with only a subset of mice 2) parasite clearance and *P. falciparum* induced inflammation are correlated 3) *P. yoelii* induces less inflammation than *P. falciparum* 4) huRBC, and the anti-inflammatory agents, induce low grade inflammation. 5) repeated administrations of huRBC, clo-lip and anti-PMN reduce inflammation and improve HuRBC grafting 6) MO/MPs are critical in controlling *P. falciparum* and huRBC grafting in NOD/SCID. A strong pro-inflammatory effect of the parasite itself and poor understanding of migration of infected and uninfected huRBC into blood stream across the peritoneum and subsequently, uneven distribution of huRBC are two barriers to achieving an improved, workable humanized mouse model. The results obtained by the IP protocols indicate that the SCID mice to study human biological processes *in vivo* need to be carefully explored and that further attempts are required to address the remaining limitations such as residual innate immunity and route of delivery of huRBC to create an optimized humanized mouse model.

The complex biological processes often require *in vivo* analysis of human cells: humanized mice or mouse-human chimeras have been developed to meet this requirement, however, with low percentage of infectivity and reproducibility supporting a long standing parasitaemia only in a proportion of animals. We have obtained improved parasitaemias based on intravenous delivery of huRBC and *P. falciparum* instead of the intraperitoneal route (IP) by testing various immunosuppressive drugs.

In essence the genetic background has also played an important role to optimize the humanized mouse model. The intravenous mouse model (NSG-IV) shows the role of ageing and inosine in controlling *P. falciparum* induced inflammation. The success of humanized mouse (NSG mice) model, with IV delivery of huRBC and *P. falciparum* in clo-lip treated (immunosuppression of macrophages) mice, was 100% in terms of infectivity and reproducibility. Synchronization, partial sequestration and receptivity to various strains of *P. falciparum* without preliminary adaptation are some of the lucrative features of the developed mouse model which is reliable and more relevant, and better meet the needs of biomedical translational research.

7. Conclusion

In conclusion, the chapter demonstrates that the careful selection of mice with combined deficiencies of adaptive and innate immunity, and the better control of innate immune defenses by improving immunomodulatory protocols and using anti-inflammatory substances, could provide a reproducible, long lasting and straightforward mouse model of

P. falciparum infection. This model may be further used to obtain malaria parasites that present a high level of resistance to different anti-malarial drugs such as artemisinin *in vivo* and *in vitro*. Interestingly, the same model could be also used to test the efficacy of new drugs, and in particular could be used to screen novel drugs for their effectiveness against ART resistant *P. falciparum* parasites

8. Abbreviations

NOD- Non-Obese Diabetic; SCID-Severe Combined Immunodeficiency; IL2Rγ- Interleukin-2 Receptor γ-chain; NK- Natural Killer; PBMCs: Peripheral Blood Monocytes; HSCs: Hematopoietic stem cells; *Rag*-Recombination-Activating Gene; IL-Interleukin; huRBCs—Human Red Blood Cells; PMN-Polymorphonuclear; SAGM- Adenine, Glucose and Mannitol solution; CL_2MDP- Dichloro-Methylene-Diphosphonate; NSG-NOD scid gamma; TGF-Tumor Growth Factor; APC-Antigen Presenting Cell; FACS- Fluorescence Activated Cell sorting; FCS- Fetal Calf Serum; FITC- Fluorescein isothiocyanate, phorbol-12-myristate-13-acetate (PMA)

9. References

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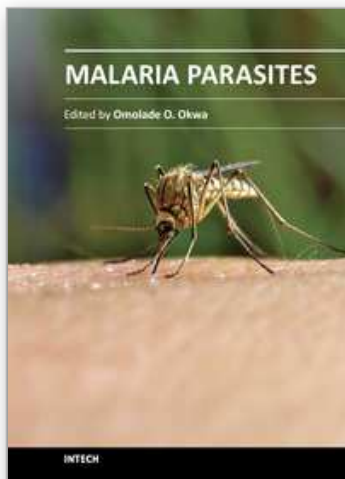
Malaria Parasites

Edited by Dr. Omolade Okwa

Malaria is a global disease in the world today but most common in the poorest countries of the world, with 90% of deaths occurring in sub-Saharan Africa. This book provides information on global efforts made by scientist which cuts across the continents of the world. Concerted efforts such as symbiont based malaria control; new applications in avian malaria studies; development of humanized mice to study P.falciparium (the most virulent species of malaria parasite); and current issues in laboratory diagnosis will support the prompt treatment of malaria. Research is ultimately gaining more grounds in the quest to provide vaccine for the prevention of malaria. The book features research aimed to bring a lasting solution to the malaria problem and what we should be doing now to face malaria, which is definitely useful for health policies in the twenty first century.

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