The unicellular eukaryotic parasite *Toxoplasma gondii* hijacks the migration machinery of mononuclear phagocytes to promote its dissemination

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*Toxoplasma gondii* is an obligate intracellular protozoan with the ability to infect virtually any type of nucleated cell in warm-blooded vertebrates including humans. *Toxoplasma gondii* invades immune cells, which the parasite employs as shuttles for dissemination by a *Trojan horse* mechanism. Recent findings are starting to unveil how this parasite orchestrates the subversion of the migratory functions of parasitised mononuclear phagocytes, especially dendritic cells (DCs) and monocytes. Here, we focus on how *T. gondii* impacts host cell signalling that regulates leukocyte motility and systemic migration in tissues. Shortly after active parasite invasion, DCs undergo mesenchymal-to-amoeboid transition and adopt a high-speed amoeboid mode of motility. To trigger migratory activation – termed hypermigratory phenotype – *T. gondii* induces GABAergic signalling, which results in calcium fluxes mediated by voltage-gated calcium channels in parasitised DCs and brain microglia. Additionally, a TIMP-1-CD63-ITGB1-FAK signalling axis and signalling via the receptor tyrosine kinase MET promotes sustained hypermigration of parasitised DCs. Recent reports show that the activated signalling pathways converge on the small GTPase Ras to activate the MAPK Erk signalling cascade, a central regulator of cell motility. To date, three *T. gondii*-derived putative effector molecules have been linked to hypermigration: Tg14-3-3, TgWIP and ROP17. Here, we discuss their impact on the hypermigratory phenotype of phagocytes. Altogether, the emerging concept suggests that *T. gondii* induces metastasis-like migratory properties in parasitised mononuclear phagocytes to promote infection-related dissemination.

**Introduction**

*Toxoplasma gondii* is a common infectious agent in humans and animals worldwide and also a model to study intracellular parasitism (Sibley, 2004; Pappas et al., 2009). The invasive tachyzoite stage of *T. gondii* is obligate intracellular. Thus, host cell invasion is essential for parasite survival. Gliding parasite motility mediates invasion of host cells but likely also interstitial movement in the microenvironment in tissues and ability to cross biological barriers (Barragan and Sibley, 2003; Sibley, 2004). Tachyzoites actively invade host cells propelled by their own actin-myosin motor with an implication of the host cell cytoskeletal machinery (Dobrovolski and Sibley, 1996; Bichet et al., 2016; Pavlou et al., 2018). The invasion process encompasses the discharge of secretory organelles.
in the host cell and results in the generation of an intracellular parasitophorous vacuole (PV; recently reviewed in Clough and Frickel, 2017). Mounting evidence show that, shortly after invasion and from within its intracellular replicative niche, the parasite secretes proteins into the host cell cytosol and nucleus, which modulate a number of host cell functions, for example, immune responses (recently reviewed in Hakimi et al., 2017).

Thus, from the parasite's entry in the ileum (recently reviewed in Delgado Betancourt et al., 2019) to its entry into the central nervous system across the blood–brain barrier with subsequent development of chronic bradyzoite-containing cysts (recently reviewed in Schluter and Barragan, 2019), the parasites invade a number of cell types, including immune cells. Early studies in rodents revealed infection of leukocytes in the intestine following oral infection and rapid presence of tachyzoites in blood and peripheral organs (Derouin and Garin, 1991; Dubey, 1997a, 1997b; Znener et al., 1998). It was subsequently demonstrated that parasitised mononuclear phagocytes transported *T. gondii* from the intestinal mucosa into the lymphatic and systemic blood circulation (Courret et al., 2006) and that the altered migratory properties of parasitised dendritic cells (DCs) promoted parasite dissemination in mice (Lambert et al., 2006).

The mononuclear phagocyte system comprises families of immune cells of diverse ontogenic origin (Guilliams et al., 2014). These include monocyte-derived cells, hematopoietic stem cell derived common dendritic cell (DC) precursors and embryonic-derived macrophages, and also microglia (Ginhoux et al., 2010). Here, we focus on (i) the cytoskeletal and migratory alterations that parasitised human and murine DCs and other mononuclear phagocytes undergo upon *T. gondii* infection, and (ii) how they impact on parasite dissemination and, ultimately, pathogenesis.

### The hypermigratory phenotype

The principal features of the hypermigratory phenotype exhibited by DCs parasitised by *T. gondii* tachyzoites have been previously reviewed (Weidner and Barragan, 2014). Briefly, standardised cellular assays define (i) enhanced motility in assays using two-dimensional (D) confinements, (ii) enhanced transmigration in transwell systems in presence or absence of endothelial cell monolayers, (iii) maintained or elevated chemotactic properties in chemotaxis assays and (iv) cytoskeletal morphological changes, for example, dissolution of actin-rich adhesion structures known as podosomes (Figure 1). More recent studies have extended these features to hypermigration in 3D matrix confinements (Kanatani et al., 2015) and revealed impaired matrix degradation and membrane redistribution of integrins in parasitised DCs (Weidner et al., 2013; Olafsson et al., 2018). In mice, adoptively transferred parasitised DCs confer exacerbated dissemination with elevated parasite loads (Lambert et al., 2006; Fuks et al., 2012; Kanatani et al., 2017). Hypermigratory responses of mononuclear phagocytes (DCs, monocytes, macrophages, microglia) appear to be a conserved feature across host species (human, mouse, bovine) and are also induced by the related coccidian *Neospora caninum* (Collantes-Fernandez et al., 2012; Garcia-Sanchez et al., 2019). Yet, while hypermigration in DCs/macrocytes/microphages/microglia is induced by all *T. gondii* and *N. caninum* strains tested to date, measurable differences exist between parasite strains/lineages in the magnitude of induction of the hypermigratory phenotype, as defined in vitro. For *T. gondii*, type I, II and III lines induce hypermigration of parasitised DCs in a genotype-related fashion in vitro (Lambert et al., 2009). In vitro in mice, findings suggest that type II strains efficiently exploited DC migration for dissemination, compared with type I strains. Similarly, differences among *N. caninum* strains were also observed in mice (Collantes-Fernandez et al., 2012). Altogether, the data suggest that shuttling of tachyzoites by parasitised DCs impacts dissemination in a strain-dependent fashion.

In addition to DCs, other mononuclear phagocytes exhibit altered migration when infected by *T. gondii*. Monocytes and macrophages exhibit hypermigratory features, hypermotility being the most pronounced (Lambert et al., 2011; Harker et al., 2013; Cook et al., 2018). Interestingly, while *T. gondii* infection upregulates the transmigration capacity of DCs and macrophages, exacerbated transmigration seems to be absent in parasitised monocytes (Lambert et al., 2011; Ueno et al., 2014; Drewry et al., 2019). Similarly, *in vitro* studies did not detect significantly elevated transmigration of T, B and NK cells upon *T. gondii* infection (Lambert et al., 2011). Yet because
T cells, NK cells, neutrophils and macrophages become infected in vivo, they probably contribute to systemic dissemination of *T. gondii* (Da Gama et al., 2004; Persson et al., 2007; Chtanova et al., 2009; Persson et al., 2009; Coombes et al., 2013). DCs can perform transmigration and reverse transmigration in vitro (D’Amico et al., 1998). In vivo, migration out of gut tissue and the peritoneal cavity involves a process of transmigration across lymphatic (or vascular) endothelium. The contribution of the different leukocyte populations is unclear, despite the relative abundance of monocytic cells in the blood compared to DCs and higher relative numbers of infected monocytic cells (Courret et al., 2006). Thus, the relative contribution of different leukocyte subtypes to each phase of *T. gondii* dissemination remains to be clarified.

Because toxoplasmosis has its most severe manifestations in the central nervous system (Schluter and Barragan, 2019), the *in vitro* hypermigratory features exhibited by parasitised primary cortical microglia are especially interesting, as these cells come into contact with *T. gondii* in the brain parenchyma (Dellacasa-Lindberg et al., 2011). In contrast, hypermigratory features were absent in primary astrocytes. It has been suggested that microglia may serve transportation functions for tachyzoites within the brain parenchyma (Dellacasa-Lindberg et al., 2011; Bhandage et al., 2019).

**Toxoplasma gondii** infection promotes migratory mesenchymal-to-ameboid transition in DCs and monocytes

Early observations of migrating leukocytes detailed changes in cell shape, which were termed ‘amoeboïd’ based on similarities with motile amöebas (de Bruyn, 1946). In contrast to other migration modes,
amoeboid motility is particularly suited for rapid locomotion in tissues and is utilised by metastasising cancer cells and rapidly migrating leukocytes (Friedl and Wolf, 2003b, 2010; Calle et al., 2006). Thus, mesenchymal-to-ameboid transition (MAT) facilitates rapid transit through interstitial tissues and also passage across biological barriers (Friedl and Wolf, 2003b; Alvarez et al., 2008; Lammermann et al., 2008). Ameboid migration of DCs is primarily integrin independent and relies chiefly on the protrusive flow of the actin cytoskeleton at the leading edge, which drives locomotion (Lammermann et al., 2008, 2009). However, DCs can also perform mesenchymal migration in the interstitial matrix by maintenance of podosomes (see Definition), irrespective of maturation status (Cougoule et al., 2018).

Dramatic morphological changes accompany T. gondii invasion of DCs. One such feature is the rapid and permanent dissolution of podosome structures (Weidner et al., 2013), which normally mediate adhesion to matrix via integrins and also concentrate matrix metalloproteinase (MMPs) for proteolytic degradation of extracellular matrix (ECM) (Figure 1). Podosome dissolution is accompanied by a redistribution of β1 (ITGB1) and β2 integrins at the cell surface of parasitised DCs (Weidner et al., 2013; Kanatani et al., 2015; Olafsson et al., 2019) and is mediated by the parasite-derived protein TgWIP, which is secreted into the host cell cytosol and interacts with the actin-regulating WAVE complex (Sangare et al., 2019). Consequently, parasitised DCs round-up, acquire an amoeboid shape and exhibit decreased adhesion in 2D and 3D matrix confinements (Kanatani et al., 2015). Importantly, this is accompanied by elevated tissue inhibitor of metalloproteinases-1 (TIMP-1) secretion (but not TIMP-2, -3, -4), which has the dual effect of inhibiting MMP activity (Olafsson et al., 2018) and activating a TIMP-1-CD63-ITGB1-FAK motogenic axis discussed below (Olafsson et al., 2019). Altogether, parasitised DCs undergo morphological and functional changes consistent with MAT and acquire features of high-speed amoeboid migration consistent with those described for activated leukocytes (Lammermann et al., 2008) and reminiscent of those described for metastatic cancer cells (Friedl and Wolf, 2003a, 2010; Lambert et al., 2017).

**Major cell signalling axes of the hypermigratory phenotype**

The onset of the hypermigratory phenotype in DCs is rapid (minutes after invasion) and depends on live intracellular tachyzoites and the discharge of parasite secretory organelles. Further, Toxoplasma-induced hypermigration is independent of TLR-MyD88 signalling and chemotaxis (Lambert et al., 2006; Fuks et al., 2012; Weidner et al., 2013; Olafsson et al., 2018). Instead, mounting evidence shows that intracellular T. gondii tachyzoites activate non-canonical motogenic signalling pathways as well as canonical signalling in parasitised DCs, which jointly promote migration (Table 1). In addition to neuronal cells, immune cells including DCs and T cells can express a functional GABAergic system (Jin et al., 2013; Barragan et al., 2015). GABAergic signalling in DCs and microglia is necessary for the induction of the hypermigratory phenotype and was recently reviewed in Bhandage and Barragan (2019). Briefly, in parasitised DCs, it was shown that T. gondii induced secretion of the neurotransmitter gamma–aminobutyric acid (GABA) and activation of its ionotropic receptor GABA(A) (Fuks et al., 2012) lead to calcium (Ca^{2+}) fluxes mediated chiefly by the voltage-gated calcium channel (VGCC) subtype CaV1.3 (Kanatani et al., 2017), which triggers hypermotility. However, signalling downstream of GABA/VGCC activation has remained elusive and is discussed below.

**VGCC-mediated Ca^{2+} influx activates the Ras-Raf-Mek-Erk MAPK cascade**

As laid out above, autocrine GABAergic signalling is activated in parasitised DCs (Fuks et al., 2012), which in turn triggers VGCC-mediated Ca^{2+} influx (Kanatani et al., 2017). More recent data revealed that Ca^{2+} influx via the VGCC subtype CaV 1.3 mediates activation of the small GTPase Ras via calmodulin (CaM) and CaM kinase II (CaMkII) signalling (Olafsson et al., 2020) (Figure 2). Ras activation ultimately leads to Erk1/2 phosphorylation via Raf-Mek. Further, Ras–Erk signalling was activated downstream of the receptor tyrosine kinase (RTK) MET, situating Ras as a central signalling node and point of convergence for VGCC and MET signalling (Olafsson et al., 2020). These data are in line with paradigms in neuronal cells, where VGCC-dependent CaM activity regulates cytoskeleton organisation and cell migration via Ras-Erk signalling (Rosen et al., 2020).
Non-canonical migratory activation of phagocytes

Table 1 | Molecular cell signalling components linked to the hypermigratory phenotype of DCs and monocytes parasitised by *T. gondii*

| Host cell/parasite | Effector molecule(s) | Signalling pathway | Function/phenotype | References |
|--------------------|----------------------|--------------------|-------------------|-----------|
| Host cell          | GABA                 | GABA synthesis and secretion, GABA(A) receptor activation | VGCC activation via membrane depolarization | Fuks et al. (2012) |
| Host cell          | Ca²⁺                | VGCC/CaV1.3 subtype activation | Influx of Ca²⁺ activates Ras GTPase via CaM-CaM kinase II | Kanatani et al. (2017); Olafsson et al. (2020) |
| Host cell          | MMPs                | Transcriptional modulation M, MMP inhibition Activates CD63-ITGB1-FAK | ECM degradation Reduced EOM proteolysis Migratory activation | Olafsson et al. (2018); Olafsson et al. (2018, 2019) |
| Host cell          | MET kinase          | Activates Ras GTPase | Migratory activation by phosphorylation of Raf-Mek-Erk | Olafsson et al. (2020) |
| Host cell          | Ras GTPase          | Converges signal from CaM-CaM kinase II and MET | | |
| Host cell          | Erk1/2              | MAPK | Regulation of migratory activation | Olafsson et al. (2020) |
| Host cell          | Integrins/ITGB1     | Adhesion Signal transduction | Reduced adhesion Cytoskeletal rearrangements Transmigration | Kanatani et al. (2015); Olafsson et al. (2019); Cook et al. (2018) |
| Host cell          | CCR7                | Signal transduction in response to CCL19/21 | Chemotaxis (in conjunction with hypermigration) Sequestration of host 14-3-3 to PVM | Fuks et al. (2012); Weidner et al. (2013) |
| Parasite           | Tg14-3-3            | Putative action on host cell MAPK | Modulation of MAPK activity | Sangare et al. (2019) |
| Parasite           | TgWIP               | Putative action on WAVE complex and SHP2 phosphatase | Modulation of actin dynamics | |
| Parasite           | ROP17               | Putative action on Rho-ROCK phosphorylation | Modulation of actin dynamics | Drewry et al. (2019) |

CaM, calmodulin; CCL, chemokine (C-C motif) ligand; CCR, chemokine receptor; ECM, extracellular matrix; Erk, extracellular signal regulated kinase; FAK, focal adhesion kinase; GABA, gamma-aminobutyric acid; GTPase, guanosine triphosphate hydrolase; ITGB1, beta 1 integrin; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; PVM, parasitophorous vacuole membrane; ROCK, Rho-associated kinase; SHP2, Src homology domain phosphatase 2; TIMP, tissue inhibitor of metalloproteinases; VGCC, voltage-gated calcium channel; WASP, family verprolin homologous protein.

Further, in lymphocytes, VGCC antagonism and agonism blocked and stimulated Erk phosphorylation, respectively (Kotturi et al., 2003), indicating the existence of this signalling axis in lymphocytes. Thus, VGCC/CaV1.3-mediated Erk activation promotes migratory activation of DCs and likely other leukocytes when parasitised by *T. gondii*.

**A role for MET signalling in DC hypermotility**

The RTK MET, also known as c-MET, scatter factor receptor or hepatocyte growth factor receptor, has been associated with cancer metastasis but also more recently with DC/Langerhans cell migration in skin immunity (Baek et al., 2012). It was recently reported that MET is activated in DCs upon *T. gondii* infection with an impact on DC hypermotility (Olafsson et al., 2020). Interestingly, secretion of METs only known ligand hepatocyte growth factor (Hgf) was not significantly elevated upon *T. gondii* infection, while inhibition of integrin (ITGB1)-linked tyrosine kinases (FAK, PYK2) led to reduced Erk activation. Phosphorylation of MET likely occurs through both Hgf ligation and transactivation via FAK/PYK2, which are activated by the TIMP-1/CD63/ITGB1/FAK axis (Olafsson et al., 2019). MET and VGCC signalling converge on Ras GTPase, which activates the Raf-Mek-Erk signalling cascade (Figure 2).
Figure 2 | Signaling pathways that mediate migratory activation of phagocytes parasitised by *T. gondii*. (1) *T. gondii* actively invades phagocytes and resides in a parasitophorous vacuole (PV). Within minutes of invasion, *T. gondii* induces a hypermigratory phenotype in host phagocytes, which is characterised by cytoskeletal reorganization and migratory activation (Weidner et al., 2013). (2) *T. gondii* infection triggers GABAergic signalling in DCs and in monocytes (Fuks et al., 2012), which leads to influx of Ca\(^{2+}\) via VGCCs (mainly CaV1.3) (Kanatani et al., 2017) and downstream activation of Ras GTPase via CaM-CaMkII signalling (Olafsson et al., 2020). ‘P’ indicates protein phosphorylation. (3) Infection leads to elevated TIMP-1 secretion, which blocks pericellular proteolysis of extracellular matrix (ECM) via the inhibition of matrix metalloproteinases (MMPs) (Olafsson et al., 2018). (4) Secreted TIMP-1 activates CD63-ITGB1-FAK-Src/Pi3k signalling leading to cytoskeletal rearrangements and shifts the cell into a hypermotile state that facilitates dissemination (Olafsson et al., 2019). (5) The receptor tyrosine kinase (RTK) Met is rapidly activated upon *T. gondii* infection, partly through cytoplasmic transactivation via FAK and Pyk2 (Olafsson et al., 2020). (6) The VGCC-CaM-CaMkII and Met signalling pathways converge on the activation of Ras GTPase (Olafsson et al., 2020). (7) Activation of Ras GTPase leads to phosphorylation of Erk1/2 MAPK via Raf-Mek. Erk1/2 phosphorylates substrate proteins in the cytoplasm and nucleus that maintain hypermotility. (8) In the nucleus of the parasitised phagocyte, transcriptional upregulation/modulation of genes implicated in hypermigration takes place. (9) Podosomes are rapidly dissolved in infected phagocytes leading to reduced ECM degradation and adhesion. (10) The infected phagocyte undergoes MAT, exhibiting integrin-independent amoeboid hypermigration. Inset images (I, II, III) represent putative parasite-derived effector molecules with attributed roles in the hypermigratory phenotype of parasitised phagocytes. The precise modes of action of these three effector molecules or of associated molecules of the secretory machinery of *T. gondii* remain to be elucidated: (I) Host 14-3-3, which regulates Ras-Raf-Mek signalling, is sequestered to the PV membrane (PVM) and *T. gondii* 14-3-3 (Tg14-3-3) localizes to the perivacuolar space (Weidner et al., 2016). (II) ROP17 regulates Rho-ROCK-dependent amoeboid migration via putative interactions with RhoGEFs (Drewry et al., 2019). (III) TgWip modulates Arp2/3-mediated F-actin branching through the WAVE complex (Sangare et al., 2019).
Non-canonical migratory activation of phagocytes

Ras-Erk MAPK signalling governs hypermigration of parasitised DCs
The mitogen-activated protein kinase (MAPK) extracellular signal regulated kinase 1/2 (p44/p42, Erk) plays central roles in cell migration through phosphorylating nuclear and cytoplasmic targets (Huang et al., 2004). The Ras-Erk signalling axis constitutes an important regulator of metazoan cell migration and its aberrant signalling is associated to cancer cell metastasis (Ehlen, 2018). Ras activates the Raf-Mek-Erk signalling cascade that regulates cell migration. As a central signalling node, an array of extracellular stimuli converge on Ras activation, including VGCC-mediated Ca$^{2+}$ influx, RTKs signalling and integrin-mediated signalling (Rosen et al., 1994; Schlaepfer et al., 1994; Giehl et al., 2000). While p38 MAPK signalling non-significantly impacts hypermigration (ten Hoeve et al., 2019), it was recently shown that Ras-Erk signalling is central to the migratory activation of parasitised DCs (Olafsson et al., 2020) (Figure 2). Moreover, a dependency on the phosphorylation of both Erk isoforms (Erk1 and Erk2) was shown, indicating a tight regulation of DC hypermigration by this signalling pathway. The Erk-dependent arm of hypermotility likely involves nuclear translocation of Erk with modulation of host transcription after the first hours of infection. However, because Erk phosphorylation coincides with the rapid onset of hypermotility, it is plausible that cytosolic Erk or Ras substrates that modulate actin dynamics, such as FAK, RhoGEFs, RhoGAPs and WAVE proteins (Wu et al., 2014; Tanimura and Takeda, 2017), sustain hypermotility shortly after invasion.

A TIMP-1-CD63-ITGB1-FAK signalling axis drives amoeboid hypermotility
The data to date show that upon Toxoplasma infection, DCs undergo MAT with reduced integrin-mediated adhesion through the dissolution of podosomes, redistribution of integrins and abrogated pericellular proteolysis (Weidner et al., 2013; Kanatani et al., 2015; Olafsson et al., 2018). Yet, secreted and membrane-bound MMPs still play a role for specific steps of the hypermigratory phenotype, such as transmigration. Consequently, MMP inhibition reduced transmigration and, to some extent, hypermotility in collagen (Olafsson et al., 2018). Seemingly paradoxical, the observed reduced matrix degradation (proteolysis) by parasitised DCs was accompanied by a significant by-stander effect on non-parasitised DCs. This effect was mediated by an elevated secretion of TIMP-1 by parasitised DCs (Olafsson et al., 2018). Further, it was shown that secreted TIMP-1 drives a motogenic CD63-ITGB1-FAK signalling axis, which promotes amoeboid hypermotility (Olafsson et al., 2019) (Figure 2). Because systemic TIMP-1 is elevated in T. gondii infected mice (Tomasik et al., 2016) with a possible impact on parasite loads in the brain (Clark et al., 2011), TIMP-1 dysregulation may have implications for immune cell responses and inflammation during toxoplasmosis. Thus, from the parasite’s perspective, host TIMP-1 upregulation may facilitate both dissemination via shuttling DCs and reduced tissue pathology, which may dampen the inflammatory effects of encephalitis.

Dual effects of integrins/ITGB1 in hypermotility and transmigration
Shortly after parasite invasion, cytoskeletal and morphological alterations take place in infected DCs with increased rounding-up, accentuated membrane veils/ruffles and, markedly, irreversible dissolution of podosome structures (Weidner et al., 2013). These changes are accompanied by a redistribution of β2 integrins (CD18, CD11c) (Weidner et al., 2013), which altogether likely contribute to reduced integrin-dependent adherence (Kanatani et al., 2015), reduced matrix proteolysis (Olafsson et al., 2018) and facilitate MAT of parasitised DCs (Olafsson et al., 2019). Similarly, in monocytic cells a dysregulation of β1 integrin (ITGB1, CD29) modulates cell surface interactions and transmigration (Harker et al., 2013; Ueno et al., 2014; Cook et al., 2018). Further, activation of CaMkII by Ca$^{2+}$ influx inhibits β1 integrin-mediated focal adhesion in osteoblasts (Millon-Fremillon et al., 2013), consistent with reduced adhesion of mononuclear phagocytes upon infection by T. gondii. Independently of adhesion, ITGB1 also mediates signal transduction in a motogenic axis promoting cell displacement, as discussed above (Olafsson et al., 2019). Thus, the data to date indicate that integrins have dual functions in the hypermigratory phenotype. Additionally, contrary to amoeboid motility, which is integrin independent in leukocytes (Lamermann et al., 2008), transmigration is considered an integrin-dependent process. Consistent with this, integrin- or intercellular cell
adhesion molecule 1 (ICAM-1) blockade inhibited transmigration of parasitised monocytes across human umbilical vein cell (HUVEC) monolayers (Ueno et al., 2014). Altogether, the data indicate that different integrins may mediate different functions related to hypermotility or transmigration, or that integrins are sequentially activated upon transmigration. This needs to be addressed in further studies in order to understand the impact of *T. gondii* infection on the transmigration of leukocytes.

**Chemotactic responses by hypermigratory Toxoplasma-infected DCs**

Early studies indicated that the onset of the hypermigratory phenotype in DCs was independent of CCR7, CCR5 and TLR-MyD88 signalling (Hitziger et al., 2005; Lambert et al., 2006). Later, it was reported that hypermotile parasitised DCs upregulate CCR7 and readily chemotax in CCL19 gradients (Fuks et al., 2012). Furthermore, CCR7 upregulation occurred in parasitised DCs in the absence of a measurable effect on by-stander non-infected DCs (Weidner et al., 2016). This indicated a dependence on live intracellular parasites for CCR7 chemotaxis and was in contrast to CCR5 downregulation, which occurred in both parasitised and non-parasitised DCs upon challenge with *T. gondii* (Weidner et al., 2013). Thus, in addition to motogenic GABAergic activation (Fuks et al., 2012; Kanatani et al., 2017), parasitised DCs undergo canonical activation and maturation events (Lambert et al., 2006). Interestingly, in this context maturation is downmodulated via sustained expression of the transcription factor early growth response-1 (Egr-1) (ten Hoeve et al., 2019). Thus, hypermigration and chemotaxis are not antithetical and may, in fact, cooperatively potentiate the migratory potential of parasitised phagocytes, and therefore also the dissemination of *T. gondii*. While a parasite-derived modulator of CCR5 has been identified (Aliberti et al., 2003), the putative *T. gondii* effector(s) that mediate CCR7 upregulation in parasitised DCs remain uncharacterised and await further investigation.

**Parasite-derived effector molecules implicated in the modulation of host cell migration**

As delineated above, the hypermigratory phenotype implicates multiple host cell signalling pathways for which the cognate parasite effectors remain unknown (Bhandage and Barragan, 2019). To date, three putative effector molecules have been implicated in the modulation of the migratory functions of parasitised phagocytes (DCs, macrophages, monocytes and microglia) (Figure 2).

Tg14-3-3 is a cytoplasmic and secreted protein with multiple functions in Apicomplexa (Assoussou et al., 2004; Zhou et al., 2005; Lorestan et al., 2012). Heterologous expression of Tg14-3-3 in primary DCs and microglia induced hypermotility (Weidner et al., 2016). Interestingly, a prominent recruitment of host cell 14-3-3 was observed around the PV in infected DCs, suggesting sequestration of host cell 14-3-3 (Weidner et al., 2016). Note that 14-3-3 regulates multiple signalling pathways by molecular sequestration in the cytosol (Hermeking, 2003). Because Ras-Raf-Mek-Erk MAPK signalling is central to hypermigration (Olafsson et al., 2020) and 14-3-3 proteins modulate this signalling axis (Rajalingam and Rudel, 2005; Yin et al., 2019), it is likely that the abundant concentration of 14-3-3 to the PV impacts on MAPK signalling. Additionally, because 14-3-3 has been shown to regulate GABA receptor function (Laffray et al., 2012), Tg14-3-3 may be involved in the regulation of GABAergic signalling in parasitised DCs. Along these lines, in *N. caninum*, Nc14-3-3 was recently associated to modulation of host immune cell responses via MAPK signalling (Li et al., 2019). TgWIP was recently identified as a rhoptry protein secreted into the host cell cytosol upon parasite invasion of DCs (Sangare et al., 2019). TgWIP interacts with the host WAVE regulatory complex and SHP2 phosphatase, both of which regulate actin dynamics. TgWIP impacts the morphology of DCs and mediates the dissolution of podosome structures, which DCs use to adhere to ECM. Further, TgWIP enhances the motility and transmigration of parasitised DCs, likely explaining its impact on systemic dissemination. *In vivo*, TgWIP-deficient parasites exhibited dramatically reduced dissemination and reduced numbers of cysts in mouse brains.

ROP17 tyrosine kinase was first identified as a secreted molecule mediating parasite survival in macrophages by phosphorylating immunity-related GTPases in conjunction with two other rhoptry proteins (ROP5, ROP18) (Etheridge et al., 2014). A recent study showed that monocytes parasitised with ROP17-deficient *T. gondii* exhibited reduced tissue
migration compared with wild-type parasites and presented an early dissemination delay (Drewry et al., 2019). It was postulated that ROP17 enhanced tissue migration of monocytes by phosphorylating Rho guanine nucleotide exchange factors (GEFs), which regulate actin nucleation via Rho-ROCK signalling. While Rho-GTPase signalling regulates cell contractility and polarity in amoeboid migration (Sit and Manser, 2011), its role in *T. gondii*-mediated hypermotility awaits further investigation.

**Concluding remarks**

The evolutionary arms race between intruding microorganisms and the immune systems of their hosts has equipped leukocytes with an arsenal of molecular mechanisms to counter pathogens. Therefore, the study of host-microbe interactions has emerged as a powerful approach to gain insight into basic cell and molecular biology. In this context, the hypermigratory phenotype implies a reprogramming of migratory functions in parasitised leukocytes and implicates a tight regulation of several signalling pathways for which the cognate parasite effectors remain unknown. Recently identified parasite-derived effectors have allowed a closer characterisation of how *T. gondii* orchestrates the hijacking of migration in parasitised cells. The conservation of the phenotype in the related species *N. caninum* and in natural host species (human, mouse, bovine) advocates for a conserved strategy by coccidian parasites. Thus, infecting immune cells and hijacking or even exacerbating their migratory properties promotes coccidian parasite dissemination. Similarly, the apicomplexan *Theileria* hijacks MAPK signalling and conveys immortalisation and altered migratory properties to infected leukocytes (Tretina et al., 2015; Latre De Late et al., 2019).

The data to date show that *T. gondii* orchestrates a migratory activation in parasitised phagocytes by acting both on central signalling nodes that regulate cell motility and on the regulation of actin dynamics. The Ras-Erk MAPK pathway plays a central role, integrating signalling from GABA/VGCC activation, tyrosine kinase signalling and integrin signalling. Thus, a signalling milieu which is consonant with paradigms in cell metastasis is starting to appear (Lambert et al., 2017). In summary, the emerging concept suggests that *T. gondii* transforms invaded mononuclear phagocytes into shuttles with metastasis-like migratory properties. The invader is transported across tissues and into the systemic circulation to facilitate pervasive dissemination to distal tissues, including the central nervous system.

**Definitions**

**Tachyzoite**: Tachyzoites are the fast-replicating stage of *T. gondii* and are associated with disease in vertebrates. In the host cell, *T. gondii* tachyzoites reside and replicate inside a parasitophorous vacuole (PV).

**Mononuclear phagocytes**: These are immune cells that constitute the mononuclear phagocyte system (MPS). Dendritic cells (DCs), monocytes, macrophages, and also microglia in the brain are members of the MPS and mediate multiple, partly overlapping, functions during immune responses (Guilliams et al., 2014).

**MAT**: Mesenchymal-to-ameboid transition occurs when cells switch between different motility modes. Cells migrating in a mesenchymal fashion typically exhibit an elongated, spindle-like shape and exert traction on their substrates via focal adhesions associated with actin-rich protrusions (lamellipodia, filopodia). In contrast, cells undergoing amoeboid migration adopt round or irregular shapes.

**Podosomes**: These are actin-rich structures found on the outer surface of the cellular membrane. Podosomes serve as sites of attachment and degradation along the ECM.

**WAVE**: The WAVE regulatory complex (WRC) controls actin cytoskeletal dynamics throughout the cell by stimulating the actin-nucleating activity of the Arp2/3 complex at distinct membrane sites.

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