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Mini review

Stem cell-derived cell cultures and organoids for protozoan parasite propagation and studying host–parasite interaction

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ABSTRACT

Possibilities to study the biology of human protozoan parasites and their interaction with the host remain severely limited, either because of non-existent or inappropriate animal models or because parasites cannot even be cultured in vitro due to strict human-host specificity or physiology. Here we discuss the prospects of using induced pluripotent stem cell (iPSC)-derived culture systems including organoids as a strategy to address many of these experimental bottlenecks. iPSCs already allow the generation of differentiated cell cultures for many human organs, and these cells and derivatives are amenable to reverse genetics in combination with advanced tools for genetic manipulation. We present examples of blood, neuron, liver, and intestine-dwelling protozoa, i.e. *Plasmodium falciparum*, *Toxoplasma gondii* and *Giardia duodenalis*, where iPSCs or organoids would allow addressing questions of cell and developmental biology, immunology, and pharmacology in unprecedented ways. Starting points and resources for iPSC experimentation are briefly discussed.

Introduction

The ability to propagate pathogens in vitro in order to study their biology and interaction with their host is a prerequisite for understanding infectious diseases, and this is as important today as it was for Robert Koch when he first identified and cultivated *Bacillus anthracis* as the etiological agent causing anthrax (Koch, 1876). The study of protozoan parasites is no exception to this. Although a substantial body of literature exists describing in vitro methods for their propagation (e.g. Taylor and Baker, 1987; Visvesvara and Garcia, 2002) a large number of problems and restrictions remain.

Some parasites cannot be cultivated in vitro at all, e.g. *Cyclospora cayetanensis* (Ortega and Sanchez, 2010); for others such as *Cryptosporidium* sp. (Arrowood, 2002) or *Giardia duodenalis* (Benere et al., 2010), existing in vitro or in vivo systems are unsatisfactory, and axenic culture systems that would allow one to dissect biochemical dependencies for growth are not available for most medically important parasites, including all Apicomplexa. Host specificity is another important obstacle against the propagation of protozoan, in particular intracellular parasites, such as *Plasmodium* sp. or *Eimeria* sp., or for studying host species-restricted life cycle segments such as sexual reproduction of the apicomplexan parasites *Toxoplasma gondii* and *Neospora caninum*, which only occur in feline or canine hosts, respectively. The recent progress in stem cell biology, i.e. the in vitro generation of pluripotent stem cells and organoids from those, holds great promise as a solution for a number of the aforementioned problems in the study of specific, mainly human host–parasite combinations. In this review we aim to highlight some of the opportunities and remaining challenges of these developments for the study of protozoan parasites by focusing on select examples.

What are stem cells, and how can they be generated in vitro?

In 1998 the propagation of pluripotent cells from human blastocysts, cultured on mouse embryonic fibroblasts in serum-containing media, was reported (Thomson et al., 1998). Thus, a protocol became available for the derivation of human – in this case embryonic – stem cells, i.e. cells with the capacity for long-term self-renewal and the potential to differentiate into the components and derivatives of all three embryonic germ layers. While this was a technical breakthrough, it immediately provoked a necessary ethical discussion (Miller and Bloom, 1998) and subsequently led to legislation in different countries to regulate when and how the technique or working with human embryonic stem cells (hESCs) would be acceptable (e.g. in Germany see http://www.rki.de/DE/Content/Kommissionen/ZES/zes_node.html). Eight years later Takahashi and Yamanaka published a protocol that allowed for the reprogramming of differentiated murine fibroblasts by expression of just four transcription factors, Oct-4, Sox-2, Krüppel-like factor 4 (Klf-4), and c-myc (Takahashi and

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Yamanaka, 2006). Thereby they were able to re-establish the intracellular signaling circuitry that induces pluripotent stem cells (iPSCs). Since then several solutions for delivering such reprogramming factors into mouse and human somatic cells have been developed even to the level of commercial products, and these include alternatives such as using small molecules and miRNAs (for review see Hanna et al., 2010; Robinton and Daley, 2012; see also Supplementary Table 1 for selected iPSC-related resources). The iPSC technology not only poses less severe ethical issues, it also offers a real possibility to study the basis of genetic diseases, because iPSC lines can be established from affected patients (Sadelain et al., 2011). Additionally, protocols to re-derive differentiated cells of different types from iPSC are rapidly being developed. Needless to say, these advances together with initiatives to establish iPSC banks (Rao et al., 2012) open opportunities to study host species- and host genotype-specific parasite interactions. Moreover, the possibility to genetically engineer human iPSCs with a high efficiency (Hockemeyer et al., 2011) in order to introduce at a defined site a transgene or disrupt the endogenous gene(s), thus creating functional mutants, will add even more impetus to this (see next section). In the context of protozoan parasitic infections, unlimited access to cells of the hematopoietic lineages, to neuronal cells, to liver and gastrointestinal cell types, or to organoids mimicking the respective organs is the promise that iPSCs of relevant host species hold (see below and Table 1). Methods for efficient transdifferentiation of readily available cells such as fibroblasts to more difficult-to-get cells complement the choices (Vierbuchen and Wernig, 2011).

Generation of human hematopoietic cells with designed genetic makeup – a novel resource for studying *Plasmodium*–erythrocyte interactions

iPSCs are an attractive platform for the propagation of human *Plasmodium* species due to the strict host cell specificity of the latter for human erythrocytes or hepatocytes (Schuster, 2002). Recent advances in the field of large-scale in vitro production of functional red blood cells (RBCs) from iPSCs (for review see Dravid and Crooks, 2011; Timmins and Nielsen, 2011) provide exciting opportunities for studying the respective host–parasite interactions. Through evolution, the burden of human malaria has resulted in the selection of a number of gene mutations in the human population that affect RBC function. These mutations lower morbidity and mortality due to malaria in the affected human beings (Bauduer, 2012; Gouagna et al., 2010) and have provided important clues to the intraerythrocytic biology of *Plasmodium falciparum*. It can be assumed that more host erythrocyte proteins are important in this context, as suggested by recent studies (Bei et al., 2010; Crosnier et al., 2011). Based on protocols described previously (Giarratana et al., 2005), these investigators used $CD34^+$ precursor stem cells purified from human bone marrow and knocked down glycophorin A and basigin, respectively, by RNAi before differentiating these cells to RBC and infecting them with *P. falciparum*. However, the potential of designing specific gene deletions, site-directed specific mutations and modifications or corrections with the aid of so-called zinc finger nucleases (ZFNs; Collin and Lako, 2011) or transcription activator-like effector nucleases (TALENs; Mussolino and Cathomen, 2012) in iPSCs (Hockemeyer et al., 2009, 2011) allows one to refine and extend the design of such experiments (see Fig. 1). The subsequent differentiation of such mutated cells into erythrocytes would greatly expand the repertoire of mutant erythrocytes amenable to functional testing. Moreover, access to human $CD34^+$ precursor stem cells or already known mutant erythrocytes from patients is limited, and iPSC-derived erythrocytes from such individuals would facilitate research on such *P. falciparum*-infected cells.

A further application of this technology is to humanize mice with such cells (Arnold et al., 2011; Legrand et al., 2009) in order to study many aspects of host–parasite relation in vivo in an unprecedented way. The generation of mice with gene defects that prevent

Table 1
Potential applications of stem cell-derived primary tissues/cells for research on the host–pathogen interaction of protozoan parasites.

Differentiated cells/tissue[a]	(Stem) Cell source and technical references[a]	Protozoan pathogen	Examples of possible application
Hemopoietic cells			
Erythrocytes	hiPSCs (Dravid and Crooks, 2011; Timmins and Nielsen, 2011)	*P. falciparum* and *P. vivax* (blood stage) *Babesia* spp.	Mutant erythrocytes for studying metabolite transport
Neuronal tissue			
Neurons	Fibroblasts (transdifferentiation) (Ambasudhan et al., 2011; Pang et al., 2011; Pfisterer et al., 2011) hESCs, hiPSCs (Lie et al., 2012)	*T. gondii*	Dissecting neuronal host–parasite interaction of the latent parasite stage (bradyzoites)
Liver			
Hepatocytes	hiPSCs, hESCs (Cai et al., 2007; Chen et al., 2012; Duan et al., 2007; Schwartz et al., 2005; Song et al., 2009; Touboul et al., 2010)	*P. falciparum* and *P. vivax*	Studying nutrient acquisition, replication, and antigen presentation in primary human (mutant) hepatocytes
	Fibroblasts (transdifferentiation) (Sekiya and Suzuki, 2011)	*Entamoeba* spp.	Revealing underlying pathogenicity factors of human liver/hepatocyte destruction
Hollow fiber/organoids	miPSCs and mESCs (Amimoto et al., 2011; Mizumoto et al., 2008)		
Intestine			
Intestinal organoids	hiPSCs, human intestinal crypts (McCracken et al., 2011; Sato et al., 2011a; Spence et al., 2011)	*G. duodenalis*	Improved in vitro culture of specific genotypes, studying virulence/pathogenicity factors
	Mouse $Lrg5^+$ cells, mouse intestinal crypts (Ootani et al., 2009; Sato et al., 2009, 2011a,b)	*Entamoeba* spp., *Balantidium* spp.	Studying pathogenicity of intestinal amoebiasis and balantidiosis
		Cryptosporidium spp., *Cyclospora* spp.	Improved in vitro culture, drug and disinfectant screening, studying biology of life cycle stages, viability assays
		T. gondii	Recapitulating host–parasite interactions at the primary infection site

[a] The different stem cell sources and differentiated cells can be combined with different protozoa, depending on the intended experiment.