Parasitol Res (2011) 108:247–252
DOI 10.1007/s00436-010-2072-y

SHORT COMMUNCATION

The hypnozoite concept, with particular reference to malaria

Miles B. Markus

Received: 3 August 2010 / Accepted: 20 September 2010 / Published online: 6 October 2010

Abstract In 1978, the nature of the hypnozoite was discussed in an article that appeared in a relatively obscure journal, which is also where the term was adopted for *Plasmodium* (a little-known fact). As a result, that commentary on the use of the word "hypnozoite" has been almost completely overlooked. Although the publication is now more than three decades old, the analysis remains valid today. It is explained in the present paper that like "merozoite" and "sporozoite", the name "hypnozoite" is applicable not only to a latent stage in the life cycle of *Plasmodium* but to some apparently dormant forms of other kinds of apicomplexan parasites as well. Merozoites of different genera of parasitic protozoa are not necessarily the same biologically and/or otherwise. Similarly, although the hypnozoite concept relates primarily to pre-merozoite stages, some atypical post-divisional apicomplexan forms might also be hypnozoites. Examples are likewise given of latent organisms that, in contrast, are clearly not hypnozoites, such as dormant merozoites in malaria infections. Lastly, the plasmodial hypnozoite is placed in context in relation to the relatively unfamiliar (nomenclaturally) malarial bradysporozoite, chronozoite, dormozoite, merophore, merosome and *x* body. This paper is based on a presentation by the author, as a Life Member of the American Society of Tropical Medicine and Hygiene, to its 59th Annual Meeting in Atlanta, Georgia, USA, 3–7 November 2010.

M. B. Markus
Imperial College London,
London, UK

M. B. Markus (✉)
School of Animal, Plant and Environmental Sciences,
University of Witwatersrand,
Private Bag 3, Wits,
Johannesburg 2050, South Africa
e-mail: medsynth@yahoo.co.uk

Introduction

Following the detection of an apparently latent, singly occurring, non-merozoite-like liver form in the life cycle of *Plasmodium*, the term "hypnozoite" was used for this stage (Krotoski et al. 1980). The word had been coined and adopted for malaria by Miles B. Markus in 1978 (see Markus 2010), at which time the existence of malarial hypnozoites was still a hypothetical notion. Three decades later, hypnozoites are widely understood to be dormant hepatic forms of certain primate malaria species. What is not general knowledge, however, is that hypnozoites also occur in the life cycles of other apicomplexan protozoa, as is true of merozoites and sporozoites; i.e. "hypnozoite" is not exclusively a malaria-associated name. The hypnozoite is discussed in a publication that has remained almost entirely unread by scientists, including malariologists, because it appeared in a local (as opposed to international) journal (Markus 1978a). The paper was, however, based on contributions to two international conferences (Markus 1978b, c).

Cystoisospora and *Plasmodium*: historical connection

Research that I carried out on the coccidian parasite *Cystoisospora felis* (synonym: *Isospora felis*—see Barta et al. 2005), while a Ph.D. student at Imperial College London, led to a detailed ultrastructural investigation in which the sporozoite-like nature of the extraintestinal stage of *Cystoisospora* of mammals was revealed for the first

time (Mehlhorn and Markus 1976). Considering that *Plasmodium* is related to *Cystoisospora* in that the former is what might be described as a blood coccidian parasite, it was suggested by Markus (1976) that a latent, sporozoite-like plasmodial form might, likewise, exist. This turned out to be the case (Krotoski et al. 1980).

Hypnozoites in life cycles of malaria parasites of primates

Plasmodium vivax and, presumably, both *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* are among the species with hypnozoites in their life cycles (Krotoski et al. 1982c, 1986; Sutherland et al. 2010). Furthermore, hypnozoites have been seen in the life cycles of the relapsing primate malaria species *Plasmodium cynomolgi* (see Krotoski et al. 1980, 1982a, b; Bray et al. 1985; Jiang et al. 1988) and *Plasmodium simiovale* (see Cogswell et al. 1991). They have been searched for, but not found, in the life cycle of the non-relapsing primate malaria parasite *Plasmodium knowlesi* (see Krotoski and Collins 1982). *Plasmodium malariae* is thought not to have a hypnozoite stage, despite the fact that parasites can persist in the host for more than 50 years (Collins and Jeffery 2007).

It has been shown that hypnozoites of *P. vivax* often have a different genotype from the parasite(s) that gave rise to the initial infection (Collins 2007; Chen et al. 2007; Imwong et al. 2007). The malarial hypnozoite poses a challenge in respect of control interventions, including the use of vaccines directed against *P. vivax* (Galinski and Barnwell 2008; Wells et al. 2010).

Hypnozoite stage terminology

It has been suggested that the word "hypnocyst" should not be applied (like "caryocyst") to the "monozoic cyst" of *Cystoisospora*, because "hypnocyst" has a prior protozoological meaning (Markus 1978a, 2010).

"Dormozoite" is a synonym for "hypnozoite" (Mehlhorn 2008). Similarly, an intracellular plasmodial "bradysporozoite" (Lysenko et al. 1977) in the liver is a "hypnozoite". The terms "dormozoite" and "bradysporozoite" are seldom used.

The name "*x* bodies" appears once (in the plural) in a paper by Shute (1946). This was in relation to, in Shute's words, "… the hypothetical stage of the parasite between the sporozoite and the erythrocytic parasite." Shute was writing in a theoretical vein, i.e. he had seen neither a pre-erythrocytic plasmodial schizont (meront) in a mammal, nor any apicomplexan hypnozoite. The latter was first identified as such in the mid-1970s (Mehlhorn and Markus 1976; Markus 1998) and the former was first found in the late 1940s (Shortt and Garnham 1948). The apicomplexan hypnozoite owes its name (Markus 1978a, 1980, 2010) not to a hypothetical concept but to the detailed laboratory and fine structural research that was carried out by Markus (1976) and Mehlhorn and Markus (1976). In view of the context of Shute's speculative discussion, which was about the presumed existence of "resting" malarial organisms, the "*x* body" is to be (retrospectively) regarded as synonymous not with a (at the time still unknown) plasmodial schizont in the liver of the primate host but, rather, with the subsequently discovered malarial hypnozoite. In a later paper by Shute, the following sentence is associated with an analysis of research results (Shute et al. 1976): "This observation makes it unlikely that some special cell "X" elsewhere [other than in the liver] may harbour either the [late tissue] stages or the sporozoite itself, during the prolonged prepatent period." Here, "X" is obviously a reference to a host cell, not to (as in Shute's earlier article) a form in the life cycle of *Plasmodium*.

Hypnozoite-related rationale

The hypnozoite is best known as the probable cause of latency and relapse in malaria. However, in addition to use of the word for the small, inactive, uninucleate liver stage of *Plasmodium*, the definition of a hypnozoite also covers (inter alia) apicomplexan "… post-divisional, dormant, sporozoite-like organisms (should any occur) that are not ultrastructurally or biologically typical merozoites …" (Markus 1978a). The extraintestinal form of *Cystoisospora belli* might be an example. Large numbers of singly occurring parasites have been found extraintestinally in persons with HIV/AIDS who were shedding *C. belli* oocysts in their faeces. Electron microscopically, the extraintestinal stage of *C. belli* is similar to the hypnozoite of *C. felis* and other species of *Cystoisospora* of cats and dogs (Mehlhorn and Markus 1976; Markus 1977, 1983; Lindsay et al. 1997a). A conspicuous feature is the crystalloid body that is characteristically found in some coccidian sporozoites but which has also been seen in post-divisional apicomplexan stages. Had very few *C. belli* parasites been present extraintestinally, their presence might have been directly ascribable (speculatively) to the ingestion of sporulated oocysts, based on knowledge concerning the presumed origin of extraintestinal forms of canine and feline species of *Cystoisospora*. The extraintestinal *C. belli* parasites can be so numerous, however, that a sporozoite origin seems unlikely, considering (partly) that both small and large inocula of cat and dog cystoisosporan oocysts lead to subsequent detection of relatively few hypnozoites, even in immunosuppressed hosts (Markus 1976; M.B.

Markus, unpublished data). Therefore, the extraintestinal *C. belli* stages might, despite their sporozoite-like ultrastructure, have gut merozoites as their origin (Lindsay et al. 1997b; Markus 2004). If so, they are post-divisional organisms (not dormant sporozoites). *C. belli* aside, it would not be incorrect to describe, as hypnozoites, quiescent merozoites which are atypical structurally (in that they are sporozoite-like) and/or biologically (Markus 1978a).

Like hypnozoites, apicomplexan merozoites of different protozoa are not always directly comparable either. For example, if a tissue cyst of *Toxoplasma* is ingested by the definitive host, the bradyzoic merozoites (cystozoic merozoites) from inside it initiate asexual multiplication. "Bradyzoic merozoite" ("cystozoic merozoite") is a little-known, informative term for "bradyzoite" ("cystozoite") (Markus 1987, 2003; Markus et al. 2004). However, when a tissue cyst of *Sarcocystis* is swallowed by the definitive host, the bradyzoic merozoites give rise directly to macrogametocytes and microgametocytes, without any asexual reproduction taking place in the host's intestine (Heydorn and Rommel 1972a, b). Thus, there is a biological difference between the bradyzoic merozoites of *Toxoplasma* and *Sarcocystis*.

Other latent forms of apicomplexan parasites

Some other known (or hypothetical/possible) inactive forms of apicomplexan protozoa within the host are considered to be sporozoite-like (thus, hypnozoite-like), whereas others are not (or are presumably not). A few examples of both are covered by the following publications: Markus (1978a), Bledsoe (1980), Beyer and Sidorenko (1984), Speer et al. (1985), Tse et al. (1986), Lindsay et al. (1988), Ball et al. (1989), Sundermann and Lindsay (1989), Telford (1989), and Bristovetzky and Paperna (1990). Also, see below under this subheading as well as under the subheading "Non-hypnozoite-associated dormancy in mammalian malaria".

Melanomacrophage aggregations in the liver of apparently blood parasite-free skinks contained hypnozoites (Koudela and Modrý 1999) that were ultrastructurally similar to those of *C. felis* in the murine host (Mehlhorn and Markus 1976) and extraintestinal stages of other cystoisosporan species (Markus 1977, 1983; Lindsay et al. 1997a). The skinks were thought to be acting as paratenic hosts for the parasite species concerned, rather than as definitive hosts. In an unrelated reptilian study, both sporozoite-like and merozoite-like (possibly resting) forms, presumed to be those of *Plasmodium sasai*, were seen in the same malaria-infected lizard host, with the former stages (in liver parenchymal cells) having been designated as hypnozoites and the latter termed "chronozoites" (Telford 1989). Later, encysted "phanerozoites" (an old name) were equated with "chronozoites" (Telford 1998; Telford and Stein 2000).

A "spring relapse" is associated with some avian haematozoa (Valkiūnas 2005). For example, it was shown by Markus (1970) to occur in woodpigeons *Columba palumbus* infected with *Haemoproteus palumbis*, but not in rooks *Corvus frugilegus* harbouring *Leucocytozoon sakharoffi* or in domestic canaries that had been used for passaging, by hypodermic syringe, of *Plasmodium subpraecox*. The spring relapse phenomenon in birds has yet to be thoroughly investigated. There is some evidence that merozoites from exoerythrocytic or erythrocytic schizonts might be responsible for relapse of avian haematozoan infections, but the role of sporozoites (if any) has not been elucidated (Valkiūnas 2005).

Hypnozoites and "relapse" in human malaria

The meanings in relation to human malaria of the terms recrudescence, recurrence, and relapse have, historically, been the subject of academic debate (Bruce-Chwatt 1984; Markus 1984; Corradetti 1985; Krotoski 1985). Current usage of these three words is outlined in a publication by the World Health Organization (2010). "Recrudescence" refers to a recurrence of asexual parasitaemia which (for any reason) originates from the same parasites that were responsible for the initial illness. A parasitaemic recrudescence is usually a consequence of malarial organisms in the bloodstream not having been completely cleared because of inadequate treatment or drug resistance. The term "recrudescence" is not used for a new infection (re-infection), as can be determined in endemic malarial areas by molecular genotyping, albeit often with considerable difficulty (Chen et al. 2007; Imwong et al. 2007; Orjuela-Sánchez et al. 2009). A recrudescence also differs from a "relapse" (see below) in *Plasmodium ovale* and *P. vivax* infections. "Recurrence" refers to renewed asexual parasitaemia, which is easily recognised if blood stages appear following drug treatment. A recurrence can be caused by a new infection, a relapse (in *P. ovale* and *P. vivax* malaria only) or a recrudescence. A "relapse" is the recurrence of asexual parasitaemia from a hypnozoite source following earlier elimination of stages in the bloodstream. Thus, the word "relapse" in regard to human malaria is reserved for (restricted to) the phenomenon of renewed asexual parasitaemia originating (via hepatic schizogony) from hypnozoites of *P. ovale* and *P. vivax*.

Non-hypnozoite-associated dormancy in mammalian malaria

Clinical *Plasmodium falciparum* disease can develop long after individuals have left endemic malarial areas (Greenwood

et al. 2008; Poilane et al. 2009; Szmitko et al. 2009; Theunissen et al. 2009). Parasites have in the meantime persisted in the body in an undetermined form(s) and site(s). Late onset of *P. falciparum* malaria has been ascribed to immune suppression (Focà et al. 2009) or loss (to an unknown extent) of partial immunity. Other speculative explanations have also been put forward. It is at present assumed (with little actual evidence to support the assumption) that hypnozoites are not involved. In this regard, it is relevant to note that Muehlenbachs et al. (2007) detected a nidus of intra-erythrocytic *P. falciparum* ring-form stages in placental tissue from a woman who did not have any other evidence of placental or peripheral blood parasitaemia. As for *P. malariae*, the persistence of parasites is mentioned earlier in this paper.

Sporozoites of *Plasmodium* have been observed in the lymphatic system soon after their inoculation into the host by the mosquito vector (Amino et al. 2006). So far, there is no indication that sporozoite-like plasmodial forms are able to remain in lymphoid tissue for any length of time, as happens in the liver in *P. vivax* infections, for instance. In chronic rodent malarial infections, parasites can be demonstrated in the lymphatic network as latent merozoites inside "merophores" (Landau et al. 1999). These stages are not hypnozoites. The question arises as to whether some malarial "merosomes" (Sturm et al. 2006; Baer et al. 2007; Stanway et al. 2009) persist as merophores.

Conclusion

In summary, the hypnozoite concept primarily concerns dormant, pre-merozoite apicomplexan organisms, but (contrary to current general understanding of the use of the term) not only those of *Plasmodium*. Instances in which the name "hypnozoite" would or might be applicable for latent apicomplexan stages are not always clear. The extraintestinal form of *C. belli* is an example of a non-malarial stage that is classified as a hypnozoite on the basis of a combination of fine structural and "behavioural" grounds, even though the origin and functional nature of this *C. belli* form are uncertain.

Much research remains to be carried out on inactive stages of various apicomplexan organisms, including *Plasmodium*. In vitro work (Hollingdale et al. 1986; Hollingdale 1992; Millet et al. 1994; Liu et al. 1995; Shu et al. 1995; House et al. 2009), imaging-associated investigations (Rankin et al. 2010), and gene expression studies (Westenberger et al. 2010) are some of the techniques that should facilitate the gaining of further insight into the biology of these enigmatic quiescent parasite forms.

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