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Invited Review

Tightly regulated migratory subversion of immune cells promotes the dissemination of *Toxoplasma gondii*

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ABSTRACT

While the spread of *Toxoplasma gondii* within the infected human or animal host is associated with pathology, the pathways of dissemination have remained enigmatic. From the time point of entry into the gut, to the quiescent chronic infection in the central nervous system, *Toxoplasma* is detected and surveyed by immune cells that populate the tissues, for example dendritic cells. Paradoxically, this protective migratory function of leukocytes appears to be targeted by *Toxoplasma* to mediate its dissemination in the organism. Recent findings show that tightly regulated events take place shortly after host cell invasion that promote the migratory activation of infected dendritic cells. Here, we review the emerging knowledge on how this obligate intracellular protozoan orchestrates the subversion of leukocytes to achieve systemic dissemination and reach peripheral organs where pathology manifests.

1. Introduction

Toxoplasma gondii infects a large portion of the human population and warm-blooded vertebrates. Following oral infection, *Toxoplasma* initially crosses the intestinal epithelium, disseminates into the tissues and traverses biological barriers such as the placenta and the blood–brain barrier (BBB) to reach immunologically privileged sites. *Toxoplasma* infection can manifest with acute symptoms or be chronically contained in the tissues, e.g. in the developing fetus, the retina and the CNS (Weiss and Dubey, 2009). It is in these loci that the parasite causes the most severe pathology: disseminated congenital infections, severe neurological complications in immune-compromised individuals and ocular pathology in otherwise healthy individuals. Thus, the pathogenesis of toxoplasmosis is linked to the passage of this obligate intracellular protozoan across normally restrictive physiological barriers.

The regulatory role of cellular barriers in homeostasis must be reconciled with the organism's need for cellular migration. Specifically, leukocyte migration is regulated by complex intrinsic and extrinsic signaling pathways and a tightly regulated process of leukocyte extravasation into the tissues, including immune-privileged sites (Ley et al., 2007). Homing cellular motility is an essential element of the immune system. By populating the circulation and the peripheral tissues, leukocytes rapidly mediate essential immune responses leading to protection and control of toxoplasmosis, albeit not clearance of the parasite in the chronically infected host. Paradoxically, it is likely this inherent migratory function of leukocytes that *Toxoplasma* utilizes to mediate its dispersion in the organism. Here, we review the on-going research on how leukocytes contribute to the systemic dissemination of *Toxoplasma* and the emerging mechanisms by which the parasite takes advantage of the migratory properties of immune cells to ensure dissemination.

2. The Trojan horse hypothesis for the spread of *Toxoplasma*

Following oral infection and subsequent release of the sporozoites from the oocyst or bradyzoites from tissue cysts, *Toxoplasma* can rapidly disseminate throughout the host. In rodents, parasites are found in distant sites, such as the spleen, within a few hours after natural infection, suggesting rapid penetration into the lymphatic system and blood. Early histopathological studies in the 1990s showed invasion of a variety of cell types in the intestine, including intra-epithelial leukocytes (Dubey et al., 1997), and rapid hematogenous spread of parasites (Derouin and Garin, 1991; Dubey, 1997; Zenner et al., 1998).

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Toxoplasma actively invades epithelial cell barriers but the precise mechanisms leading to systemic dissemination during natural infections have remained elusive. In vitro and ex vivo experimental systems demonstrated that *Toxoplasma* tachyzoites can migrate across cellular barriers, powered by gliding motility and by utilizing a paracellular transfer route (Barragan and Sibley, 2002; Barragan et al., 2005). Thus, direct tissue penetration by extracellular parasites early during infection is likely. In the intestinal tissues, resident and non-resident leukocytes become parasitized (Courret et al., 2006; Gregg et al., 2013). Consequently, the transfer of the parasite into the circulation is dependent on either direct tissue penetration by motile parasites or host cell-mediated transfer (Lambert and Barragan, 2010).

The rapid and efficient dissemination of parasites of different genotypes (Hitziger et al., 2005), together with the ability to infect migratory cells of the immune system, led us and others to hypothesize a Trojan horse type of mechanism underlying the circulatory spread of parasites mediated by permissive leukocytes. Mounting evidence indicates that dendritic cells (DCs) play a significant role in this mode of dissemination (Courret et al., 2006; Lambert et al., 2006; Bierly et al., 2008). Similar to DCs, monocytes have been shown to transport tachyzoites to the brain (Courret et al., 2006; Lachenmaier et al., 2011) and T cells have been reported to shuttle parasites in the circulation, albeit at a later stage of infection (Persson et al., 2007; Chtanova et al., 2009). In addition, macrophages (Da Gama et al., 2004; Lambert et al., 2011), neutrophils (Norose et al., 2008; Coombes et al., 2013) and natural killer (NK) cells (Persson et al., 2009) have been suggested to contribute to parasite dissemination. Thus, monocytic cells, DCs and neutrophils may be primary targets of *Toxoplasma* invasion, while lymphocytes take on an increasing portion of the parasite burden as the infection progresses (Gregg et al., 2013). Yet, the infection frequencies of specific leukocyte populations in the blood or in the tissues may not necessarily reflect their relative roles in the delivery of parasites to target organs. The relative impact of individual leukocyte types in the dissemination of *Toxoplasma* over the course of infection needs further investigation.

3. Approaching a definition of a hypermigratory phenotype in *Toxoplasma*-infected DCs

Due to their inherent migratory properties, DCs represent an appealing target for host cell-mediated dissemination of *Toxoplasma*. Upon infection of DCs by tachyzoites, a series of tightly regulated events takes place as revealed by cellular assays; (i) within minutes after invasion, DCs exhibit profound morphological changes and cytoskeletal rearrangements impacting cell adhesion (Weidner et al., 2013); (ii) this dramatic feature is accompanied by a hypermotility phenotype characterized by enhanced random directional locomotion in motility assays (Fuks et al., 2012); (iii) the *Toxoplasma*-infected DCs exhibit enhanced transmigration ability across membrane filters and polarized and non-polarized cell monolayers (Lambert et al., 2006, 2009; Collantes-Fernandez et al., 2012); (iv) also, hypermotile *Toxoplasma*-infected DCs can acquire the ability to chemotax in vitro, resulting in increased velocities of DCs towards a chemokine gradient (Fuks et al., 2012; Weidner et al., 2013).

Recent data have revealed that *Toxoplasma* orchestrates a tightly regulated migratory activation of DCs and the observed in vitro features (i–iv) likely reflect different or partly overlapping facets of this activation. The co-existence of several or all of these features in *Toxoplasma*-infected DCs, i.e. rapid morphological transformation, altered adhesive properties, hypermotility, transmigration and responsiveness to chemokines, may be termed the 'hypermigratory phenotype' (Fig. 1).

Toxoplasma-induced hypermotility or enhanced transmigration capacity has been documented in a variety of DC subtypes, including human monocyte-derived DCs, primary human blood DCs, murine bone-marrow derived DCs and murine intestinal DCs. Additionally, both human and murine macrophages, as well as primary murine microglia, exhibit hypermotility and enhanced transmigration following *Toxoplasma* infection (Lambert et al., 2006, 2009, 2011; Dellacasa-Lindberg et al., 2011). Yet, extrapolations of defined in vitro features to putative effects in vivo require cautious interpretation. Hypothetically, hypermotility may reflect an enhanced crawling ability in tissues, while transmigration, especially across endothelial cell monolayers, may reflect an enhanced ability to perform extravasation. Responsiveness to chemokines in vitro may reflect a maintained or enhanced migratory ability of infected DCs. While this awaits confirmation in vivo, it is also pertinent to determine the precise cellular and molecular events behind the migratory activation of infected leukocytes.

4. Dissecting the cellular and molecular events behind the hypermigratory phenotype

4.1. Effects on the DC cytoskeleton following invasion by *Toxoplasma*

Upon maturation, DCs undergo a series of changes that are necessary for antigen processing, homing to lymph nodes and antigen presentation. Cell migration requires dynamic rearrangement of the actin cytoskeleton as well as the loss of actin-rich structures known as podosomes. Because podosomes limit fast migration by their strong interactions with the extracellular matrix, it is believed that DCs need to dissolve their podosomes during maturation, e.g. after encountering microbial antigens or lipopolysaccharide (LPS) (van Helden et al., 2006, 2010; Friedl and Weigelin, 2008; Lammermann et al., 2008). The loss of podosomes allows DCs to switch from a strongly adhesive state to a highly migratory phenotype. In addition, the formation of filopodia and lamellipodia are linked to the protrusive activity of DCs. In agreement with the role of DCs as Trojan horses, it was recently found that DCs infected with *Toxoplasma* undergo rapid cytoskeletal changes, marked by the loss of podosomes and a rounded morphology (Fig. 1A). In contrast to observations with LPS-matured DCs (van Helden et al., 2010), the *Toxoplasma*-induced cytoskeletal reorganization was extremely rapid (evident 5–10 min after invasion) and was not dependent upon Toll-like receptor 4 (TLR4) signaling or prostaglandin E_2 (PGE_2) secretion (Weidner et al., 2013). This observation is consistent with the findings that the induction of the hypermigratory phenotype is MyD88-independent (Lambert et al., 2006), and does not require *de novo* protein synthesis from either the host cell or the parasite (Weidner et al., 2013).

The rapid cytoskeletal rearrangements coincide in time, with the onset of a hypermotility phenotype (Fig. 1C). Both the morphological changes and the induction of a hypermotility phenotype are dependent upon a live parasite and an active invasion event. In contrast, inhibition of parasite invasion and rhoptry secretion abrogated the onset of morphological changes and hypermotility (Weidner et al., 2013). The inability of heat-inactivated tachyzoites and soluble *Toxoplasma* antigen (STAg) to induce detectable phenotypic effects indicates that the form of delivery of putative parasite-derived effector molecules into the host cell may be crucial. Also, the hypermotility phenotype in DCs perseveres throughout the infection (>24 h), raising the question whether maintained discharge of secretory organelles after invasion is required (Lambert et al., 2006; Fuks et al., 2012).