Differentiation and apoptosis in the sleeping sickness inducing parasite *Trypanosoma brucei*

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Eukaryotic parasites still cause fatal diseases (malaria, sleeping sickness, Kala Azar, Chagas disease etc.) which costs millions of deaths yearly and are hard to control because of insufficient drugs and fast developing resistance mechanisms. We investigate life cycle progression and differentiation processes in *Trypanosoma brucei,* the causative agent of sleeping sickness. Our results show that this parasite despite of being a protozoan, behaves as a concerted population which communicates by small signalling molecules.

Slender parasites are rapidly dividing and thus responsible for the observed increase of parasitemia. They constantly produce a factor of unknown chemical structure, which at a certain cell density reaches a threshold concentration and triggers formation of metabolic and morphological changes within the parasite, thus leading to stumpy form trypanosomes. These are cell cycle arrested and very sensitive against prostaglandin D_2, which is also produced by trypanosomes. PGD_2 and metabolites thereof induce cell death of the stumpy form accompanied by typical hallmarks of apoptosis like chromatin condensation, ROS formation and loss of mitochondrial membrane potential. In this way stumpy parasites are removed and the parasitemia decreases. This mechanism ensures a longer survival of the host and increases the chance of the parasite to be taken up by the tsetse fly to complete its life cycle.

Keywords African trypanosomes, sleeping sickness, differentiation, apoptosis

1. Introduction

Until some decades ago the consensus thinking by many biochemists and medical doctors was that the number of diseases threatening human life and comfort, would constantly decline with the advancement of biosciences and medical care techniques. Indeed, today we are able to treat a vast number of former deadly diseases and fatal epidemic plagues due to the development of more or less ideal drugs and adequate medical treatment, at least in first and second world countries. The great challenges that remain are especially viral infections (caused by emerging of new (e.g. HIV, Ebola) and/or zoonotic viruses (e.g. bird flu)) and so called "life style-dependent" diseases, like obesity with all its devastating consequences, diabetes type II and several forms of cancer. Following identification of a virus, respective infections can either be avoided by safety measures or treated by supporting/stimulating the host's immune system or by inhibiting virus replication. Life style-depending diseases, on the long run, should be omitted by socio-ecological training and education. However, throughout the decades, infections by eukaryotic parasites, either protozoa or metazoa, have not lost their utterly devastating impact on human health and are still threatening hundreds of million of people worldwide, causing millions of deaths yearly and having a tremendous influence on the lagged development of 3rd world countries within the tropical belt on earth. Infections by these parasites are not easily eradicated primarily because of the climatic and geographic conditions, leaving aside (in some cases) political ignorance, social upheavals, adverse cultural traditions, insufficient hygiene measures and medical incompetence. Thus treatment depends on drugs

directed against parasite specific cellular behaviour or metabolism, which –in contrast to bacteria– is often very similar to the respective counterpart of the mammalian host. Interestingly, most of the currently used drugs against protozoa have been developed empirically many decades ago and show an often unknown mode of action and rather inacceptable side effects [1]. In addition, emergence of drug resistance is often readily observed in the field, probably because of the extended co-evolution between parasite and host and the evolutionary pressure on the parasite to survive under adverse circumstances, and limits the use of these medicines significantly.

In order to fight malaria, sleeping sickness, leishmaniases and related parasitic diseases, new concepts based on deeper insights into parasite specific cell development and regulation. We therefore investigate life cycle progression and the molecular basis for differentiation processes in African trypanosomes, causative agents of sleeping sickness in humans and nagana in cattle. Sleeping sickness is caused by two sub-species of the *Trypanosoma brucei* group, *T. b. gambiense* (chronic form) and *T. b. rhodesiense* (acute form). The third sub-species, *T. b. brucei*, which is not human infective due to a so called "trypanolytic factor" prevalent in human serum [2], is very similar on the cellular and metabolic level and thus ideally suited for *in vitro* experiments. Sleeping sickness can be treated using suramin (developed 1916), which is effective against the blood stages but cannot cross the blood-brain barrier, and melarsoprol (developed 1940); effective against all trypanosome stages but highly toxic to the patients, leading to about 5% deaths of treated people [3, 4]. Eflornithine (developed 1985) is effective only against *T. gambiense* and thus of limited use.

2. Life cycle and differentiation

The life cycle of African trypanosomes has been extensively studied and consists of three distinct stages within both, the mammalian host and the insect vector (Fig. 1) [5]. Briefly for the human infective trypanosomes *T. b. gambiense* and *T. b. rhodesiense*: upon feeding on an infected mammal, trypanosomes are taken up with the blood meal by the tsetse fly. Within the digestive tract of the fly procyclic parasites are generated. They populate the midgut of the insect and give rise to formation of the epimastigote form found primarily in the salivary gland. Eventually, the human infective metacyclic form appears and is transferred during the next blood meal. Within human blood, the cell cycle arrested metacyclic form spontaneously converts to the fast dividing slender form which finally, via a poorly defined intermediate stage, differentiates to the cell cycle arrested stumpy form.

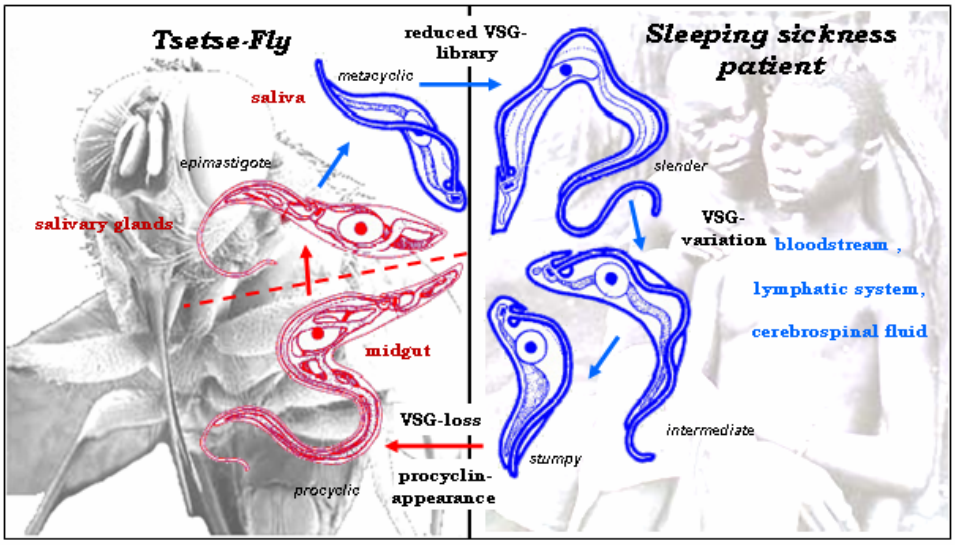

Fig.1: Life cycle of Trypanosoma brucei (modified after Vickerman, 1969).

During the bloodmeal of a tsetse-fly, metacyclic parasites enter the human bloodstream. Spontaneously this form changes into the slender blood form, rapidly dividing and therefore responsible for the increase of the parasitemia. At high cell densities, slender parasites differentiate into the stumpy form, passing an intermediate stage. Only these forms can survive in the midgut of the tsetse fly and transform into procyclic trypanosomes, loosing their VSG-coat, changing it into procyclin and fully developing their mitochondrion. When the procyclics leave the midgut and enter the salivary glands, they differentiate into the epimastigotis form and at last into metacyclics, already expressing a certain VSG-variant and thus being infectious for humans.

During the abidance in the human body, the parasite protects itself against the host immune system by covering its surface with a specific protein called *variant surface glycoprotein* (VSG). This coat protects the parasite against all immune competent cells and the complement system (Fig. 2), but not against VSG-specific antibodies. Some 1000 different VSG genes are encoded within the trypanosome genome, which are sequentially expressed to keep the parasite one step ahead of antibody production. Only one VSG gene is expressed at a given time due to its location in the one active expression site. This transcription unit is arranged in clusters with several other *expression site associated genes* (ESAGs) which are cotranscribed [6]. During infection, the expressed VSG gene copy is replaced by a copy of another VSG gene or a switch to another expression site occurs, giving rise to a population expressing another VSG coat. Thus, upon appearance of VSG specific antibodies, part of the parasite's population is killed, while trypanosomes expressing another VSG coat can grow up again. Since trypanosomes divide by binary fission, this is sort of a clonal selection.

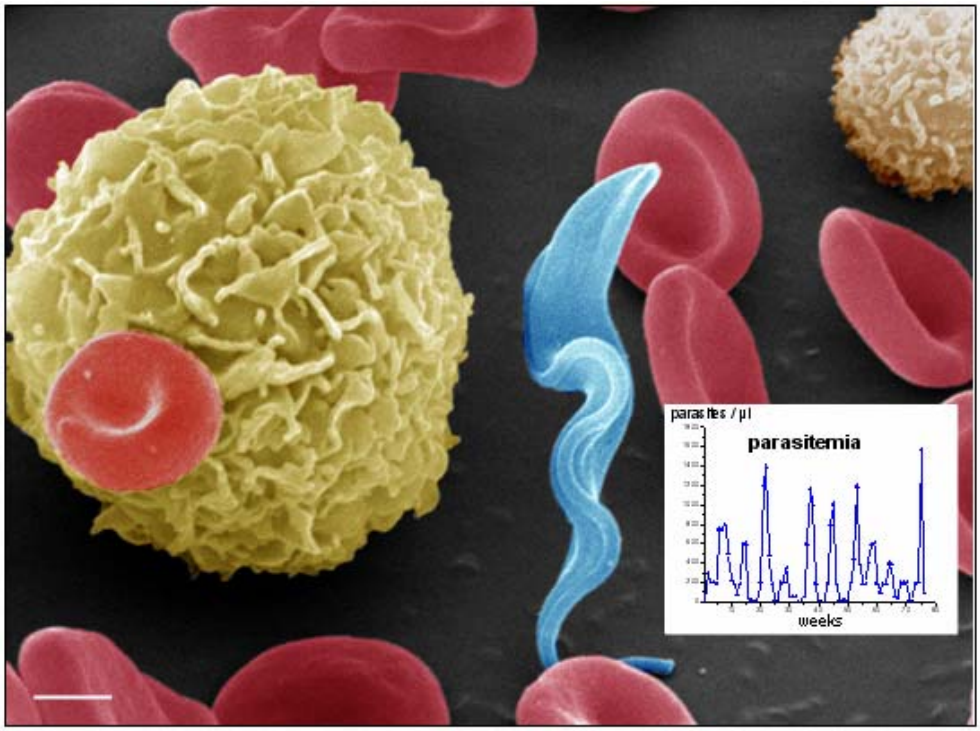

Fig. 2: Scanning electron micrograph of infected blood, showing a slender trypanosome beside erythrocytes and immune competent lymphocytes.

From the onset of infection, blood trypanosomes have to defend themselves against the immune system of their host. For this purpose they form a densely packed surface coat consisting of identical copies of a glycoprotein called VSG (variant surface glycoprotein). As soon as antibodies against this VSG are formed, the population density declines, giving rise to trypanosomes expressing a different VSG

molecule. This subpopulation growths until new antibodies are formed. This way an oscillating parasitemia occurs. Inset: Time course of infection in a male patient (after [48]. (bar = 2 μm).

It is generally accepted that the stumpy form is pre-adapted for survival in the insect vector and transforms to the procyclic form. Although it is possible to induce slender to stumpy and further to procyclic conversion *in vitro*, little is known about the molecular triggers, which intracellularly induce and perform the differentiation processes. Some years ago we questioned whether differentiation from slender to stumpy is controlled by the parasite itself or by environmental changes within the infected host. For this purpose trypanosomes have been grown in axenic culture until they reached the stationary phase, i.e. cells ran into a cell cycle arrest. These cultures have been spun down and the clear supernatant (the so called conditioned medium) was analysed for differentiation inducing activity. Using 50% fresh and 50% conditioned medium, slender trypanosomes could not divide anymore but differentiated readily, as judged by biochemical and morphological criteria [7]. As shown by us and others, a soluble stumpy induction factor (SIF) accumulates in the culture medium which, reaching a threshold concentration, leads to formation of the stumpy form [8, 9]. Unfortunately, the chemical identity of this factor has not been elucidated so far, mainly due to the complexity of the culture media, which depends on fetal calf serum of undefined composition. On the other hand, secretion of differentiation inducing factors are not unique to trypanosomes but have been observed by several protozoa [10, 11], leaving aside plant cells (indol derivatives) and higher eukaryotes (cytokines).

3. Prostaglandins and apoptosis

In search for secreted molecules of trypanosome origin, we investigated production and release of prostaglandins (PGs). PGs comprise a family of structurally related bioactive lipid mediators inducing a plethora of physiological effects especially on smooth muscle contraction and during inflammation. Interestingly, African trypanosomes produce PGD_2, PGE_2 and $PGF_{2\alpha}$ from arachidonic acid [12, 13] which may influence the host organism, since accumulation of PGD_2 and PGE_2 within the serum coincides remarkably with symptoms of sleeping sickness such as pain, fever, immunosuppression, extended sleepiness during the day etc. Analysing, whether PGs are responsible for differentiation from slender to stumpy blood forms, we observed, however, that PGD_2 induce cell death rather than differentiation [14, 15]. Since prostaglandins are highly hydrophobic, it was logical to assume that these compounds just accumulate within membranes thus causing cell damage and finally necrosis. Surprisingly, however, this effect was specific for PGD_2, while PGE_2 and $PGF_{2\alpha}$ (although highly related structurally) had no effect on cell growth when applied in similar or even higher concentrations (Fig. 3). Based on these results we became interested in the type of cell death induced and performed staining protocols using propidium iodide (indicative for necrosis) and monodansylcadaverine (indicative for autophagy). For this purpose trypanosomes were grown under culture conditions for 36 h in the presence or absence of PGD_2 and subjected to the respective staining protocol and microscopical analyses thereafter. Compared with untreated control cells, staining in both cases was negligible (below 5%) and within the area of standard deviation [14]. In contrast, positive control cells treated either with digitonin to induce necroses or starved by the absence of serum to induce autophagy, where about 95% positive for the respective staining method. We were thus prompted to consider apoptosis, a self-induced cell death mechanism used by metazoan organisms to get rid of unwanted or for the survival of the whole organism dangerous individual cells [15]. In view of the Darwinian theory it seems kind of contradictory to assume apoptosis for single cell organisms. However, there is a rapidly growing field in literature showing the appearance of hallmarks of apoptosis when investigating cell death mechanisms in protozoa [16]. In case of PGD_2-induced cell death in trypanosomes, we too found clear indications for an apoptotic mechanism. Among those were: 1) maintenance of plasma membrane integrity; 2) exposition of phoshatidylserine in the outer leaflet of the plasma membrane; 3) loss of the mitochondrion membrane potential; 4) nuclear chromatin condensation and segmentation of the nucleus; 5) DNA degradation; and 6) morphological alterations on the level of electron microscopy (Fig. 3) [14]. On the other hand, classical apoptosis depends on activation of caspases by limited proteolysis. Protozoa do not contain caspases and the only

homologous genes within the trypanosome's genome are metacaspases, which so far seem not to be involved in apoptosis in African trypanosomes [16 - 18]. Without the involvement of caspases, a molecular mechanism for apoptosis in trypanosomes remains obscure. We have however shown that PGD_2 induced apoptosis depends on a functioning protein synthesis and is readily abolished by cycloheximide, a translation inhibitor. In addition, following induction of apoptosis, the intracellular level of reactive oxygen species (ROS) increased significantly and apoptosis was inhibited using N-acetyl-l-cysteine or glutathione, i.e. compounds which reduce ROS formation considerably [15]. Interestingly, caspase-independent apoptosis was recently discovered in metazoa as well, which was also ROS dependent [19], and ROS seem to be involved in most cases, where programmed cell death was investigated in protozoa [20]. So, although the molecular players of apoptosis in protozoa are still unknown and the individual steps of the process remains to be elucidated, experimental evidence clearly supports the idea that protozoa as well as metazoa contain an inherited program to cease their own life.

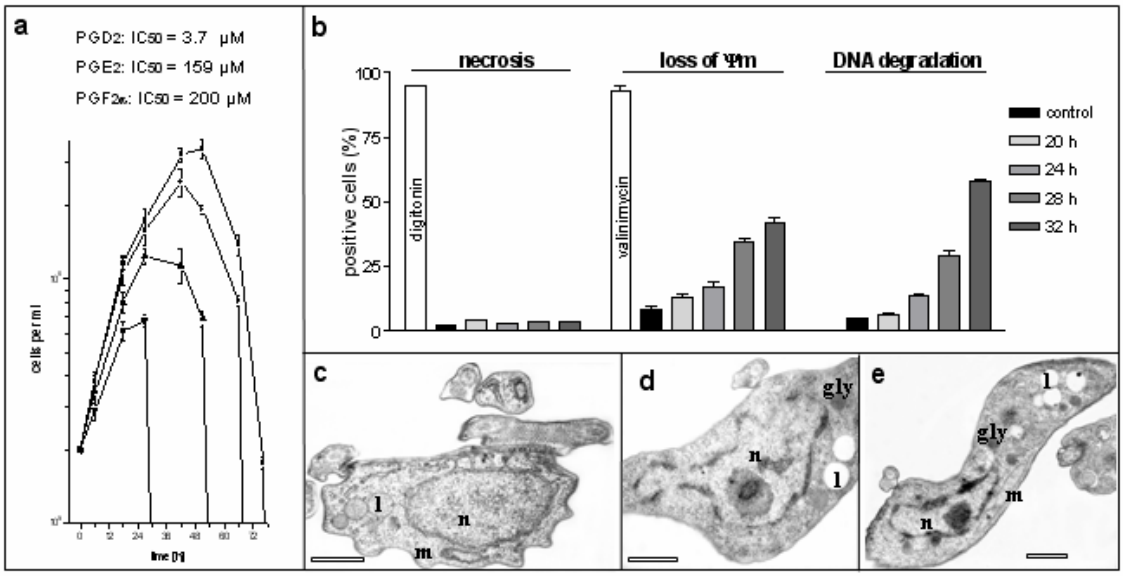

Fig. 3: PGD_2 secreted by the trypanosomes cause PCD of the parasite.
(a) Addition of different PGD_2-concentrations to blood trypanosomes (strain MITat1.2) caused a drecrease of cell density in vitro. (■) control, (●) 2.5 µM PGD_2, (▲) 5 µM PGD_2, (▼) 10 µM PGD_2.
(b) FACS analyses of trypanosomes treated with 5 µM PGD_2 revealed no increase of membrane permeability, indicative for necrosis (digitonin = positive control), but a time dependent loss of mitochondrial membrane potential (valinomycin = positive control) and DNA-degradation.
(c-e) Transmission electron microscopy. (c) non-treated control cell, (d and e) cells treated with 5 µM PGD_2 showed an increase of lysosomes, a dilatated mitochondrion and chromatine-condensation. (gly = glycosome, l = lysosome, m = mitochondrion, n = nucleus; bars = 0.5 µm)

4. Regulation of differentiation

Although Vickerman described different blood forms already in 1965 as pleomorphism [5], little is known about the mechanism responsible for these changes. Nevertheless, during the course of infection high percentages of slender forms alternate with those of stumpy cells. Remarkably, stumpy parasites occur mainly at peaks of parasitemia, suggesting that stumpy formation depend on cell density. It was indeed proven that a high cell density triggers differentiation from the dividing slender form to the cell cycle arrested stumpy form *in vitro* [7, 8, 9]. Cell density dependent differentiation is also supported by statistical analysis of the cell type composition during infections [21], indicating a critical cell density, so-called quorum, necessary for cell-cell communication. These investigations proposed the

accumulation of a chemical factor (SIF) within the blood of the mammalian host, which eventually induced formation of stumpy forms [7, 22]. SIF could act in a way of quorum sensing, already investigated in different bacteria like proteobacteria or streptomyces ssp. These prokaryotes synthesise small molecules like homoserine lactones or γ-butyrolactones, which bind to certain target proteins [23]. These targets are either transmembrane sensor kinases or intracellular receptors, leading to the transcription of respective target genes. Since transcriptional control seems to play only a marginal role in trypanosomes [24], other ways of signal transduction are more likely responsible for the reported changes of morphology, enzyme composition and cell cycle arrest [5, 22, 25]. A starting point in solving the differentiation mechanism could be the involvement of cAMP, which was described as a second messenger involved in stumpy formation. Treatment of slender form trypanosomes with membrane permeable cAMP analoga, led to the induction of cell cycle arrest and stumpy morphology *in vitro* [22, 26]. The genome of *Trypanosoma brucei* encodes about 100 adenylyl cyclases, which convert ATP to cAMP. However, only one of them is exclusively expressed in the slender form and could therefore be responsible for the initiation of differentiation. This enzyme, called ESAG4 because it is one of the expression site associated genes linked to the expression of a variant surface glycoprotein (VSG), is located on the flagellar surface of the parasite [27]. It contains a single transmembrane domain that connects the catalytic site with a large extracellular domain, which probably works as a receptor. Upon binding of a so far unknown ligand, ESAG4 forms a dimer, which seems to be a prerequisite for activation [28].

Another signal transduction way, likely to be involved in regulation of cell cycle, cell growth and differentiation, are mitogene activated protein kinases. Normally MAPKs are responsible for phosphorylation of several proteins like cell surface proteins, cytoskeletal proteins, metabolic enzymes, components of signal transduction pathways, or factors responsible for transcription, mRNA stability and/or translation [29]. Null mutants of one of the trypanosomal MAPKs, the TbMAPK5, showed an optimization of differentiation into stumpy forms [30]. This led to the suggestion that SIF could also act as a negative regulator of TbMAPK5, which normally acts antagonistically to the cAMP-pathway. Nevertheless, TbMAPK5 turned out to be only one of several factors that have to act in concert.

Prostaglandins communicate with their target cells either by reacting with the respective cell surface receptor, usually a G-protein associated membrane protein with 7 transmembrane helices, or with intracellular receptors like the peroxisome proliferator activated receptor gamma (PPARγ). A highly effective agonist of PPARγ is troglitazone, a member of the thiazolidinedione family. Members of this family are often used for experimental analyses of prostaglandin effects on target cells, but have also been employed to treat metabolic disorders like diabetes type II [31]. In addition, thiazolidinediones are proven inducers of differentiation in higher eukaryotes by activating PPARγ [32], ERK's (extracellular-signal regulated kinases) [33, 34] or other regulatory proteins [35, 36]. In our hands, troglitazone induced slender to stumpy differentiation as judged by the observed metabolic and morphological changes; cell cycle arrest, a typical feature of stumpy cells, was not observed, however [37]. Nevertheless, troglitazone treated cells, in contrast to the untreated control cells transformed readily to the procyclic insect form, following exposition to an *in vitro* transformation protocol [37]. From these results, we cannot rule out the possibility that transformation from blood to insect midgut forms do not depend on cell cycle arrested stumpy forms but on intermediate forms, which show a more developed mitochondrion (as a prerequisite for procyclic formation) but are not yet blocked in cell division. As an approach to address this question experimentally, the expression profiles of cells during these stages were investigated. The results confirmed the involvement of ESAG4 as a differentiation inductor, but demonstrated also a remarkably up-regulation of ESAG8, another member of the expression site associated genes. This non-membrane protein is thought to have a regulatory function during cell cycle progression and in energy metabolism due to its primary location in the nucleolus on the one hand, and to its structure on the other hand [6], which contains 18 leucine rich repeats and a ring finger motif [38], both of them important for protein-protein and protein-DNA interactions. There is indeed experimental evidence indicating that some interaction partners of ESAG8 exist (e.g. PIE8 [39] and TbPUF1 [40], but so far no clear function has been assigned.

5. Life cycle progression and cell density regulation

During our survey to investigate the molecular triggers of differentiation during life cycle progression, we discovered two low molecular weight compounds which are formed and secreted by trypanosomes and similar to cytokines in higher eukaryotes have an explicit impact on cell function and morphology: PGD_2 induces apoptosis, SIF leads to the induction of stumpy formation. The question is, if these processes might be linked to each other.

Existence of apoptosis is an altruistic form of cell death which, in a single cell organism such as trypanosomatids, would appear counter-intuitive if we consider these parasites as self-contained organisms that compete for nutrients and growth factors with their companion cells. However, as we know from studies of free living bacteria such as *Pseudomonas aeruginosa*, these organisms are capable of quorum sensing, whereby all individual cells secrete a signal factor (homoserine lactone derivatives, HSL), which induces a common genetic response in the population once a threshold concentration of HSL is reached [41]. Similar factors have been analysed in protozoan organisms [8, 10, 11, 22]. The ability of these organisms to sense their environment suggests that the parasitic protozoa are able to interact as complex communities, making apoptosis an important instrument to ensure a controlled and persistent parasitaemia.

Trypanosomes show an oscillating parasitaemia in the mammalian host with regularly increasing and decreasing population densities. A population decrease depends primarily on the formation of specific antibodies against existing VSG molecules. Due to antigenic variation, population density increases again, with the appearance of parasites expressing a so far unrecognized VSG molecule [42, 43]. However, an oscillating growth curve was also obtained in culture media without any antibodies present when the conditioned medium was replaced at regular intervals against fresh culture media [8] and in immuno-suppressed mice [21]. These observations suggest that quorum sensing is an active and essential mechanism for the regulation of parasite growth.

The differentiation factor is constantly secreted and accumulates in the media or in blood and once a certain threshold concentration is reached, differentiation from slender to stumpy parasites is initiated. However, blood form ‘stumpy’ parasites cannot divide and antigenic variation stops [22, 44]. This differentiation from a dividing slender to a non-dividing stumpy form is accompanied by morphological and biochemical changes, so that different populations exist in an infected mammal: distinct slender and stumpy blood forms with a less defined intermediate form in between. It is the general consensus that the slender form assures a persistent parasitaemia, while the stumpy form promotes propagation of the trypanosome life cycle in the tsetse fly. However, this perception ignores the fact that the mammalian host may be overgrown by the parasite entering hypoglycaemic crisis accompanied by oxygen depletion and metabolic acidosis [45].

In addition to cell density-dependent (quorum sensing) differentiation, population density seems to be controlled by programmed cell death of the stumpy form. This mechanism would ensure an immediate decrease in population density, while the appearance of relevant antibodies could take more than a week. We have been able to demonstrate that PGD_2 induces apoptosis especially in stumpy parasites [14, 15]. In addition, it might also be that host derived PGD_2 increases in response to increased parasitaemia [46, 47], leading to the same result. Independent of its origin, PGD_2 appears to be a physiological inducer of PCD in *T. brucei*, which in turn leads to a model for cell density regulation in African trypanosomes: slender parasites secrete a differentiation factor, which by an autocrin or paracrin mechanism induces differentiation. Stumpy parasites are pre-adapted for transmission and respond to PGD_2 with apoptosis. In this way, survival of the parasite would be ensured, since differentiation depends on the density of the slender form and will not occur if their number decreases through differentiation. The number of stumpy parasites may decrease due to apoptosis, but will be constantly replaced due to the oscillating parasitaemia. This controlled mechanism of cell density regulation by the parasite can be seen as altruistic as removal of stumpy forms ensures a persistent infection.

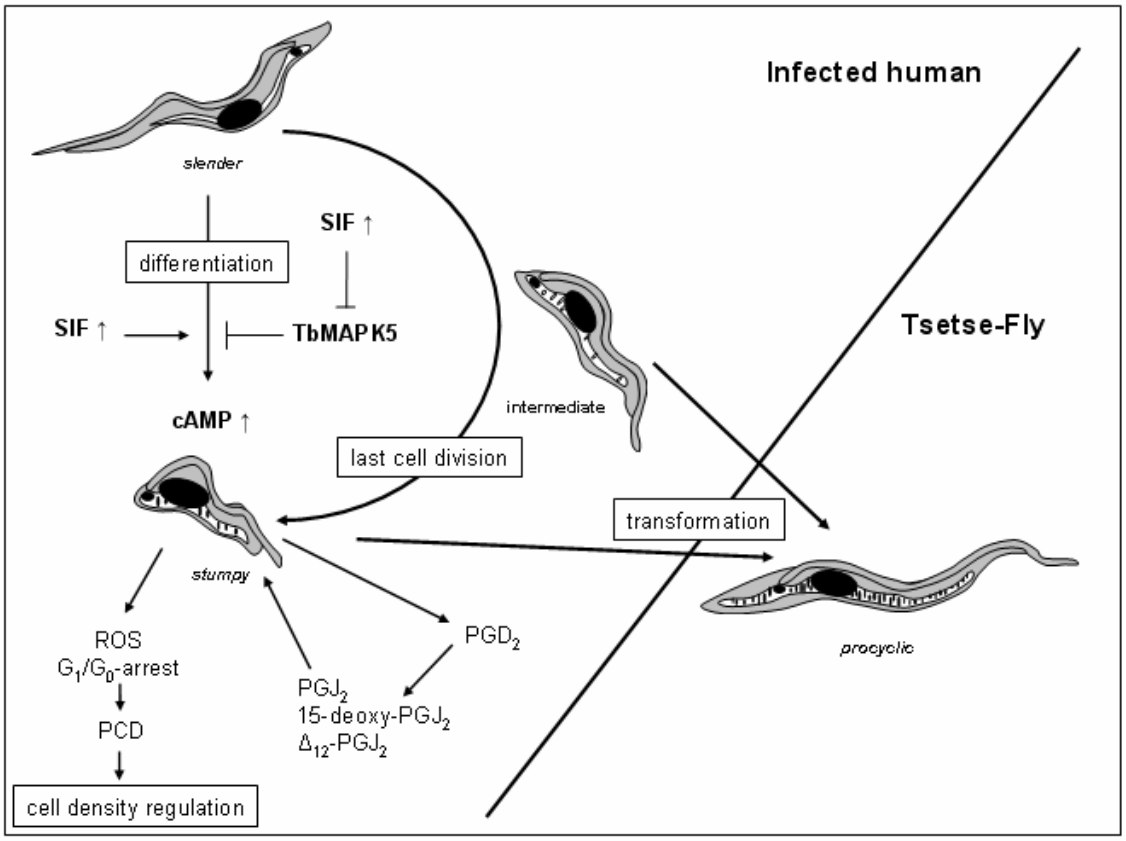

Fig. 4: Hypothesis on cell-density regulation based on our current knowledge.
When present in a critical cell concentration, slender trypanosomes produce a chemical compound, called stumpy induction factor (SIF) leading to an increase in intracellular cAMP concentration and thus triggering the formation of cell cycle arrested stumpy forms via an intermediate stage which are both able to transform into procyclic parasites within the midgut of the tsetse-fly. In contrast to this factor, the TbMAPK5 inhibits stumpy formation and promotes growth of the parasite. The stumpy form, preferentially occurs at higher cell densities, is cell cycle arrested and produces PGD_2. The latter is rapidly metabolised into several other derivatives within the blood. These prostaglandins induce formation of reactive oxygen species (ROS) within the parasite and cause programmed cell death specifically of the stumpy form. In this way a decrease of parasitemia beside the host's immune system occurs.

6. Outlook

Protozoan induced diseases like malaria, sleeping sickness, leishmaniasis and others are still a major health problem worldwide, especially in the tropical belt. The involved parasites are not self-contained individuals but form a population that acts as an entity via cross-talk using signalling molecules like SIF and PGD_2 in trypanosomes and xanthurenic acid in plasmodium. Successful survival for these parasites depends on two main problems: 1) they should not "overgrow" the host, as its premature death would limit the time for uptake by the respective insect, needed for transmittance; 2) they have to survive despite existence of a very broad and highly sensitive immune system within the host. Quorum sensing induced apoptosis seems to be a simple but well developed strategy to regulate population density in a parasite controlled way independent of acceptable losses due to the immune system. Hiding within host cells (Plasmodium, American trypanosomes, Leishmania) or antigenic variation (African trypanosomes)

are mechanisms to escape from the immune system. We need to identify the complex regulation of their respective life cycles in order to understand the rational underlying the evolved mechanisms to ensure survival. The involved parasite specific signalling pathways should be vulnerable to treatment leading to non-dividing cells which should be recognized by the immune system.

References

[1] http://www.who.int/mediacentre/factsheets/fs259/en/
[2] E. Pays, B. Vanhollebeke, L. Vanhamme, F. Paturiaux-Hanocq, D.P. Nolan and D. Pérez-Morga, Nat. Rev. Microbiol., **4**, 6 (2006)
[3] A.J. Nok, Parasitology Research., **90**, 1, (2003)
[4] J. Robays, P. Lefèvre, P. Lutumba, S. Lubanza, V. Kande Betu Ku Mesu, P. Van der Stuyft and M. Boelaert, Trop. Med. Int. Health., **12**, 2, (2007)
[5] K. Vickerman, Nature, **208**, 5012, (1965)
[6] E. Pays, S. Lips, D. Nolan, L. Vanhamme and D. Pérez-Morga, Mol. Biochem. Parasitol., **114**, 1, (2001)
[7] B. Hamm, A. Schindler, D. Mecke and M. Duszenko, Mol. Biochem. Parasitol., **40**, 1, (1990)
[8] F. Hesse, P.M. Selzer, K. Muhlstadt and M. Duszenko, Mol. Biochem. Parasitol., **70**, 1-2, (1995)
[9] B. Reuner, E. Vassella, B. Yutzy and M. Boshart, Mol. Biochem. Parasitol., **90**, 1, (1997)
[10] T. Saito, G.W. Taylor, J.C. Yang, D. Neuhaus, D. Stetsenko, A. Kato and R.R. Kay, Biochim. Biophys. Acta., **1760**, 5, (2006)
[11] O. Billker, V. Lindo, M. Panico, A.E. Etienne, T. Paxton, A. Dell, M. Rogers, R.E. Sinden and H.R. Morris, Nature, **392**, 6673, (1998)
[12] B.K. Kubata, M. Duszenko, Z. Kabututu, M. Rawer, A. Szallies, K. Fujimori, T. Inui, T. Nozaki, K. Yamashita, T. Horii, Y. Urade and O. Hayaishi, J. Exp. Med., **192**, 9, (2000)
[13] K.B. Kilunga, T. Inoue, Y. Okano, Z. Kabututu, S.K. Martin, M. Lazarus, M. Duszenko, Y. Sumii, Y. Kusakari, H. Matsumura, Y. Kai, S. Sugiyama, K. Inaka, T. Inui and Y. Urade, J. Biol. Chem., **280**, 28, (2005)
[14] K. Figarella, M. Rawer, N.L. Uzcategui, B.K. Kubata, K. Lauber, F. Madeo, S. Wesselborg and M. Duszenko, Cell Death Differ., **12**, 4, (2005)
[15] K. Figarella, N.L. Uzcategui, A. Beck, C. Schoenfeld, B.K. Kubata, F. Lang and M. Duszenko, Cell Death Differ., **13**, 10, (2006)
[16] A. Szallies, B.K. Kubata and M. Duszenko, FEBS Lett., **517**, 1-3, (2002)
[17] A. Szallies, Doctoral thesis, University of Tuebingen, Tuebingen, (2003)
[18] M.J. Helms, A. Ambit, P. Appleton, L. Tetley, G.H. Coombs and J.C. Mottram, J. Cell Sci., **119**, Pt 6, (2006)
[19] M. Blank, and Y. Shiloh, Cell Cycle, **6**, 6, (2007)
[20] S.C. Welburn, E. Macleod, K. Figarella and M. Duzensko, Parasitology, **132**, Suppl:S7-S18, (2006)
[21] J.R. Seed and S.J. Black, J. Parasitol., **83**, 4, (1997)
[22] E. Vassella, B. Reuner, B. Yutzy and M. Boshart, J. Cell Sci., **110**, 21, (1197)
[23] K.L. Visick and C. Fuqua, J. Bacteriol., **187**, 16, (2005)
[24] C.E. Clayton, EMBO J., **21**, 8, (2002)
[25] J.W. Priest and S.L. Hajduk, J. Bioenerg. Biomembr., **26**, 2, (1994)
[26] T. Breidbach, E. Ngazoa and D. Steverding, Exp. Parasitol., **101**, 4, (2002)
[27] P. Paindavoine, S. Rolin, S. Van Assel, M. Geuskens, J.C. Jauniaux, C. Dinsart, G. Huet and E. Pays, Cell Biol., **12**, 3, (1192)
[28] B. Bieger and L.O. Essen, EMBO J., **20**, 3, (2001)
[29] K.L. Guan, Cell Signal., **6**, 6, (1994)
[30] D. Domenicali-Pfister, G. Burkard, S. Morand, C.K. Renggli, I. Roditi and E. Vassella, Eukaryot Cell, **5**, 7, (2006)
[31] A.K. Hihi, L. Michalik and W. Wahli, Cell. Mol. Life Sci., **59**, 5, (2002)
[32] T.M. Willson, M.H. Lambert and S.A. Kliewer, Annu. Rev. Biochem., **70**, (2001)
[33] G. He, Y.M. Sung and S.M. Fischer, Prostaglandins Leukot. Essent. Fatty Acids., **74**, 3, (2006)
[34] F. Turturro, R. Oliver 3rd, E. Friday, I. Nissim and T. Welbourne, Am. J. Physiol. Cell. Physiol., **292**, 3, (2007)
[35] J.R. Weng, C.Y. Chen, J.J. Pinzone, M.D. Ringel and C.S. Chen, Endocr. Relat. Cancer., **13**, 2, (2006)
[36] J.R. Colca, W.G. McDonald, D.J. Waldon, J.W. Leone, J.M. Lull, C.A. Bannow, E.T. Lund and W.R. Mathews, J. Physiol. Endocrinol. Metab., **286**, 2, (2004)
[37] V. Denninger, K. Figarella, C. Schönfeld, S. Brems, C. Busold, F. Lang, J. Hoheisel and M. Duszenko, Exp. Cell Res., **313**, 9, (2007)
[38] P. Revelard, S. Lips, and E. Pays, Nucleic Acids Res., **18**, 24, (1990)

[39] D. Perez-Morga and E. Pays, Mol. Biochem. Parasitol., **101**, 1-2, (1999)
[40] M. Hoek, T. Zanders and G.A. Cross, Mol. Biochem. Parasitol., **120**, 2, (2002)
[41] E.P. Greenberg, J. Clin. Invest., **112**, 9, (2003)
[42] G.A. Cross, Proc. R. Soc. Lond. B. Biol. Sci., **202**, 1146, (1978)
[43] D. Barry and M. Carrington, The Trypanosomiasis (Maudlin, I et al., eds). CABI Publishing, pp. 25-38, (2005)
[44] A. Amiguet-Vercher, D. Pérez-Morga, A. Pays, P. Poelvoorde, H. Van Xong, P. Tebabi, L. Vanhamme and E. Pays, Mol. Microbiol., **51**, 6, (2004)
[45] T. von Brand, Biochemistry of Parasites, Academic Press, (1976)
[46] V.W. Pentreath, K. Rees, O.A. Owolabi, K.A. Philip and F. Doua, Trans. R. Soc. Trop. Med. Hyg., **84**, 6, (1990)
[47] R.A. Alafiatayo, M.R. Cookson and V.W. Pentreath, Parasitol. Res., **80**, 3, (1994)
[48] R. Ross and D. Thomson, Proc. Roy. Soc. (London), Series B. **82**, (1910)