Chapter 14
Transmembrane Kinases and Their Role in *Entamoeba histolytica* Pathogenesis

Nathaniel C.V. Christy and William A. Petri Jr.

Abstract The transmembrane kinases (TMKs) of *Entamoeba histolytica* are a recently discovered family of cell-surface proteins that bear sequence similarity to the intermediate subunit of the Gal/GalNAc adherence lectin. The TMKs have been implicated in growth response to serum components as well as the phagocytosis of host cells and bacteria, processes that are critical to amebic pathogenesis during infection of the human host. Members of the TMK family appear to function variously as active kinases or pseudo-kinases depending on the structure of their intracellular domains and likely interact with intracellular signaling networks during signal transduction.

14.1   Introduction

"It would, perhaps, be one step towards the establishment of a rational mode of treatment in this very destructive form of dysentery, if the nature of the connection subsisting between the affection of the bowels and that of the liver were clearly ascertained, and the manner in which the one supervenes to the other were closely observed." (Annesley, p. 385) This quote, from an 1841 description of hepatic dysentery in India by the physician James Annesley, highlights the perplexing dual nature of infection of both the intestine and the liver in human hosts by *Entamoeba histolytica* [1]. Known today as amebiasis, this disease has been observed since ancient times [2], but Annesley's report was an early observation of the link between liver abscesses and the pathology seen during intestinal dysentery caused by *E. histolytica*. The molecular and cellular mechanisms that permit *E. histolytica* to infect such distinct physiological niches of the host remain in part a mystery to this day. Because of the nature of *E. histolytica* as a single-celled organism, the receptors and cell signaling components on the surface of the parasite represent an important interface with the host during survival and replication in differing compartments. Regardless of its location, the parasite must be able to adhere to host cells and

N.C.V. Christy • W.A. Petri Jr. (✉)

Division of Infectious Diseases and International Health, University of Virginia, Carter-Harrison Bldg, Rm 1709A, 345 Crispell Drive, Charlottesville, VA 22908, USA
e-mail: wap3g@virginia.edu

compartments such as the colonic epithelium, induce widespread contact-dependent host cell death, and ingest these cells and other host components to survive, as well as to respond to a myriad of other signals that inform the parasite as to its external environment [3, 4]. It can easily be speculated that the parasite modulates its survival during different stages of host exposure at least partially through its diverse repertoire of cell-surface sensors and their ability to multitask in these various environments. Although a number of amoebic surface molecules that are important in pathogenicity have been discovered and characterized, the discovery of a novel family of cell-surface proteins known as the transmembrane kinases (TMK) promises to shed further light on how *E. histolytica* interacts with the host during its varied stages of infection. This section focuses on the characterization to date of members of the TMK family as signaling molecules during amebic pathogenesis as well as speculate on the signaling mechanisms that TMK family members may use during signal transduction.

14.2 Discovery of the TMK Family

14.2.1 Initial Characterization

The discovery of the TMK family stems from a key event that has itself contributed to our understanding of the pathogenic biology of *E. histolytica*: the identification of the amebic cell-surface Gal/GalNAc adherence lectin. It was known that pathogenicity was linked with the ability of trophozoites to adhere to mucins in the colonic epithelium and that this was likely caused by a parasite galactosamine-binding lectin [5]. However, the identity of this molecule remained undiscovered until the purification of the galactose/*N*-acetyl-D-galactosamine (Gal/GalNAc)-specific lectin [6, 7]. The 260-kDa Gal/GalNAc lectin complex was also found to be an immunodominant surface antigen expressed during parasitic infection of both the intestine and the liver [8]. This adhesin was further characterized as having two unique subunits: a "heavy" lectin (Hgl) of 170 kDa and a "light" subunit (Lgl) of 35 kDa [9]. The Hgl lectin was observed to have a cysteine-enriched extracellular region with a carbohydrate recognition domain (CRD) [10]. A third "intermediate" subunit (Igl) of 150 kDa was also discovered by biochemical purification [11]. When purified together, both the Hgl and Igl subunits were able to bind galactose and galactosamine-containing residues via the cysteine-rich regions of their extracellular domains [12, 13]. In contrast to the biochemical approaches used to discover the Gal/GalNAc lectin, the recent rapid expansion of genetic information leading to the completion of sequencing of the *E. histolytica* genome [14] also led to the identification of a large family of more than 80 putative cell-surface proteins during sequence analysis of the Igl lectin subunit [11]. These open reading frames (ORF) were unique in that they shared significant sequence similarity to the Igl lectin with a number of CXXC-based motifs in their extracellular domains.

Group[a]	Transmembrane kinase no.	Size[b]	No. of amino acids, TM to kinase[c]	Signature motif in kinase	No. of amino acids, kinase to end[d]	No. of motifs[e]			
						CXC	CXXC	CXXXC	CXXCXXGYY
A	4, 17, 23, 25, 52, 53, 55, 61, 65, 68, 69, 72, 85	517–532	220	CC(I/V)KITDFGTSR[f]	40	4	4/5	3/4	3
B1	5, 12, 43, 76, 86, 91, 95, 100, 101, 103, 104	897–916	135	KLTDFGS(A/S)R[g]	0	1	25	2	9
B2	2, 8, 10, 11, 14, 15, 31, 36, 41, 62, 74, 75, 77, 87, 88, 92, 94, 105	822–1762	133	KLTDFGS(A/S)R[h]	0	0	90	0	25
B3	21, 28, 29, 30, 32, 35, 37, 38, 42, 48, 51, 96	830–2117	145	KLTDFGS(A/S)R	0	0	24	0	7
C	9, 13, 39, 60, 63, 71	547–624	160	C(A/G)KLTDFGTC[i]	59–82	0	33	0	11
D1	3, 18, 40, 56, 70, 79	520–619	234	PITAKVTDFGTS	63	3	5	3	7
D2	19, 27, 44, 46, 50, 57, 64, 67, 82, 97, 98	399–614	233	V(T/V)(C/X)KV(T/S)DFGTS	55	4	5	3	9
E	22, 54, 66	401–412	150	AKLSDFGTSR	60–97	1	0	0	0
F	34, 48, 59, 80	231–368	24	VKVSDFGLS and WXAPE	0	0	0	0	0

[a] Group G transmembrane kinases are diverse and include kinases 1, 6, 16, 24, 47, 49, 73, 79, with no common signature motif or other features within the group or with other groups.
[b] Number of amino acid residues in the extracellular domain.
[c] Amino acids from the C terminus of the transmembrane domain to the N-terminal glycine of the kinase domain.
[d] Amino acids from 10 residues after the conserved arginine to the stop codon.
[e] Number of times each motif is found in the protein sequence. The number of CXC, CXXC, CXXXC, and CXXCXXGYY motifs may vary between individual family members. Values with a slash indicate that some family members have 4 or 5, or 3 or 4, of the motif.
[f] Group A TMKs can additionally be identified by a moderately conserved (K/R)XXXDI(E/N)I(Y/F)KQQQPXYYYYIXGSXXXPXKXX(K/R)Y motif C-terminal to the transmembrane domain.
[g] Group B1 TMKs can be identified by a KRKEKKEREKTTIFKTITQSNI(K/R)FI(S/P)LGDG sequence after their transmembrane domain.
[h] Group B2 TMKs can be identified by a PV(N/G)(Q/E/K)E(S/T)(K/R)DL(L/I)CIGNXXKXXXKVQ sequence after their transmembrane domain.
[i] Group C TMKs can be identified by a RRR(K/R)XXKXXXXXIKPF(H/K)VSSD(L/V)ELXLL sequence following the transmembrane domain.

Fig. 14.1 Properties of the domains of the *Entamoeba histolytica* transmembrane kinase groups. (From Beck et al. [16])

However, this tentative grouping as a gene family did not point to functional attributes other than the cysteine-rich regions that indicated a potential to sense the extracellular environment, suggested by the limited similarity of the Igl subunit to the variant surface proteins (VSP) of *Giardia* [11, 15]. Beck et al. subsequently conducted an exhaustive phylogenetic analysis on this gene family and found that many of its members contained a putative kinase domain [16]. Further, these genes were organized into six main families and nine distinct groups or subgroups (groups A, B1, B2, B3, C, D1, D2, E, and F), according to common signature motifs in the kinase domain as the number and composition of CXXC repeats in each gene varied (Fig. 14.1), although differences in the extracellular domains could distinguish between subgroups. Known collectively as the transmembrane kinase family (TMK), the common features of the TMKs included a long extracellular domain, a single-pass transmembrane domain, and an intracellular kinase-containing region (Fig. 14.2). Beck et al. then determined the transcript levels of various TMKs by both oligoarray and reverse transcriptase-polymerase chain reaction (RT-PCR) and found that members of each group were expressed during mid-log-phase growth though expression varied widely for different TMKs. Polyclonal antibodies raised to the kinase domains of TMK members showed localization of multiple kinases to the plasma membrane, confirming surface expression. Also, although many of the newly identified TMKs had all the major conserved features of an active kinase domain, some members were missing one or more, raising the possibility that these TMKs could serve as pseudo-kinases or be implicated in antigenic variation and immune evasion by the parasite [16].
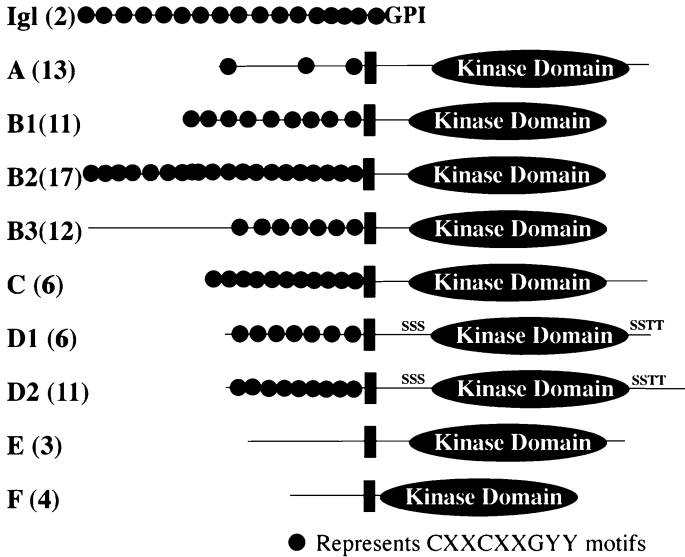
Fig. 14.2 Organization of the transmembrane kinase (TMK) family. Diagram of Igl and the TMKs shows the approximate sizes of the different proteins and the distribution of the CXXCXXGYY motifs in the extracellular domain (*black circle*). The CXXCXXGYY motifs are part of a larger motif, CXXCXXG(Y)(Y/F)(L/V/F/Y/M)-Polar–Polar, which also can begin with CXC instead of CXXC. GPI represents a putative GPI anchor. *Black rectangle* indicates a transmembrane domain, and *black oval* indicates a putative kinase domain. Serine (SSS)- and serine/threonine (SSTT)-rich regions found in groups D1 and D2 are shown. Numbers in *brackets* indicate numbers of known family members. (From Beck et al. [16])

14.2.2   Brief Survey of the E. histolytica *TMKs in Comparison to Other Receptor Kinase Families*

The discovery of the TMK family in the genome of *E. histolytica* was exciting because similar receptor kinase families are well known in a number of other organisms. These other receptor/kinase families point to potential roles for the TMKs. For example, the high number of TMK family members in the amoebic genome is similar to that seen in plant species. More than 400 genes termed plant receptor kinases (PRKs) have been identified in the *Arabidopsis* genome [17]. The PRKs are primarily serine/threonine kinases resembling the transforming growth factor (TGF)-β receptors but do not share similarity in their extracellular domains, indicating that they may have a wide range of ligand recognition specificities and functional differences, including the recognition of ligands from both endogenous plant and exogenous bacterial produced sources [18]. As is discussed further, evidence thus far indicates that different TMK families may also have varying functions and roles.

The human genome encodes another group of receptor kinases, the receptor tyrosine kinases (RTKs) with 58 individual members in 20 subfamilies that have a similar structure composed of an ecto-domain, a single transmembrane domain, and a kinase- or pseudo-kinase-containing intracellular region [19]. One of the most well known metazoan RTK members is the epidermal growth factor receptor (EGFR), which controls growth and proliferation in response to extracellular ligand binding-induced stimuli from circulating growth factors. The cysteine-rich extracellular domains of the RTK family members contain a high number of repeated motifs that promote dimerization and mediate ligand binding. As noted above, the *E. histolytica* TMKs were originally identified and classified by similar cysteine-rich repeats in their ecto-domains; it is reasonable to suspect that these repeats serve a similar function for both ligand binding and dimerization in the TMK family. Following homo-dimerization or dimerization with other family members, the EGFR autophosphorylates to become enzymatically active on other substrates and to serve as a docking site for adaptor proteins such as the SH2 domain-containing Grb2 [20]. Although no easily identified docking sites have been noted on the intracellular regions of amebic TMKs, it is possible that some docking proteins (e.g. SH2 domain-containing) are present during TMK signaling in *E. histolytica* [14].

Aside from the RTKs, amebic TMK members may also signal similarly to receptor serine/threonine kinases (RSTKs) such as the TGF-β receptor superfamily, the different members of which recruit and phosphorylate each other in sequence as a heteromeric complex after ligand binding [21]. A cursory evaluation of the intracellular regions of one TMK member, TMK39, and the TGF-β receptor I shows a region of alignment of 31 % amino acid identity, with similarity of 48 %, likely caused by the commonality of the kinase regions of these proteins. Such TMK-containing oligomeric complexes may be found to be present in the cell-surface lipid rafts described by Mittal et al. [22].

Finally, several other protozoal species maintain families of cell-surface proteins that may bear functional similarity to the TMK family. Although *Entamoeba* species including *E. histolytica* and *E. dispar* are relatively unique among single-celled protozoa in expressing TMKs with kinase domains, some TMK members may be similar to the kinase-deficient VSPs of *Giardia* species and trypanosomatids, briefly mentioned previously, in their provision for immune evasion by the alternate expression of different cysteine-rich ecto-domain-containing members of the VSP families [15, 23, 24].

14.3 Functional Characterization of the TMKs

14.3.1 Role in Growth Response

The first evidence of a functional role for a transmembrane kinase member emerged during studies by Mehra et al. on the B1 family of TMKs [25]. The Mehra et al. analysis utilized the newly published genome data for *E. histolytica*, which identified a total of 90 TMKs; 28 constituting the B1 family [14]. However, Mehra et al.

showed by RT-PCR that only a few of these were expressed and when antibodies were raised to the B1.I subfamily, these TMKs exhibited localized expression to the amebic surface at uroid and pseudopod regions in a manner similar to that seen by Beck et al. (discussed earlier). *E. histolytica* trophozoites expressing a C-terminus-truncated version of the B1.I TMK (also known as EhTMKB1-2) that lacked its cytoplasmic kinase domain allowed Mehra et al. to study the function of this TMK in a dominant-negative model system, finding that amoebic growth was significantly abrogated; this was the first indication that the intracellular kinase domain was critical in the function of a TMK and that signaling through phosphorylation might be a critical component of receptor activity by the TMKs. Interestingly, the B1.I TMK dominant-negative-expressing amoebae also showed a very poor growth recovery after restoration from a serum starvation state, suggesting that these cells lacked a normal response to a factor in the serum medium component. The studies of Mehra et al. were especially significant in that they showed at least one TMK family member to bear a *functional* as well as structural resemblance to higher metazoan growth factor receptors such as the EGFR family [25].

A later study by Shrimal et al. found that another member of the B1 TMK family, EhTMKB1-9, had higher expression levels than EhTMKB1-2 or any other B1 TMKs and was also localized to the surface of trophozoites [26]. Interestingly, the fraction of this receptor expressed in comparison to other B1 family members was proportional to the restoration of serum following serum starvation of *E. histolytica* trophozoites. The Shrimal et al. studies showed that this was the result of control by an upstream repressive promoter. Further, in a dominant-negative model system, this serum response modulated growth of the parasite and had effects on target cell adhesion and killing, possibly secondarily caused by the growth defect. Finally, EhTMKB1-9 was also shown to be an active kinase with both serine/threonine and tyrosine specificity, the first such instance of a TMK predicted kinase activity being confirmed in vitro, although an amebic substrate has not yet been found. The latest studies by Shrimal et al. have identified unsaturated fatty acid lipids in serum as the regulator of the promoter element controlling EhTMKB1-9 [27].

Work by Abyankar et al. showed that the EhTMKB1-9 dominant-negative model conferred a defect in colitis during *E. histolytica* mouse infection, although liver abscess formation was not changed [28]. This study also found that EhTMKB1-9 showed a small but significant defect or increase in erythrophagocytosis and fluid-phase pinocytosis during dominant-negative and healthy full-length expression, respectively, although these results were not directly linked to a binding/receptor activity by this TMK.

14.3.2   Control of Phagocytosis Mechanisms

E. histolytica has the opportunity to phagocytose extracellular targets in different host environments ranging from bacteria and epithelial host cells in the intestine to erythrocytes and other host cells during dissemination to sites such as the liver [29, 30]. The propensity for ingestion has been shown to be uniquely correlated

with the pathogenicity of the parasite [31, 32]. *E. histolytica* also has recognizable internal signaling machinery for the control of the initiation and maturation of phagocytosis [33–35]. However, only a few amoebic receptors that actually recognize extracellular targets have been identified, and little information exists to link such receptors with known intracellular signaling components. Further, *E. histolytica* trophozoites are likely to maintain a multiplicity of receptors engaging unique signaling pathways for the ingestion of differing targets similar to mechanisms by metazoan professional phagoctyes such as macrophages [36]. To identify new receptor candidates, multiple groups performed proteomic analyses of early time-point phagosomes utilizing mass spectrometry approaches [37–39]. Boettner et al. found that a TMK B3 family member, christened PATMK, was present at early timepoints (5 and 10 min) of purified phagosomes, indicating it may serve as a cell-surface receptor [40]. Antibodies to the extracellular region of PATMK showed that it was present on the cell surface and confirmed that it internalized with ingested carboxylate-modified beads. Rates of erythrocyte ingestion by trophozoites were inhibited in multiple experiments, including antibody pre-incubation, the expression of a dominant-negative version of PATMK, and reduced native protein expression through an interfering short hairpin RNA strategy. The dominant-negative PATMK-expressing cells also exhibited lower pathogenicity in a mouse intestinal colitis model but were still not deficient in liver abscess formation.

In a comprehensive study of TMK gene expression, Buss et al. used laser capture microdissection in conjunction with microarray analysis to determine the concurrent presence of TMK family member transcripts in a single cell [41]. Multiple TMK genes were expressed at the same time in individual cells, indicating to the authors that the individual TMK members served functional roles versus contributing to antigenic variation by the parasite. Two TMK members, TMK54 (E family) and TMK39 (C family), were then compared in localization, expression, and function. When stained with specific antibodies, TMK54 showed a general distribution on the cell surface whereas TMK39 was present in punctate spots across the plasma membrane. Also, in dominant-negative-expressing cell lines for both TMKs, disruption of TMK54 but not TMK39 led to a deficiency in growth. TMK39 dominant-negative expression singularly led to a defect in phagocytosis. This inhibition was specifically limited to apoptotic Jurkat T lymphocytes and not erythrocytes, indicating that TMK39 might serve as a receptor with recognition for specific targets. Utilizing the same dominant-negative model, further studies by Christy et al. on TMK39 showed that it also mediated the ingestion of bacteria [42]. The ingestion defect of bacteria by the TMK39 dominant-negative cells was matched by a twofold increase in bacterial adhesion, which was the first evidence of direct binding to an extracellular target by a TMK member, further supporting its role as a cell-surface receptor.

14.4 Perspective

From its origins from the sequencing of the genome of *Entamoeba histolytica*, the discovery of the transmembrane kinase family has informed our understanding of how this parasite successfully interacts with and responds to the myriad of differing
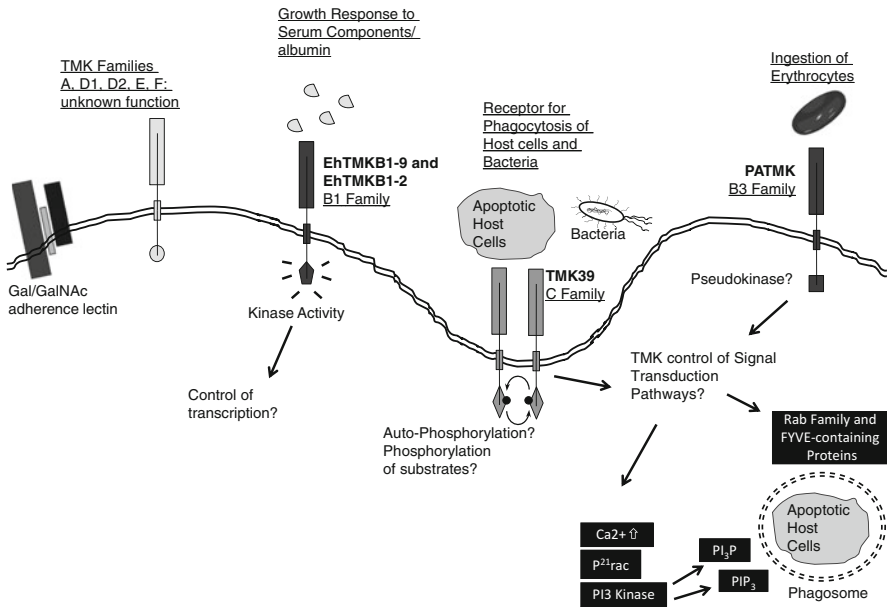
Fig. 14.3 Function and signal Transduction by the transmembrane kinases. Members of the TMK families A, D, E, and F do not have assigned function or ligand recognition. EhTMKB1-9 and EhTMKB1-2 have been implicated in growth response to serum components. EhTMKB1-9 is an active kinase and may phosphorylate amoebic substrates. TMK39 regulates the phagocytosis of apoptotic host cells and bacteria and may autophosphorylate as part of its activation. PATMK does not have all the requisite elements of an active kinase but regulates the internalization of erythrocytes and may function as a pseudokinase. It is likely that these transmembrane kinases interact with known amoebic intracellular signaling networks that control processes such as the development of phagosomesy

environments in its host (Fig. 14.3). The discovery that several TMK members play a key role during growth and response to serum components is not surprising given the structural similarity of the TMK family to metazoan growth factor receptors such as the EGFR [43]. That other TMKs are critical in the regulation of phagocytosis and may serve as direct receptors during this process is also interesting because the extracellular domains of some TMKs bear similarity to that of several macrophage scavenger receptors. The EGFR receptor itself has been shown to modulate phagocytosis, although this is a nonclassical role for this receptor [44]. Studies thus far have used the dominant-negative model of receptor characterization to great effect, which points to the key role that the intracellular kinase domains of TMK members are likely to play in signal transduction, although it is also possible that some TMKs function as pseudo-kinases, in a fashion similar to the metazoan HER3 receptor [45]. In this model, TMKs with and without kinase activity could function as hetero-oligomeric receptors similar to the HER family, with varying signaling outcomes based on combinations of different receptors [46, 47]. Future studies on these

and other remaining questions concerning the function and role of the transmembrane kinase family promise to greatly enhance our understanding of the structure and biological role of the cell-surface receptors of *E. histolytica*.

References

1. Annesley J (1841) Researches into the causes, nature, and treatment of the more prevalent diseases of India, and of warm climates generally. Longman, Brown, Green, and Longmans, London
2. Bryan C (1974) Ancient Egyptian medicine: the papyrus ebers. Ares, Chicago
3. Petri WA Jr, Ravdin JI (1987) Cytopathogenicity of *Entamoeba histolytica*: the role of amebic adherence and contact-dependent cytolysis in pathogenesis. Eur J Epidemiol 3:123–136
4. Huston CD, Boettner DR, Miller-Sims V, Petri WA (2003) Apoptotic killing and phagocytosis of host cells by the parasite *Entamoeba histolytica*. Infect Immun 71:964–972
5. Ravdin JI, Guerrant RL (1981) Role of adherence in cytopathogenic mechanisms of *Entamoeba histolytica*. Study with mammalian tissue culture cells and human erythrocytes. J Clin Invest 68:1305–1313
6. Ravdin JI, Murphy CF, Salata RA, Guerrant RL, Hewlett EL (1985) *N*-Acetyl-D-galactosamine-inhibitable adherence lectin of *Entamoeba histolytica*. I. Partial purification and relation to amoebic virulence in vitro. J Infect Dis 151:804–815
7. Petri WA, Smith RD, Schlesinger PH, Murphy CF, Ravdin JI (1987) Isolation of the galactose-binding lectin that mediates the in vitro adherence of *Entamoeba histolytica*. J Clin Invest 80:1238–1244
8. Petri WA Jr, Broman J, Healy G, Quinn T, Ravdin JI (1989) Antigenic stability and immuno-dominance of the Gal/GalNAc adherence lectin of *Entamoeba histolytica*. Am J Med Sci 297:163–165
9. Petri WA Jr, Chapman MD, Snodgrass T, Mann BJ, Broman J, Ravdin JI (1989) Subunit structure of the galactose and *N*-acetyl-D-galactosamine-inhibitable adherence lectin of *Entamoeba histolytica*. J Biol Chem 264:3007–3012
10. Dodson JM, Lenkowski PW Jr, Eubanks AC, Jackson TF, Napodano J, Lyerly DM, Lockhart LA, Mann BJ, Petri WA Jr (1999) Infection and immunity mediated by the carbohydrate recognition domain of the *Entamoeba histolytica* Gal/GalNAc lectin. J Infect Dis 179:460–466
11. Cheng XJ, Hughes MA, Huston CD, Loftus B, Gilchrist CA, Lockhart LA, Ghosh S, Miller-Sims V, Mann BJ, Petri WA, Tachibana H (2001) Intermediate subunit of the Gal/GalNAc lectin of *Entamoeba histolytica* is a member of a gene family containing multiple CXXC sequence motifs. Infect Immun 69:5892–5898
12. Pillai DR, Wan PS, Yau YC, Ravdin JI, Kain KC (1999) The cysteine-rich region of the *Entamoeba histolytica* adherence lectin (170-kilodalton subunit) is sufficient for high-affinity Gal/GalNAc-specific binding in vitro. Infect Immun 67:3836–3841
13. Cheng XJ, Tsukamoto H, Kaneda Y, Tachibana H (1998) Identification of the 150-kDa surface antigen of *Entamoeba histolytica* as a galactose- and *N*-acetyl-D-galactosamine-inhibitable lectin. Parasitol Res 84:632–639
14. Loftus B, Anderson I, Davies R, Alsmark UCM, Samuelson J et al (2005) The genome of the protist parasite *Entamoeba histolytica*. Nature (Lond) 433:865–868
15. Adam RD, Aggarwal A, Lal AA, De La Cruz VF, McCutchan T, Nash TE (1988) Antigenic variation of a cysteine-rich protein in *Giardia lamblia*. J Exp Med 167:109–118
16. Beck DL, Boettner DR, Dragulev B, Ready K, Nozaki T, Petri WA (2005) Identification and gene expression analysis of a large family of transmembrane kinases related to the Gal/GalNAc lectin in *Entamoeba histolytica*. Eukaryot Cell 4:722–732
17. Shiu S-H, Bleecker AB (2001) Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. Proc Natl Acad Sci USA 98:10763–10768

18. Cock JM, Vanoosthuyse V, Gaude T (2002) Receptor kinase signalling in plants and animals: distinct molecular systems with mechanistic similarities. Curr Opin Cell Biol 14:230–236

19. Robinson DR, Wu YM, Lin SF (2000) The protein tyrosine kinase family of the human genome. Oncogene 19:5548–5557. doi:10.1038/sj.onc.1203957

20. Jorissen RN, Walker F, Pouliot N, Garrett TP, Ward CW, Burgess AW (2003) Epidermal growth factor receptor: mechanisms of activation and signalling. Exp Cell Res 284:31–53

21. Massagué J (2012) TGFβ signalling in context. Nat Rev Mol Cell Biol 13:616–630

22. Mittal K, Welter BH, Temesvari LA (2008) *Entamoeba histolytica*: lipid rafts are involved in adhesion of trophozoites to host extracellular matrix components. Exp Parasitol 120:127–134

23. Nash TE (2002) Surface antigenic variation in *Giardia lamblia*. Mol Microbiol 45:585–590

24. Donelson JE (2003) Antigenic variation and the African trypanosome genome. Acta Trop 85:391–404

25. Mehra A, Fredrick J, Petri WA, Bhattacharya S, Bhattacharya A (2006) Expression and function of a family of transmembrane kinases from the protozoan parasite *Entamoeba histolytica*. Infect Immun 74:5341–5351

26. Shrimal S, Bhattacharya S, Bhattacharya A (2010) Serum-dependent selective expression of EhTMKB1-9, a member of *Entamoeba histolytica* B1 family of transmembrane kinases. PLoS Pathog 6:e1000929

27. Shrimal S, Saha A, Bhattacharya S, Bhattacharya A (2012) Lipids induce expression of serum-responsive transmembrane kinase EhTMKB1-9 in an early branching eukaryote *Entamoeba histolytica*. Sci Rep 2

28. Abhyankar MM, Shrimal S, Gilchrist CA, Bhattacharya A, Petri WA Jr (2012) The *Entamoeba histolytica* serum-inducible transmembrane kinase EhTMKB1-9 is involved in intestinal amebiasis. Int J Parasitol Drugs Drug Resist 2:243–248

29. Mirelman D, Feingold C, Wexler A, Bracha R (1983) Interactions between *Entamoeba histolytica*, bacteria and intestinal cells. Ciba Found Symp 99:2–30

30. Boettner DR, Huston CD, Sullivan JA, Petri WA (2005) *Entamoeba histolytica* and *Entamoeba dispar* utilize externalized phosphatidylserine for recognition and phagocytosis of erythrocytes. Infect Immun 73:3422–3430

31. Rodríguez MA, Orozco E (1986) Isolation and characterization of phagocytosis- and virulence-deficient mutants of *Entamoeba histolytica*. J Infect Dis 154:27–32

32. Hirata KK, Que X, Melendez-Lopez SG, Debnath A, Myers S, Herdman DS, Orozco E, Bhattacharya A, McKerrow JH, Reed SL (2007) A phagocytosis mutant of *Entamoeba histolytica* is less virulent due to deficient proteinase expression and release. Exp Parasitol 115:192–199

33. Ghosh SK, Samuelson J (1997) Involvement of p21racA, phosphoinositide 3-kinase, and vacuolar ATPase in phagocytosis of bacteria and erythrocytes by *Entamoeba histolytica*: suggestive evidence for coincidental evolution of amebic invasiveness. Infect Immun 65:4243–4249

34. Powell RR, Welter BH, Hwu R, Bowersox B, Attaway C, Temesvari LA (2006) *Entamoeba histolytica*: FYVE-finger domains, phosphatidylinositol 3-phosphate biosensors, associate with phagosomes but not fluid filled endosomes. Exp Parasitol 112:221–231

35. Byekova YA, Powell RR, Welter BH, Temesvari LA (2010) Localization of phosphatidylinositol (3,4,5)-trisphosphate to phagosomes in *Entamoeba histolytica* achieved using glutathione *S*-transferase- and green fluorescent protein-tagged lipid biosensors. Infect Immun 78:125–137

36. Plüddemann A, Neyen C, Gordon S (2007) Macrophage scavenger receptors and host-derived ligands. Methods 43:207–217

37. Okada M, Huston CD, Mann BJ, Petri WA Jr, Kita K, Nozaki T (2005) Proteomic analysis of phagocytosis in the enteric protozoan parasite *Entamoeba histolytica*. Eukaryot Cell 4:827–831

38. Okada M, Huston CD, Oue M, Mann BJ, Petri WA Jr, Kita K, Nozaki T (2006) Kinetics and strain variation of phagosome proteins of *Entamoeba histolytica* by proteomic analysis. Mol Biochem Parasitol 145:171–183

39. Marion S, Laurent C, Guillén N (2005) Signalization and cytoskeleton activity through myosin IB during the early steps of phagocytosis in *Entamoeba histolytica*: a proteomic approach. Cell Microbiol 7:1504–1518
40. Boettner DR, Huston CD, Linford AS, Buss SN, Houpt E, Sherman NE, Petri WA (2008) *Entamoeba histolytica* phagocytosis of human erythrocytes involves PATMK, a member of the transmembrane kinase family. PLoS Pathog 4:e8
41. Buss SN, Hamano S, Vidrich A, Evans C, Zhang Y, Crasta OR, Sobral BW, Gilchrist CA, Petri WA (2010) Members of the *Entamoeba histolytica* transmembrane kinase family play non-redundant roles in growth and phagocytosis. Int J Parasitol 40:833–843
42. Christy NCV, Buss SN, Petri WA Jr (2012) Common pathways for receptor-mediated ingestion of *Escherichia coli* and LDL cholesterol by *Entamoeba histolytica* regulated in part by transmembrane kinase 39. Int J Parasitol 42:393–400
43. Lemmon MA, Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. Cell 141:1117–1134
44. Galán JE, Pace J, Hayman MJ (1992) Involvement of the epidermal growth factor receptor in the invasion of cultured mammalian cells by *Salmonella typhimurium*. Nature (Lond) 357:588–589
45. Shi F, Telesco SE, Liu Y, Radhakrishnan R, Lemmon MA (2010) ErbB3/HER3 intracellular domain is competent to bind ATP and catalyze autophosphorylation. Proc Natl Acad Sci USA 107:7692–7697
46. Soler M, Mancini F, Meca-Cortés O, Sánchez-Cid L, Rubio N, López-Fernández S, Lozano JJ, Blanco J, Fernández PL, Thomson TM (2009) HER3 is required for the maintenance of neuregulin-dependent and -independent attributes of malignant progression in prostate cancer cells. Int J Cancer 125:2565–2575
47. Gregory CW, Whang YE, McCall W, Fei X, Liu Y, Ponguta LA, French FS, Wilson EM, Earp HS 3rd (2005) Heregulin-induced activation of HER2 and HER3 increases androgen receptor transactivation and CWR-R1 human recurrent prostate cancer cell growth. Clin Cancer Res 11:1704–1712