Influence of Paclitaxel on Parasitemia and Survival of *Plasmodium berghei* Infected Mice

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Key Words

Malaria • Cell volume • Phosphatidylserine • Apoptosis
• Cell death • Eryptosis • Taxol • Docetaxel

Abstract

Paclitaxel triggers suicidal erythrocyte death or eryptosis, characterized by exposure of phosphatidylserine at the erythrocyte surface and cell shrinkage. Eryptosis of infected erythrocytes may delay development of parasitemia and thus favourably influence the course of malaria. The present study explored whether paclitaxel influences *in vitro* parasite growth and eryptosis of *Plasmodium falciparum* infected human erythrocytes and *in vivo* parasitemia and survival of *Plasmodium berghei* infected mice. Phosphatidylserine exposing erythrocytes were identified utilizing annexin V binding and erythrocyte volume was estimated from forward scatter in FACS analysis. *In vitro* infection of human erythrocytes with *P. falciparum* increased annexin binding and decreased forward scatter, effects augmented in the presence of paclitaxel ($\geq 0.01 \mu\text{M}$). Paclitaxel ($\geq 0.01 \mu\text{M}$) significantly decreased intraerythrocytic DNA/RNA content and *in vitro* parasitemia. In *Plasmodium berghei* infected mice parasitemia was sig-

nificantly decreased (from 55.8% to 28.6% of circulating erythrocytes 20 days after infection) and mouse survival significantly enhanced (from 0% to 69.23% 25 days after infection) by administration of 8.5 mg/kg.b.w. of paclitaxel intraperitoneally from the eighth day of infection. In conclusion, paclitaxel decreases parasitemia and enhances survival of *P. berghei* infected mice, an effect, which may be due to stimulation of eryptosis and/or a direct toxic effect on the parasite.

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Introduction

Paclitaxel and the related drug docetaxel are anticancer drugs employed for the treatment of malignancies [1-4]. Paclitaxel is also used for the prevention of restenosis [5]. The substances may lead to cellular growth arrest [6]. They are considered to be primarily effective through interference with the cytoskeleton [7, 8] and p53 signaling [9] as well as stimulation of caspases [10, 11]. Paclitaxel similarly elicits eryptosis [12], the suicidal death of erythrocytes. Eryptosis is characterized by cell shrinkage and cell membrane scrambling leading to exposure of phosphatidylserine at

the cell surface [13-17]. Cell membrane scrambling is triggered by increased cytosolic Ca^{2+} activity [13, 15, 16, 18] and ceramide [19]. Ca^{2+} enters into erythrocytes through Ca^{2+} permeable cation channels which are activated by osmotic shock, oxidative stress and energy depletion [18, 20-22]. Ca^{2+} further activates Ca^{2+} sensitive K^{+} channels [23, 24] leading to exit of KCl and osmotically obliged water and thus to cell shrinkage [25]. Ca^{2+} could similarly stimulate apoptosis of nucleated cells [26]. Paclitaxel triggers eryptosis by increase of cytosolic Ca^{2+} activity, ceramide formation and activation of calpain [12].

Macrophages are equipped with receptors specific for phosphatidylserine [27, 28], and phagocytose phosphatidylserine exposing erythrocytes [29, 30], which are thus rapidly eliminated from circulating blood.

Accelerated eryptosis underlies several anemic conditions [31], such as sickle-cell anemia [32], beta-thalassemia [17], glucose-6-phosphate dehydrogenase (G6PD)-deficiency [17], phosphate depletion [33], iron deficiency [34], Hemolytic Uremic Syndrome [35], sepsis [36], malaria [37] and Wilson disease [38]. Moreover, enhanced eryptosis is stimulated by hemolysin Kanagawa [39], listeriolysin [40], IgA antibodies [41], azathioprine [42], selenium compounds [43], platelet activating factor [44], lipopeptides [45], cadmium ions [46], ciglitazone [47], curcumin [48], amyloid peptides [49], valinomycin [50], retinoic acid [51], amiodarone [52], anandamide [53], copper ions [38] and paclitaxel [12].

During malaria, stimulation of eryptosis may result in the clearance of infected erythrocytes prior to the development of trophozoites with the ability to intoxicate macrophages [54]. Accelerated eryptosis has thus been considered a protective mechanism in malaria [37]. Accordingly, sickle-cell trait, beta-thalassemia-trait, homozygous Hb-C and G6PD-deficiency lead to premature senescence and/or eryptosis upon infection with *Plasmodium* thus leading to accelerated clearance of ring stage-infected erythrocytes [17, 55-59].

The present study explored whether paclitaxel triggers phosphatidylserine exposure of *Plasmodium falciparum* (*P. falciparum*) infected erythrocytes and influences parasitemia and survival of *Plasmodium berghei* (*P. berghei*) infected mice.

Materials and Methods

Animals, cells and solutions

Animal experiments were performed according to German animal protection law and approved by the local authorities (registration number PY 2/06). Healthy SV129/J wild type mice

(aged 4 months, both male and female) were used for the experiments. Mouse erythrocytes were drawn from animals by retroorbital venopuncture or by incision of the tail vein. Human erythrocytes were drawn from healthy volunteers.

Experiments were performed at 37°C in Ringer solution containing (in mM) 125 NaCl, 5 KCl, 1 MgSO_4 , 32 N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES)/NaOH (pH 7.4), 5 glucose, 1 CaCl_2 . Paclitaxel was added to the NaCl Ringer at final concentrations varying from 0.001 μM to 1 μM (Sigma, Taufkirchen, Germany). For *in vitro* paclitaxel treatment, the final haematocrit was adjusted to 0.3%.

Determination of phosphatidylserine exposure

FACS analysis was performed as described [18]. After incubation in the presence or absence of paclitaxel, suspensions of noninfected erythrocytes were stained with annexin V-FLUOS (Roche, Mannheim, Germany), suspensions of *Plasmodium falciparum* infected erythrocytes were stained with annexin V-APC (BD Biosciences Pharmingen, Heidelberg, Germany) and/or with the DNA/RNA specific dye Syto16 (Molecular Probes, Göttingen, Germany) to identify phosphatidylserine exposing and infected erythrocytes, respectively. For annexin binding, erythrocytes were washed, resuspended in annexin-binding buffer (Ringer solution containing 5 mM CaCl_2 , pH 7.4), stained with annexin V-APC (dilution 1:20) or annexin V-FLUOS (dilution 1:100), incubated for 20 min at room temperature, and diluted 1:5 with annexin binding buffer. Syto16 (final concentration of 20 nM) was added directly to the diluted erythrocyte suspension or co-incubated in the annexin binding buffer. Cells were analyzed by flow cytometry (FACS-Calibur, Becton Dickinson, Heidelberg, Germany) in FL-1 for Syto16 or annexin V-FLUOS fluorescence intensity (detected at 530 nm) and in FL-4 for annexin V-APC fluorescence intensity (detected at 660 nm).

In vitro cultivation of Plasmodium falciparum

For infection of human erythrocytes the human pathogen *Plasmodium falciparum* strain BinH [60] was grown *in vitro* [61] in human erythrocytes. Parasites were cultured as described earlier [62, 63] at a haematocrit of 2% and a parasitemia of 2-10% in RPMI 1640 medium supplemented with Albumax II (0.5%; Gibco, Karlsruhe, Germany) in an atmosphere of 90% N_2 , 5% CO_2 , 5% O_2 .

In vivo proliferation of Plasmodium berghei

P. falciparum is not pathogenic in mice and thus, the *in vivo* experiments in mice have been performed with *P. berghei*, which is not pathogenic to human erythrocytes and cannot be used for *in vitro* experiments. The mice were infected with *Plasmodium berghei* ANKA-parasitized mouse erythrocytes (1×10^6) which were injected intraperitoneally [64] into wildtype mice with free access to water. The treated group additionally received paclitaxel (8.54 mg/kg b.w.) intraperitoneally from the eighth day of infection. Blood was collected from the mice 8 days after infection by incision of the tail. Parasitemia was determined by DNA/RNA specific fluorescence dye Syto16 staining detected by FL-1 in FACS analysis. The erythrocytes were stained with 1 μM Syto 16 in PBS and incubated for 20 min

at 37°C. The percentage of stained/infected erythrocytes was determined within the total population of erythrocytes.

In vitro growth assays of *P. falciparum* infected human erythrocytes

The *Plasmodium falciparum* BinH strain was cultured and synchronized to the ring stage by sorbitol treatment as described previously [65]. For the *in vitro* growth assay, synchronized parasitized erythrocytes were aliquoted in 96-well plates (200 μ l aliquots, 1% haematocrit, 0.5–2% parasitemia) and grown for 48 h in the presence or absence of paclitaxel (0.001 μ M–1 μ M) and/or docetaxel (0.001 μ M–1 μ M). The parasitemia was assessed at time 0 and after 48 h of culture by flow cytometry. Parasitemia was defined by the percentage of erythrocytes stained with the DNA/RNA specific fluorescence dye Syto16.

To estimate DNA/RNA amplification, the culture was ring stage-synchronized, and re-synchronized after 6 h of culture (to narrow the developmental parasite stage), aliquoted (200 μ l aliquots, 2% haematocrit and 10% parasitemia) and cultured for further 16 h in the presence or absence of paclitaxel (0.001 μ M–1 μ M). Thereafter, the DNA/RNA amount of the parasitized erythrocytes was determined by Syto16 fluorescence as a measure of intraerythrocytic parasite copies.

Statistics

Data are expressed as arithmetic means $\pm$ SEM and statistical analysis was made by paired or unpaired t-test or ANOVA using Tukey's test as post hoc test, where appropriate. $p < 0.05$ was considered as statistically significant.

Results

Annexin V binding has been utilized to identify phosphatidylserine exposing erythrocytes in FACS analysis. In freshly drawn human blood the percentage of annexin-binding erythrocytes was low ($0.91 \pm 0.07\%$, $n = 6$). Incubation of noninfected human erythrocytes in Ringer solution increased the percentage of annexin binding cells within 24 hours to $2.98 \pm 0.29\%$ ($n = 6$). Infection with *P. falciparum* for 24h significantly enhanced the percentage of annexin binding human erythrocytes (Fig. 1). The presence of paclitaxel significantly enhanced the annexin binding of both infected erythrocytes and the co-incubated noninfected erythrocytes (Fig. 1). The effect reached statistical significance at 0.001 μ M and 0.1 μ M paclitaxel in the infected and noninfected erythrocytes, respectively. Thus, paclitaxel potentiated the scrambling effect of infection on phosphatidylserine exposure.

Incubation of non-infected erythrocytes with lower concentrations of paclitaxel for extended periods tended to increase annexin binding, an effect, however, not reach-

Fig. 1. Effects of paclitaxel on phosphatidylserine exposure of *P. falciparum* infected and noninfected human erythrocytes: Arithmetic means $\pm$ SEM ($n=10$) of annexin binding of infected (closed symbols) and co-incubated noninfected (open symbols) erythrocytes before and after 24h treatment with paclitaxel as a function of the paclitaxel concentration. * indicates significant difference ($p < 0.05$) from absence of paclitaxel, # indicates significant difference to noninfected erythrocytes.

ing statistical significance. The annexin binding of non-infected erythrocytes after 48h incubation was 3.90 ± 0.44 , 4.02 ± 0.23 , 4.21 ± 0.38 and 4.6 ± 0.21 ($n = 8$ each) at 0, 0.001, 0.01, 0.05 μ M of paclitaxel respectively.

Intraerythrocytic DNA amplification in erythrocytes was slightly, but significantly decreased by paclitaxel (≥ 0.01 μ M) (Fig. 2A). *In vitro* growth of *Plasmodium falciparum* in human erythrocytes was similarly decreased at paclitaxel concentrations (≥ 0.01 μ M) (Fig. 2B). The *in vitro* growth of *Plasmodium falciparum* in human erythrocytes in the presence of docetaxel or the combination of both docetaxel and paclitaxel was similar to that of Paclitaxel (Fig. 2 C,D).

The forward scatter, which reflects the cell volume was at early stages of infection similar, at later stages significantly larger in infected than in noninfected erythrocytes. Paclitaxel slightly decreased the forward scatter, an effect reaching statistical significance at 1 μ M concentration both in the early and late stage *P. falciparum* infected erythrocytes (Fig. 3 A, B).

To determine the influence of paclitaxel treatment on the course of malaria *in vivo*, mice were infected with *Plasmodium berghei*. Parasitemia was less than 10% 8 days after infection but gradually increased the next 10 days (Fig. 4). Without paclitaxel treatment, the percentage of parasitized erythrocytes increased up to 55.8% within 20 days. Following treatment with

Fig. 2. Effects of paclitaxel on intraerythrocytic amplification and *in vitro* growth of the parasite: A. Intraerythrocytic DNA amplification of *P. falciparum* infected human erythrocytes after 24h treatment with paclitaxel as a function of the paclitaxel concentration (arithmetic means $\pm$ SEM, n=8). * indicates significant difference ($p < 0.05$) from absence of paclitaxel. B. *In vitro* growth of *P. falciparum* in human erythrocytes after 48h treatment with paclitaxel as a function of paclitaxel concentration (arithmetic means $\pm$ SEM, n=8). * indicates significant difference ($p < 0.05$) from absence of paclitaxel. C. *In vitro* growth of *P. falciparum* in human erythrocytes after 48h treatment with docetaxel as a function of docetaxel concentration (arithmetic means $\pm$ SEM, n=8). * indicates significant difference ($p < 0.05$) from absence of docetaxel. D. *In vitro* growth of *P. falciparum* in human erythrocytes after 48h treatment with a combination of paclitaxel and docetaxel (1:1 ratio of the total drug concentration) as a function of total drug concentration (arithmetic means $\pm$ SEM, n=8). * indicates significant difference ($p < 0.05$) from absence of drugs.

8.5 mg/kg b.w. paclitaxel intraperitoneally, there was significant decrease in the parasitemia (28.6% within 20 days, Fig. 4A, B).

Most importantly, paclitaxel treatment increased the survival of *Plasmodium berghei* infected mice. Whereas all nontreated animals died within 30 days after the infection, 69% of the paclitaxel treated animals survived the infection for more than 30 days (Fig. 4C).

Discussion

The present study reveals a significant favorable effect of paclitaxel on the course of malaria. Following infection with *Plasmodium berghei* the increase of parasitemia was significantly less rapid in paclitaxel treated mice than in untreated mice. Moreover, paclitaxel significantly increased the survival of *Plasmodium berghei* infected mice. Similar to what has been shown earlier [64],

untreated mice all die from infection with *Plasmodium berghei*. In contrast, some 2/3 of the paclitaxel treated mice survived the first 30 days following infection. Most of those mice recovered from the infection. Thus, paclitaxel treatment indeed favourably modifies the course of malaria.

The effect of paclitaxel could be related to the ability of the drug to induce eryptosis [12]. Paclitaxel has previously been shown to induce eryptosis by increasing cytosolic Ca^{2+} activity, stimulating ceramide formation and activating calpain [12]. Paclitaxel is effective in both, infected and noninfected cells. The combination of infection and paclitaxel exposure leads to excessive scrambling of the cell membrane. The subsequent phosphatidylserine exposure at the cell surface is an "eat me" signal for macrophages [29, 30]. Paclitaxel further decreases cell volume, which is presumably due to increase of cytosolic Ca^{2+} activity, activation of Ca^{2+} sensitive K^+ (GARDOS) channels [25], K^+ exit,

Fig. 3. Effects of paclitaxel on forward scatter of infected and noninfected human erythrocytes: A. Arithmetic means $\pm$ SEM (n=6) of forward scatter of the early stage- *P. falciparum* infected erythrocytes (closed symbols) and noninfected (open symbols) erythrocytes before and after 48h treatment with paclitaxel as a function of the paclitaxel concentration. * indicates significant difference ($p < 0.05$) from absence of paclitaxel. B. Arithmetic means $\pm$ SEM (n=6) of forward scatter of late stage- *P. falciparum* infected erythrocytes (closed symbols) and noninfected (open symbols) erythrocytes before and after a 24h treatment with paclitaxel as a function of the paclitaxel concentration. * indicates significant difference ($p < 0.05$) from absence of paclitaxel, # indicates significant difference to non-infected erythrocytes.

hyperpolarization of the erythrocyte membrane potential and Cl^- exit [66]. The exit of KCl is followed by osmotically obliged water and thus leads to cell shrinkage [67]. Phosphatidylserine exposure and cell shrinkage are the hallmarks of eryptosis [68].

Infection of erythrocytes with *Plasmodium falciparum* similarly triggers eryptosis [69, 70]. *Plasmodia* impose oxidative stress to the host erythrocyte leading to activation of the Ca^{2+} permeable cation channels [65] with subsequent cell membrane scrambling and phosphatidylserine exposure at the erythrocyte surface [71].

The favourable effect of paclitaxel on the course of malaria may partially result from a direct toxic action on the pathogen, as paclitaxel slightly, but significantly decreased the intraerythrocytic DNA/RNA content.

Fig. 4. Parasitemia and survival of *Plasmodium berghei* infected mice: A: Original histograms of parasitemia in untreated animals (upper panels) and animals treated from day 10 until day 21 with 8.5 mg/kg b.w. paclitaxel i.p. (lower panels) 10 (left panels) and 20 (right panels) days after infection with *Plasmodium berghei*. B: Arithmetic means $\pm$ SEM of parasitemia in mice without treatment (open circles, n=8) or with 8.5 mg/kg b.w. paclitaxel i.p. (closed circles, n=13) as a function of days after infection with *Plasmodium berghei*. * indicates significant difference from the untreated animals. C: Survival of mice without treatment (open circles) or with 8.5 mg/kg b.w. paclitaxel i.p. (closed circles) as a function of days after infection with *Plasmodium berghei*.

Alternatively, the favourable effect may result from the scrambling effect of paclitaxel, which decreases the life span of infected erythrocytes in circulating blood. Iron deficiency [69] and treatment with lead [70] have similarly been shown to accelerate eryptosis of infected erythrocytes and are thus similarly protective against a severe course of malaria.

In conclusion, paclitaxel augments eryptosis of infected erythrocytes, thus fostering the clearance of infected erythrocytes from circulating blood. Accordingly, paclitaxel favourably influences the course of malaria in

mice. Future studies have to explore, whether paclitaxel is similarly effective in the treatment of malaria in man.

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References

- Chang JC, Wooten EC, Tsimelzon A, Hilsenbeck SG, Gutierrez MC, Tham YL, Kalidas M, Elledge R, Mohsin S, Osborne CK, Chamness GC, Allred DC, Lewis MT, Wong H, O'Connell P: Patterns of resistance and incomplete response to docetaxel by gene expression profiling in breast cancer patients. *J Clin Oncol* 2005;23:1169-1177.
- McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, Clarke-Pearson DL, Davidson M: Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. *N Engl J Med* 1996;334:1-6.
- Pietras K, Rubin K, Sjoblom T, Buchdunger E, Sjoquist M, Heldin CH, Ostman A: Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. *Cancer Res* 2002;62:5476-5484.
- Saville MW, Lietzau J, Pluda JM, Feuerstein I, Odom J, Wilson WH, Humphrey RW, Feigal E, Steinberg SM, Broder S, : Treatment of HIV-associated Kaposi's sarcoma with paclitaxel. *Lancet* 1995;346:26-28.
- Heldman AW, Cheng L, Jenkins GM, Heller PF, Kim DW, Ware M, Jr., Nater C, Hruban RH, Rezai B, Abella BS, Bunge KE, Kinsella JL, Sollott SJ, Lakatta EG, Brinker JA, Hunter WL, Froehlich JP: Paclitaxel stent coating inhibits neointimal hyperplasia at 4 weeks in a porcine model of coronary restenosis. *Circulation* 2001;103:2289-2295.
- Blagosklonny MV, Darzynkiewicz Z, Halicka HD, Pozarowski P, Demidenko ZN, Barry JJ, Kamath KR, Herrmann RA: Paclitaxel induces primary and postmitotic G1 arrest in human arterial smooth muscle cells. *Cell Cycle* 2004;3:1050-1056.
- Ferlini C, Raspaglio G, Mozzetti S, Cicchillitti L, Filippetti F, Gallo D, Fattorusso C, Campiani G, Scambia G: The seco-taxane IDN5390 is able to target class III beta-tubulin and to overcome paclitaxel resistance. *Cancer Res* 2005;65:2397-2405.
- Fuchs DA, Johnson RK: Cytologic evidence that taxol, an antineoplastic agent from *Taxus brevifolia*, acts as a mitotic spindle poison. *Cancer Treat Rep* 1978;62:1219-1222.
- Cassinelli G, Supino R, Perego P, Polizzi D, Lanzi C, Pratesi G, Zunino F: A role for loss of p53 function in sensitivity of ovarian carcinoma cells to taxanes. *Int J Cancer* 2001;92:738-747.
- Park SJ, Wu CH, Gordon JD, Zhong X, Emami A, Safa AR: Taxol induces caspase-10-dependent apoptosis. *J Biol Chem* 2004;279:51057-51067.
- von Haefen C, Wieder T, Essmann F, Schulze-Osthoff K, Dorken B, Daniel PT: Paclitaxel-induced apoptosis in BJAB cells proceeds via a death receptor-independent, caspases-3/-8-driven mitochondrial amplification loop. *Oncogene* 2003;22:2236-2247.
- Lang PA, Huober J, Bachmann C, Kempe DS, Sobiesiak M, Akel A, Niemoeller OM, Dreischer P, Eisele K, Klari BA, Gulbins E, Lang F, Wieder T: Stimulation of erythrocyte phosphatidylserine exposure by paclitaxel. *Cell Physiol Biochem* 2006;18:151-164.
- Berg CP, Engels IH, Rothbart A, Lauber K, Renz A, Schlosser SF, Schulze-Osthoff K, Wesselborg S: Human mature red blood cells express caspase-3 and caspase-8, but are devoid of mitochondrial regulators of apoptosis. *Cell Death Differ* 2001;8:1197-1206.
- Brand VB, Sandu CD, Duranton C, Tanneur V, Lang KS, Huber SM, Lang F: Dependence of *Plasmodium falciparum* in vitro growth on the cation permeability of the human host erythrocyte. *Cell Physiol Biochem* 2003;13:347-356.
- Bratosin D, Estaquier J, Petit F, Arnoult D, Quatannens B, Tissier JP, Slomianny C, Sartiaux C, Alonso C, Huart JJ, Montreuil J, Ameisen JC: Programmed cell death in mature erythrocytes: a model for investigating death effector pathways operating in the absence of mitochondria. *Cell Death Differ* 2001;8:1143-1156.
- Daugas E, Cande C, Kroemer G: Erythrocytes: death of a mummy. *Cell Death Differ* 2001;8:1131-1133.

- 17 Lang KS, Roll B, Myssina S, Schittenhelm M, Scheel-Walter HG, Kanz L, Fritz J, Lang F, Huber SM, Wieder T: Enhanced erythrocyte apoptosis in sickle cell anemia, thalassemia and glucose-6-phosphate dehydrogenase deficiency. *Cell Physiol Biochem* 2002;12:365-372.
- 18 Lang KS, Duranton C, Poehlmann H, Myssina S, Bauer C, Lang F, Wieder T, Huber SM: Cation channels trigger apoptotic death of erythrocytes. *Cell Death Differ* 2003;10:249-256.
- 19 Lang KS, Myssina S, Brand V, Sandu C, Lang PA, Berchtold S, Huber SM, Lang F, Wieder T: Involvement of ceramide in hyperosmotic shock-induced death of erythrocytes. *Cell Death Differ* 2004;11:231-243.
- 20 Bernhardt I, Weiss E, Robinson HC, Wilkins R, Bennekou P: Differential effect of HOE642 on two separate monovalent cation transporters in the human red cell membrane. *Cell Physiol Biochem* 2007;20(5):601-606.
- 21 Duranton C, Huber SM, Lang F: Oxidation induces a Cl(-)-dependent cation conductance in human red blood cells. *J Physiol* 2002;539:847-855.
- 22 Foller M, Kasinathan RS, Koka S, Lang C, Shumilina E, Birnbaumer L, Lang F, Huber SM: TRPC6 contributes to the Ca(2+) leak of human erythrocytes. *Cell Physiol Biochem* 2008;21(1-3):183-192.
- 23 Bookchin RM, Ortiz OE, Lew VL: Activation of calcium-dependent potassium channels in deoxygenated sickled red cells. *Prog Clin Biol Res* 1987;240:193-200.
- 24 Brugnara C, de Franceschi L, Alper SL: Inhibition of Ca(2+)-dependent K⁺ transport and cell dehydration in sickle erythrocytes by clotrimazole and other imidazole derivatives. *J Clin Invest* 1993;92:520-526.
- 25 Lang PA, Kaiser S, Myssina S, Wieder T, Lang F, Huber SM: Role of Ca2+-activated K⁺ channels in human erythrocyte apoptosis. *Am J Physiol Cell Physiol* 2003;285:C1553-C1560.
- 26 McConkey DJ, Orrenius S: The role of calcium in the regulation of apoptosis. *Biochem Biophys Res Commun* 1997;239:357-366.
- 27 Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM: A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* 2000;405:85-90.
- 28 Henson PM, Bratton DL, Fadok VA: The phosphatidylserine receptor: a crucial molecular switch? *Nat Rev Mol Cell Biol* 2001;2:627-633.
- 29 Boas FE, Forman L, Beutler E: Phosphatidylserine exposure and red cell viability in red cell aging and in hemolytic anemia. *Proc Natl Acad Sci U S A* 1998;95:3077-3081.
- 30 Yamanaka M, Eda S, Beppu M: Carbohydrate chains and phosphatidylserine successively work as signals for apoptotic cell removal. *Biochem Biophys Res Commun* 2005;328:273-280.
- 31 Lang F, Gulbins E, Lerche H, Huber SM, Kempe DS, Foller M: Eryptosis, a window to systemic disease. *Cell Physiol Biochem* 2008;22(5-6):373-380.
- 32 Wood BL, Gibson DF, Tait JF: Increased erythrocyte phosphatidylserine exposure in sickle cell disease: flow-cytometric measurement and clinical associations. *Blood* 1996;88:1873-1880.
- 33 Birka C, Lang PA, Kempe DS, Hoefling L, Tanneur V, Duranton C, Nammi S, Henke G, Myssina S, Krikov M, Huber SM, Wieder T, Lang F: Enhanced susceptibility to erythrocyte "apoptosis" following phosphate depletion. *Pflugers Arch* 2004;448:471-477.
- 34 Kempe DS, Lang PA, Duranton C, Akel A, Lang KS, Huber SM, Wieder T, Lang F: Enhanced programmed cell death of iron-deficient erythrocytes. *FASEB J* 2006;20:368-370.
- 35 Lang PA, Beringer O, Nicolay JP, Amon O, Kempe DS, Hermle T, Attanasio P, Akel A, Schafer R, Friedrich B, Risler T, Baur M, Olbricht CJ, Zimmerhackl LB, Zipfel PF, Wieder T, Lang F: Suicidal death of erythrocytes in recurrent hemolytic uremic syndrome. *J Mol Med* 2006;84:378-388.
- 36 Kempe DS, Akel A, Lang PA, Hermle T, Biswas R, Muresanu J, Friedrich B, Dreischer P, Wolz C, Schumacher U, Peschel A, Gotz F, Doring G, Wieder T, Gulbins E, Lang F: Suicidal erythrocyte death in sepsis. *J Mol Med* 2007;85:273-281.
- 37 Lang F, Lang PA, Lang KS, Brand V, Tanneur V, Duranton C, Wieder T, Huber SM: Channel-induced apoptosis of infected host cells-the case of malaria. *Pflugers Arch* 2004;448:319-324.
- 38 Lang PA, Schenck M, Nicolay JP, Becker JU, Kempe DS, Lupescu A, Koka S, Eisele K, Klarl BA, Rubben H, Schmid KW, Mann K, Hildenbrand S, Heftner H, Huber SM, Wieder T, Erhardt A, Haussinger D, Gulbins E, Lang F: Liver cell death and anemia in Wilson disease involve acid sphingomyelinase and ceramide. *Nat Med* 2007;13:164-170.
- 39 Lang PA, Kaiser S, Myssina S, Birka C, Weinstock C, Northoff H, Wieder T, Lang F, Huber SM: Effect of Vibrio parahaemolyticus haemolysin on human erythrocytes. *Cell Microbiol* 2004;6:391-400.
- 40 Foller M, Shumilina E, Lam R, Mohamed W, Kasinathan R, Huber S, Chakraborty T, Lang F: Induction of suicidal erythrocyte death by listeriolysin from *Listeria monocytogenes*. *Cell Physiol Biochem* 2007;20:1051-1060.
- 41 Attanasio P, Shumilina E, Hermle T, Kiedaisch V, Lang PA, Huber SM, Wieder T, Lang F: Stimulation of eryptosis by anti-A IgG antibodies. *Cell Physiol Biochem* 2007;20(5):591-600.
- 42 Geiger C, Föller M, Herrlinger KR, Lang F: Azathioprine-induced Suicidal Erythrocyte Death. *Inflammatory Bowel Diseases* 2008;14(8):1027-32.
- 43 Sopjani M, Föller M, Gulbins E, Lang F: Suicidal death of erythrocytes due to selenium-compounds. *Cell Physiol Biochem* 2008;22(5-6):387-394.
- 44 Lang PA, Kempe DS, Tanneur V, Eisele K, Klarl BA, Myssina S, Jendrosseck V, Ishii S, Shimizu T, Waidmann M, Hessler G, Huber SM, Lang F, Wieder T: Stimulation of erythrocyte ceramide formation by platelet-activating factor. *J Cell Sci* 2005;118:1233-1243.
- 45 Wang K, Mahmud H, Föller M, Biswas R, Lang KS, Bohn E, Götz F, Lang F: Lipopeptides in the triggering of erythrocyte cell membrane scrambling. *Cell Physiol Biochem* 2008;22(5-6):381-386.
- 46 Sopjani M, Föller M, Dreischer P, Lang F: Stimulation of eryptosis by cadmium ions. *Cell Physiol Biochem* 2008;22(1-4):245-252.
- 47 Niemoeller OM, Mahmud H, Föller M, Wieder T, Lang F: Ciglitazone and 15d-PGJ2 induced suicidal erythrocyte death. *Cell Physiol Biochem* 2008;22(1-4):237-244.
- 48 Bentzen PJ, Lang E, Lang F: Curcumin induced suicidal erythrocyte death. *Cell Physiol Biochem* 2007;19:153-164.
- 49 Nicolay JP, Gatz S, Liebig G, Gulbins E, Lang F: Amyloid induced suicidal erythrocyte death. *Cell Physiol Biochem* 2007;19:175-184.
- 50 Schneider J, Nicolay JP, Foller M, Wieder T, Lang F: Suicidal erythrocyte death following cellular K⁺ loss. *Cell Physiol Biochem* 2007;20:35-44.
- 51 Niemoeller OM, Foller M, Lang C, Huber SM, Lang F: Retinoic acid induced suicidal erythrocyte death. *Cell Physiol Biochem* 2008;21(1-3):193-202.
- 52 Nicolay JP, Bentzen PJ, Ghashghaieina M, Wieder T, Lang F: Stimulation of erythrocyte cell membrane scrambling by amiodarone. *Cell Physiol Biochem* 2007;20(6):1043-1050.
- 53 Bentzen PJ, Lang F: Effect of anandamide on erythrocyte survival. *Cell Physiol Biochem* 2007;20(6):1033-1042.
- 54 Schwarzer E, Turrini F, Ulliers D, Giribaldi G, Ginsburg H, Arese P: Impairment of macrophage functions after ingestion of *Plasmodium falciparum*-infected erythrocytes or isolated malarial pigment. *J Exp Med* 1992;176:1033-1041.

- 55 Ayi K, Turrini F, Piga A, Arese P: Enhanced phagocytosis of ring-parasitized mutant erythrocytes: a common mechanism that may explain protection against falciparum malaria in sickle trait and beta-thalassemia trait. *Blood* 2004;104:3364-3371.
- 56 Cappadoro M, Giribaldi G, O'Brien E, Turrini F, Mannu F, Ulliers D, Simula G, Luzzatto L, Arese P: Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)-deficient erythrocytes parasitized by *Plasmodium falciparum* may explain malaria protection in G6PD deficiency. *Blood* 1998;92:2527-2534.
- 57 de Jong K, Emerson RK, Butler J, Bastacky J, Mohandas N, Kuypers FA: Short survival of phosphatidylserine-exposing red blood cells in murine sickle cell anemia. *Blood* 2001;98:1577-1584.
- 58 Kean LS, Brown LE, Nichols JW, Mohandas N, Archer DR, Hsu LL: Comparison of mechanisms of anemia in mice with sickle cell disease and beta-thalassemia: peripheral destruction, ineffective erythropoiesis, and phospholipid scramblase-mediated phosphatidylserine exposure. *Exp Hematol* 2002;30:394-402.
- 59 Kuypers FA, Yuan J, Lewis RA, Snyder LM, Kiefer CR, Bunyaratvej A, Fucharoen S, Ma L, Styles L, de Jong K, Schrier SL: Membrane phospholipid asymmetry in human thalassemia. *Blood* 1998;91:3044-3051.
- 60 Binh VQ, Luty AJ, Kremsner PG: Differential effects of human serum and cells on the growth of *Plasmodium falciparum* adapted to serum-free in vitro culture conditions. *Am J Trop Med Hyg* 1997;57:594-600.
- 61 Huber SM, Uhlemann AC, Gamper NL, Duranton C, Kremsner PG, Lang F: *Plasmodium falciparum* activates endogenous Cl(-) channels of human erythrocytes by membrane oxidation. *EMBO J* 2002;21:22-30.
- 62 Jensen JB, Trager W: *Plasmodium falciparum* in culture: establishment of additional strains. *Am J Trop Med Hyg* 1978;27:743-746.
- 63 Trager W, Jensen JB: Human malaria parasites in continuous culture. *Science* 1976;193:673-675.
- 64 Huber SM, Duranton C, Henke G, Van De SC, Heussler V, Shumilina E, Sandu CD, Tanneur V, Brand V, Kasinathan RS, Lang KS, Kremsner PG, Hubner CA, Rust MB, Dedek K, Jentsch TJ, Lang F: *Plasmodium* induces swelling-activated ClC-2 anion channels in the host erythrocyte. *J Biol Chem* 2004;279:41444-41452.
- 65 Duranton C, Huber S, Tanneur V, Lang K, Brand V, Sandu C, Lang F: Electrophysiological properties of the *Plasmodium falciparum*-induced cation conductance of human erythrocytes. *Cell Physiol Biochem* 2003;13:189-198.
- 66 Myssina S, Lang PA, Kempe DS, Kaiser S, Huber SM, Wieder T, Lang F: Cl-channel blockers NPPB and niflumic acid blunt Ca(2+)-induced erythrocyte 'apoptosis'. *Cell Physiol Biochem* 2004;14:241-248.
- 67 Lang F, Busch GL, Ritter M, Volkl H, Waldegger S, Gulbins E, Haussinger D: Functional significance of cell volume regulatory mechanisms. *Physiol Rev* 1998;78:247-306.
- 68 Lang KS, Lang PA, Bauer C, Duranton C, Wieder T, Huber SM, Lang F: Mechanisms of suicidal erythrocyte death. *Cell Physiol Biochem* 2005;15:195-202.
- 69 Koka S, Foller M, Lamprecht G, Boini KM, Lang C, Huber SM, Lang F: Iron deficiency influences the course of malaria in *Plasmodium berghei* infected mice. *Biochem Biophys Res Commun* 2007;357:608-614.
- 70 Koka S, Huber SM, Boini KM, Lang C, Foller M, Lang F: Lead decreases parasitemia and enhances survival of *Plasmodium berghei*-infected mice. *Biochem Biophys Res Commun* 2007;363:484-489.
- 71 Tyurina YY, Tyurin VA, Zhao Q, Djukic M, Quinn PJ, Pitt BR, Kagan VE: Oxidation of phosphatidylserine: a mechanism for plasma membrane phospholipid scrambling during apoptosis? *Biochem Biophys Res Commun* 2004;324:1059-1064.