するための，生化学・細胞生物学的解析の必要性は日々高まっている．そのような中でわれわれは赤痢アメーバの病原機構を，主に細胞内小胞輸送という観点から眺めて研究を行っている．

細胞内小胞輸送（膜輸送，メンブレントラフィック）は真核生物にとって必須のシステムであり，その根幹は真核生物全般に保存されている．しかしながら，進化の過程で早期に真核生物の主幹から分岐した赤痢アメーバ原虫ではその輸送システムが大きく変化している．赤痢アメーバの感染症病原体としての重要性に加え，このユニークな進化上の位置が，赤痢アメーバの細胞内小胞輸送の研究を生物学的観点から重要なものにしている．また感染病原体に特異的・選択的に存在する分子機構の解明は，薬剤やワクチンなどの新しい標的を提供する可能性をもっている．基礎生物学における重要な発見は常に臨床応用への可能性を秘めている．本稿では赤痢アメーバの病原機構における小胞輸送の役割に関して，特に病原因子の輸送機構についてわれわれの研究を中心に紹介したい．

1 赤痢アメーバの病原因子と病原機構における小胞輸送の関与

1）確立された病原因子

赤痢アメーバ症の主要な症状（粘血性下痢，肝膿瘍）の成立に関与する病原性因子として主に3つの分子が精力的に研究されている．①宿主組織との接着に関与するGal/GalNAc特異的レクチン（Gal/GalNAc specific lectin），②タンパク質分解酵素であるシステインプロテアーゼ（cysteine protease：CP），③膜穿孔性ペプチドであるアメーバポア（amoebapore：AP）である．赤痢アメーバの腸管上皮への接着にはレクチンが，組織侵入・障害にはCPが，宿主細胞や細菌の貪食胞での消化にはAPやCPが機能している[6]．また病態形成に直接関与しないが，感染伝播に重要なシスト形成も病原機構の一部と考えられ，主要な研究テーマとなっている．

2）病原因子と小胞輸送

リボソームで合成されたタンパク質は，一部は細胞質内にとどまり，一部はさまざまな経路を経て，細胞内の特殊なオルガネラや細胞表面，ときに細胞外へと輸送される．レクチン・CP・APを含む病原因子の機能発現のみならず，シスト化における細胞のリモデリングやシスト壁の合成・輸送においても適切な細胞内輸送は不可欠である．よって小胞輸送経路の統合的理解が病原機構全体の解明に資すると考えられる．

2 ゲノムからわかるユニークな小胞輸送機構

1）Rab低分子量GTPaseの多様化

赤痢アメーバ全ゲノムの解読は終了している[4) 5)]．そこでまずゲノム情報から予測可能な小胞輸送の分子機構に関して概説する．真核生物で中心的な小胞輸送制御分子としてRab低分子量GTPase※2がある．酵母では11，多細胞生物であるヒトでは60を超えるRabが存在する．しかし，単細胞生物である赤痢アメーバには91ものRab遺伝子が存在する（図1）[4) 7)]．このうち6グループ計21のRab（Rab1, 2, 5, 7, 8, 11）は他種生物と相同性をもつオーソログと予想される．一方，9グループ計30のRab（RabC, D, F, I, K, L, M, N, P）ならびにグループを形成しない40のRabは赤痢アメーバに選択的に保存していた．DNAマイクロアレイによる解析から栄養体で70％以上のRabが有意に発現していることが確認されている（中野ら未発表）．また他種生物と高い相同性を示すオーソログでも，その局在・機能がモデル生物とは異なることが示された（本稿3参照）．赤痢アメーバに保存されたRabは進化的に初期に確立したRabであると予測されるが，依然として進化の選択圧に影響されている可能性がある．また，単細胞生物における多様なRabは他の貪食性の寄生性原虫（膣トリコモナス）や自由生活性原虫（ゾウリムシやテトラヒメナ）でも発見されており，Rabの多様性が原虫の貪食を選択圧として生まれた可

※1　アメーバ赤痢（赤痢アメーバ症）

感染は赤痢アメーバ原虫のシスト（嚢子）に汚染した水・食物などの経口摂取で起こる．大腸で脱嚢した栄養体が組織に接着・侵入し，感染者の5～10％に大腸粘膜の破壊，大腸炎や粘血性の下痢を起こす．一部で肝臓・肺・脳などに播種し，膿瘍を生じ，重篤化する．また，感染者の一部は無症状シストキャリアとなり，次の感染源となる．

※2　Rab低分子量GTPase

20～25kDaのグアニンヌクレオチド結合タンパク質．Ras, Rho/Rac, Ran, Sar/Arf, Rabなどが知られ，さまざまな細胞内現象における制御分子として使われる．Rabは特に小胞輸送において重要であり，GTPに結合した活性化型とGDPに結合した非活性型で異なる分子（＝エフェクター）に結合し，特異的な機能を発揮する．

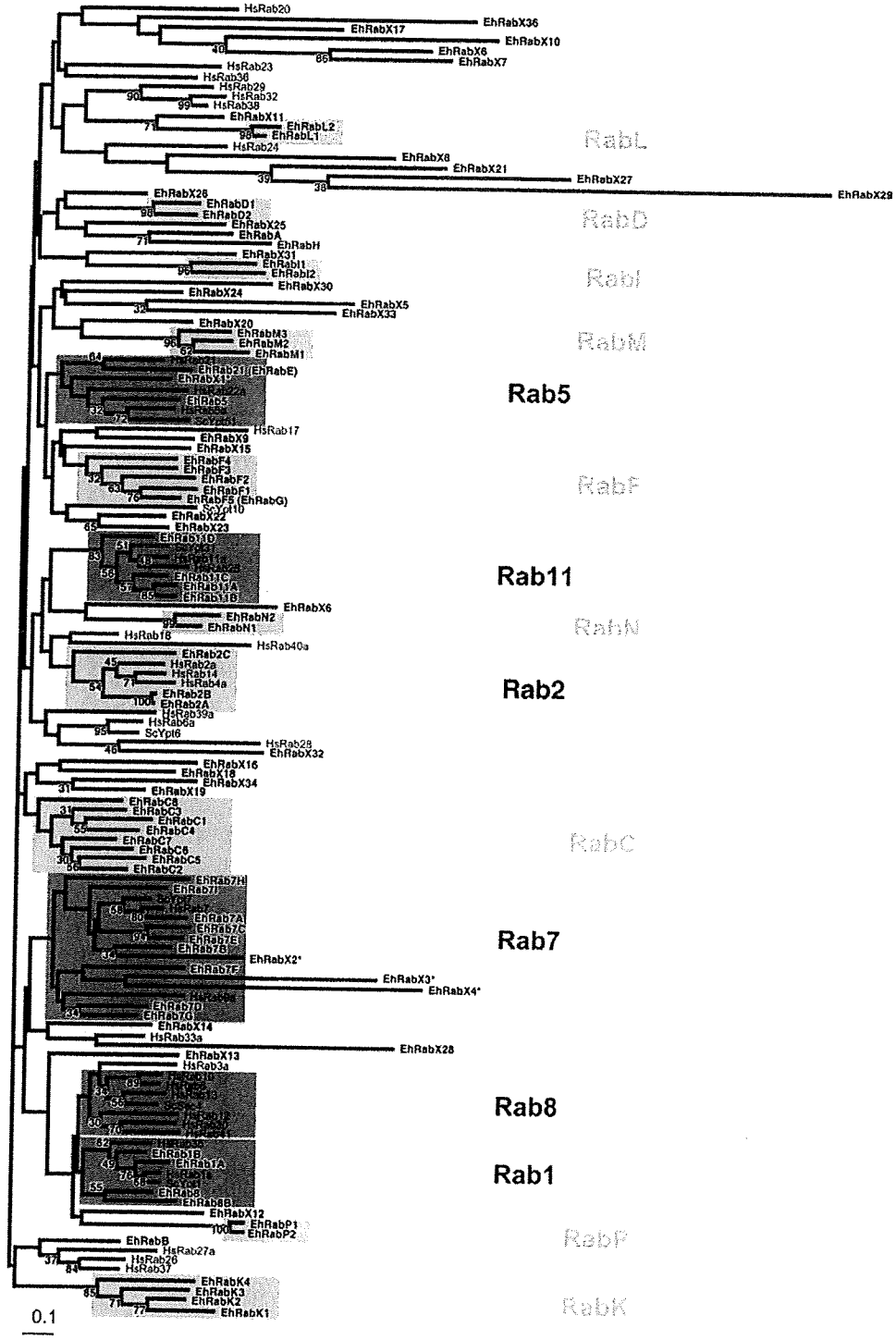

図1　赤痢アメーバRabの系統樹

赤痢アメーバゲノムデータベースから取得したRab遺伝子情報をもとに系統樹を作成した．黒字はヒトや酵母で保存されているグループ，グレーの字は赤痢アメーバ独自に形成されていたグループ．大部分のRabが赤痢アメーバ特異的に進化していることがわかる．30％以上のブートストラップ値を図中に示し，図下のスケールバーはアミノ酸置換に基づく距離を示す（文献7より転載）

能性が示唆される（中野ら，未発表）．また，赤痢アメーバの細胞骨格制御の特殊性がRab依存的な小胞輸送制御系に多様性を与えたのかもしれない（本稿**2**-3）参照）．

2）Rabエフェクターオーソログの欠損

Rabの機能は特異的エフェクターによって規定される．酵母・ヒトなどではRab5やRab7にそれぞれEEA1，Rabaptin-5，Rabex-5やRILP，Rabring7，Vps34というエフェクターが存在することが知られている．これらがRab5やRab7と結合することで，それぞれ初期エンドソーム，後期エンドソームに特異的な分子が動員され，コンパートメントの成熟が起こる[8)9)]．赤痢アメーバにもRab5やRab7オーソログが保存しているが，上記エフェクターについてはホスファチジルイノシトール3-キナーゼ（phosphatidylinositol 3-kinase：PI3K）であるVps34以外保存されていない．よって，赤痢アメーバにおけるRab依存的小胞輸送の調節機構は種特異的に分化していると考えられる（本稿**3**参照）．

3）細胞骨格の構成・制御因子の保存

活発に運動と捕食を行う赤痢アメーバにおいて，細胞骨格の制御は重要である．赤痢アメーバゲノムには細胞骨格分子のうちアクチンと微小管が存在するが，微小管を介した輸送に必須なモーター分子やその制御分子が存在しないことから，主要な細胞骨格はアクチンであると考えられている[5)]．アクチン制御分子はほぼすべて保存されているにもかかわらず，アクチン重合の重要な制御分子であるWiskott-Aldrich Syndrome Protein（WASP）のホモログを欠損している．しかし最近，WASPのC末側に存在するG-アクチンとArp2/3複合体に結合するドメイン（verprolin homology, cofilin homology and acidic motif：VCA）が単体でArp2/3依存的アクチン重合を促進できること，VCAドメインをもついくつかのタンパク質が赤痢アメーバに存在していることが明らかになり，このギャップが埋められようとしている．

4）オートファジーの単純化

近年盛んに研究されているオートファジーの過程も赤痢アメーバでユニークに進化している．細胞質タンパク質やオルガネラをリソソームへ送るこのシステムは栄養飢餓時の応答のみならず，細胞分化時のリモデリングにおいても重要な役割をもつことがわかっている．オートファジーの過程は，①開始シグナル，②preautophagosomal structure（PAS）形成，③オートファゴソーム膜形成にかかわる2つのユビキチン様因子の結合，④ホスファチジルイノシトール-3-リン酸（phosphatidylinositol 3-phosphate：PI3P）形成の制御系の大きく4つの分子群の関与が知られる．赤痢アメーバには他種生物で①，②，④の過程に関与することの知られる遺伝子のほとんど（Atg1, 2, 6, 9, 13, 14, 17, 18, 29）が存在しない．さらに③の過程にあるAtg8とAtg12という2つのユビキチン様タンパク質の結合系のうちAtg8系（Atg3, 4, 7, 8）しか保存されていない．このことはオートファジーあるいはその元となる細胞機能において，Atg8とその修飾機構が初期から保存されていたことを示唆している．オートファジー，ならびにAtg8の遺伝子発現がシスト化過程で強く誘導されること，PI3K阻害剤がシスト化とオートファゴゾーム形成の両者を同時に阻害することが*Entamoeba*のシスト化のモデルである*E. invadens*で示されている[10)]．シスト化に伴う細胞構造のリモデリングに原始的なオートファジーが関与するのか，あるいは，Atg8経路がオートファジー様の新規な分子機構として働いているのかまだ明らかでないが，いずれにせよシスト化にかかわる重要な機構であると考えられる．

3 病原因子にかかわる小胞輸送経路

赤痢アメーバゲノムからいくつかの小胞輸送関連分子や機構が特異的な進化を遂げたことを紹介した．次に，病原性・組織傷害において中心的な働きをするCPの輸送にかかわる分子機構について解説する．

1）一般的なリソソーム酵素輸送系

他種生物ではCPなどのリソソーム酵素は一般にどのように輸送されるのだろうか？ 代表的な，マンノース-6-リン酸（mannose 6-phospate：M6P）とその受容体（M6P receptor：MPR）を介した系を図2に示す．リソソームに輸送されるプロテアーゼは，まずN末端のシグナル配列により選別されSec61複合体により小胞体内部へ移送され，さらに，ゴルジ体でM6P修飾を受ける．これがトランスゴルジネットワーク（TGN）でMPRに認識され，アダプタータンパク質（アダプター）複合体とクラスリンにより濃縮，出芽し，輸送小胞が形成される．輸送小胞はエンドソー

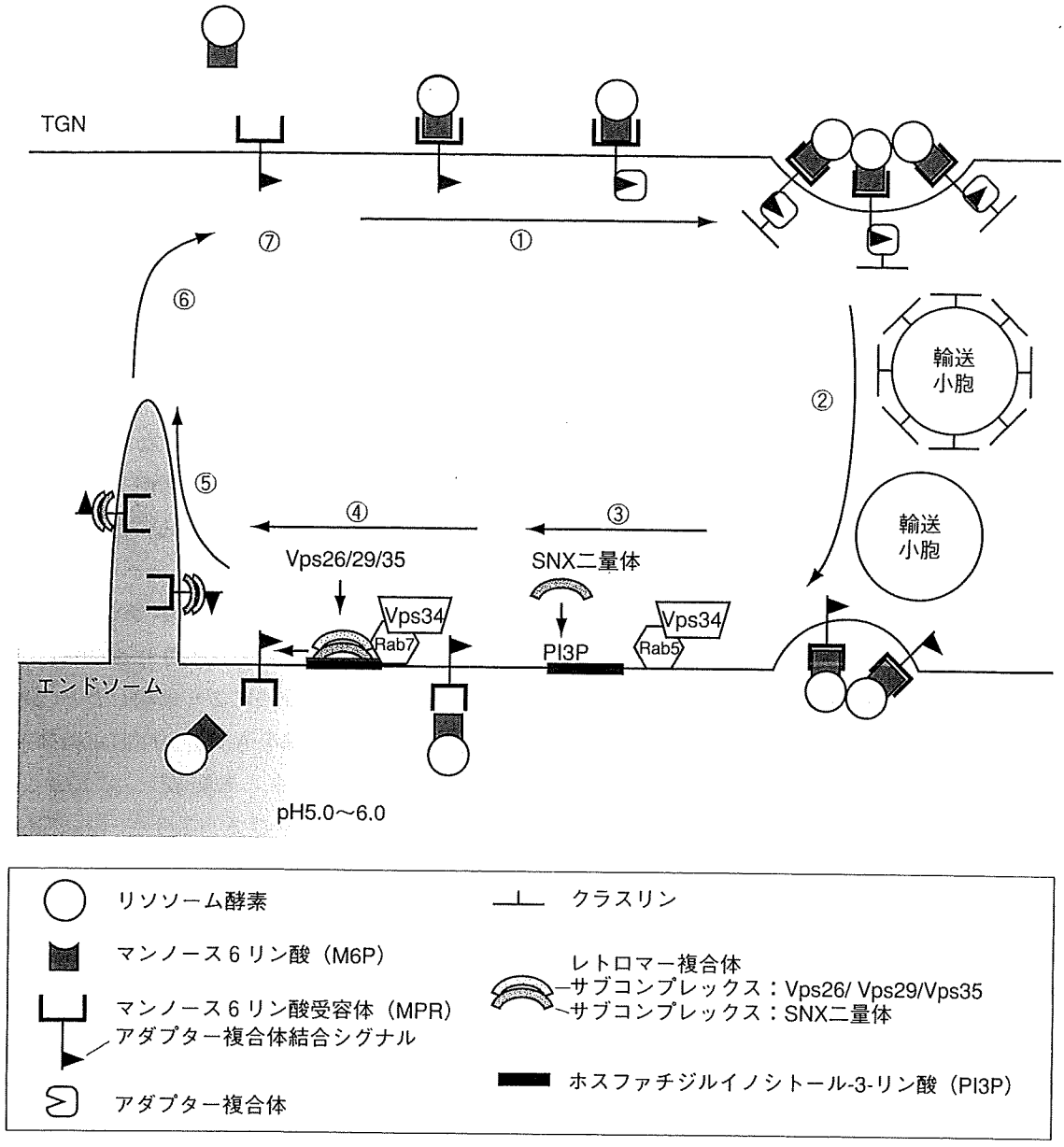

図２　他種生物におけるリソソーム酵素輸送系

MPRを介したリソソーム酵素輸送系．①M6P修飾を受けたリソソーム酵素はMPRによって認識され，MPRの細胞質側にアダプタータンパク質複合体が動員される．②アダプタータンパク質複合体を介してクラスリンが動員され，MPRとリソソーム酵素が濃縮された輸送小胞が形成される．クラスリンコートが外れ，エンドソーム膜と結合，融合する．③初期エンドソームではRab5とそのエフェクター（Vps34）の働きでPI3Pが産生され，そこにレトロマー複合体の一部であるSNX二量体が動員される．④エンドソームに運ばれたリソソーム酵素は後期エンドソームへの成熟に伴う酸性化（pH5.0～6.0）によりMPRから外れる．⑤後期エンドソームにはRab7が動員され，引き続きVps34の働きによりPI3Pを産生しSNX二量体の安定化を図るとともにVps26, 29, 35からなるサブコンプレックスの動員を行う．⑥積み荷を降ろしたMPRの細胞質側にレトロマー複合体が結合，チューブ状のエンドソームを形成，TGNへの逆行輸送を行う．⑦MPRがTGNにリサイクルされ，次の輸送に備える

ム膜に結合・融合し，エンドソームは成熟・酸性化する．エンドソームの酸性化に伴い，プロテアーゼはMPRから解離する．プロテアーゼはリソソームに留まる一方で，MPRはレトロマー複合体によりTGNへ再び回収される．すなわち，この輸送機構はプロテアーゼに付加されるM6Pをタグ（荷札）とした受容体依存的認識・輸送システムである．

2）Rab7ファミリーとリソソーム形成における役割

Rab7は一般に後期エンドソームやリソソームに局在し，エンドソームからリソソームへの成熟過程（あるいは輸送）を制御する．赤痢アメーバでは病原因子APやCPがリソソームで貯蔵され機能すること，さらに，貪食と貪食胞成熟が病原性に必須であることから，Rab7の機能解明が病原機構の理解に重要であった．赤痢アメーバには9つのRab7アイソタイプ（Rab7A～I）が存在する[11]．そのうちRab7AとRab7Bの局在と機能は明らかになっており，その働きは他種生物と異なるものであった[11) 12)]．Rab7Aは定常状態でリソソームに局在せず，貪食開始時に貪食胞と独立して形成される前貪食胞（prephagosomal vacuole：PPV，赤痢アメーバに特異的に観察される）とファゴリソソームに局在した．PPVはAPやCPを含んでいたことから，Rab7AがPPV，貪食胞へのリソソーム酵素の輸送に重要な分子であることが示唆された．一方Rab7Bはリソソームに恒常的に存在していた[11]．Rab7Bの活性化型変異体を強制発現させるとドミナントネガティブ効果が観察され，リソソームの減少とCPの過剰な分泌が観察された[11]．以上の結果から，Rab7A，Rab7Bはリソソーム形成において哺乳動物等とまったく異なった機能を担うこと，アイソタイプは機能的棲み分けをすることが示唆された．

3）Rab7A結合タンパク質：レトロマー複合体

Rab7AがPPV，貪食胞へのリソソーム酵素輸送を調節することが明らかになったので，Rab7Aのエフェクター分子を探索した[12]．GTP型Rab7A特異的に結合する分子群をタンパク質相互作用をもとに精製したところ，Vps26, Vps29, Vps35の3分子が同定された．これらは酵母やヒトでレトロマーと呼ばれる5分子からなる複合体の一部であった（図3）．レトロマー複合体は他種生物においてMPRに結合し，MPRのエンドソームからTGNへの逆行輸送を行う分子である（図2）．赤痢アメーバでは5分子のうち，精製・同定された3分子しかゲノム上に保存されておらずSNX※3分子を欠いていた．レトロマーと結合するMPRのホモログも存在しなかった．またRab7Aとレトロマー複合体との結合はVps26の種特異的なC末端延長配列を介しており，全く前例がなかった．赤痢アメーバにおいてRab7Aの強発現により起こるCP分泌の低下がVps26との共発現でキャンセルされたことから，Rab7Aとレトロマー複合体の相互作用がCP輸送を調節することが示唆された（図3）[11) 13)]．他種生物ではMPRとVps35が結合することが示されているため，これに代わるCP受容体の同定が重要となった．最近，哺乳動物でRab7とレトロマー複合体とが，Rab7とVps35との直接的な結合により相互作用することが報告された[14]．

4）CP受容体の同定

赤痢アメーバのCP遺伝子の中でも発現量が多く，病原性への直接的な関与の示唆されるCP5に結合する分子を，生化学的な手法により単離したところ，シグナル配列と膜貫通領域をもつ約100 kDaの分子が同定された（中田-津久井，未発表）．このタンパク質（CPBF1）はゲノム中でファミリー（cysteine protease binding protein family：CPBF）として存在しており，ほとんどが細胞質側にYxxLというアダプター複合体との結合モチーフを保存していた．赤痢アメーバにはアダプターとクラスリンが保存されており，これらの分子メカニズムが予想通り共同してCP輸送にかかわるのか，赤痢アメーバ特異的役割を果たすのか，今後の解析が必要であるが，CP受容体の発見はCP輸送の分子メカニズムの解析を大きく進めると期待される．

5）CP分泌の主要調節因子Rab11

赤痢アメーバのCP輸送を調節するRabが明らかになる一方で，細胞外への分泌を調節する分子は未同定であった．通常リサイクルエンドソームに局在し，輸送分子のリサイクルに関与するとされるRab11は赤痢アメーバに4種類のアイソタイプ（Rab11A～D）と

※3 SNX

sorting nexinの略称．ホスファチジルイノシトール-3-リン酸（PI3P）に結合するphox homology（PX）ドメインと，弓状の構造を取り湾曲した膜に結合するbin-amphiphysin-rvs（BAR）ドメインを有するタンパク質．この特徴からPI3P依存的に膜に動員され，曲率を上げることで膜をチューブ化する．SNX1, 2, 5, 6がレトロマー複合体のサブコンプレックスになりうる．

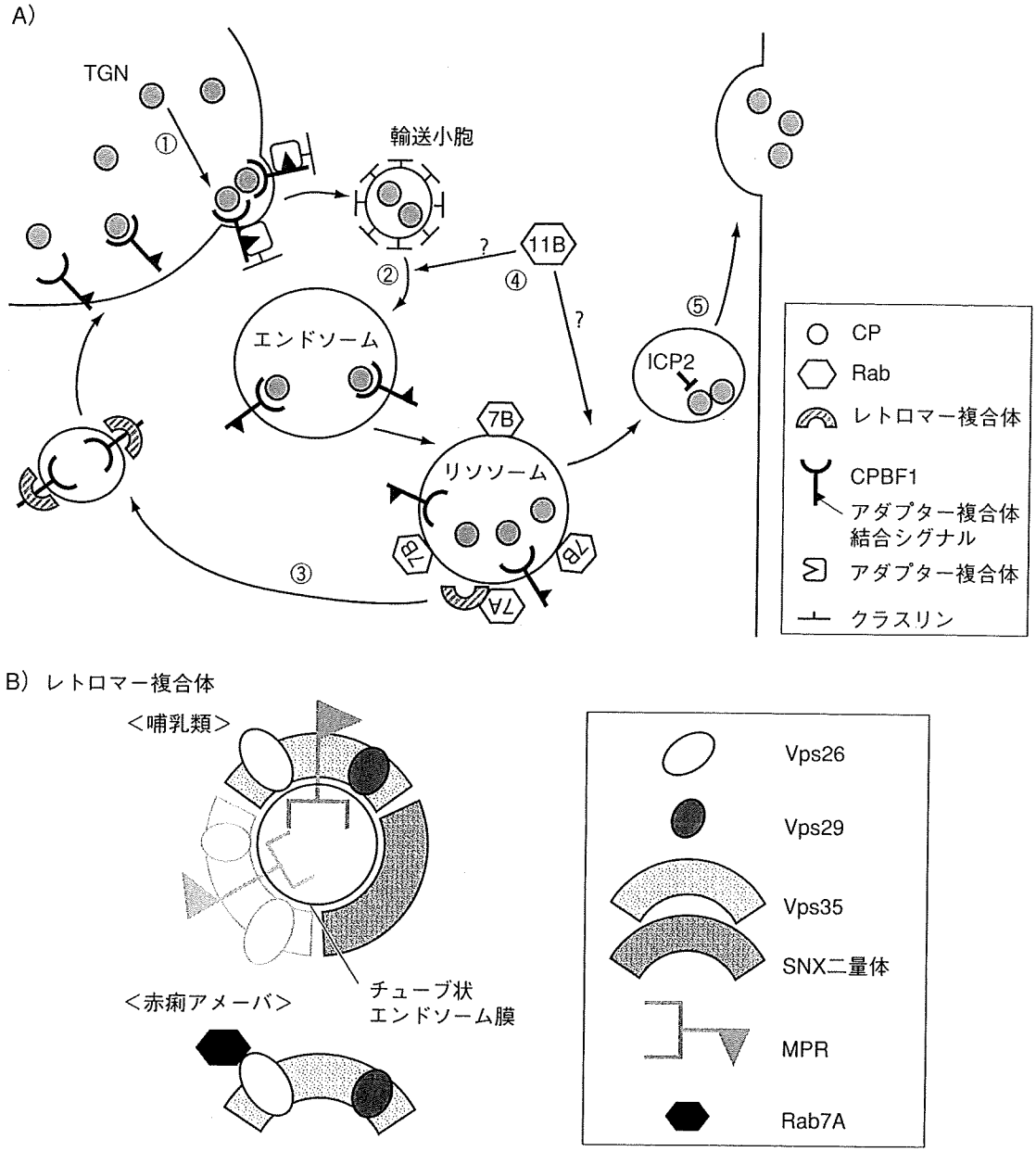

図3　赤痢アメーバにおけるCP輸送関与分子

A）赤痢アメーバのCP輸送．CPは小胞体で合成されゴルジ体を通過し，TGNへ送られる（①）．②CPはさらにTGNからエンドソームへアダプター複合体結合モチーフをもつCPBF1によりリソソームへと輸送され，③CPBF1はリソソームから再びTGNへリサイクルされると考えられる．この逆行輸送はRab7Aとレトロマー複合体により制御されると予想されるが，現在CPBF1とレトロマーの直接の結合を示唆する結果はない．また約半数のCPで糖鎖修飾部位が予想されていないことから，糖鎖非依存的輸送機構も存在すると考えられる．さらに赤痢アメーバレトロマー複合体は膜結合サブユニットであるSNXを欠いており，小胞膜への結合様式は不明である．また，細胞外へはリソソームを経て分泌されるか，あるいはポストゴルジ輸送小胞から直接分泌されるかは不明である．④Rab11Bは細胞外への分泌を正に制御するが，作用点は不明である．⑤リソソームにはICP2があり，CPの活性ならびに輸送を負に制御していると考えられる．ICP1については細胞質に存在する分子であることから，小胞体への移行時など，TGNに入る前の段階のCP活性と輸送を制御すると考えている．B）哺乳類と赤痢アメーバでのレトロマー複合体の相違．哺乳類ではレトロマー複合体は5分子からなる．立体構造解析から5分子が会合することでエンドソームをらせん状に取り囲みチューブ状に形成する働きがあることが明らかにされた．またVps35が受容体分子と直接結合する．赤痢アメーバでは5分子のうち3分子（Vps26, Vps29, Vps35）が保存されており，Rab7AがVps26と直接結合し，CP輸送に関与していた．しかしチューブ状の形態をとることに重要なSNX分子のホモログがないことから，その輸送機構はユニークなものと考えられる．最近動物細胞でRab7とレトロマー複合体の関係が解明され，Vps35とRab7が結合することが示された

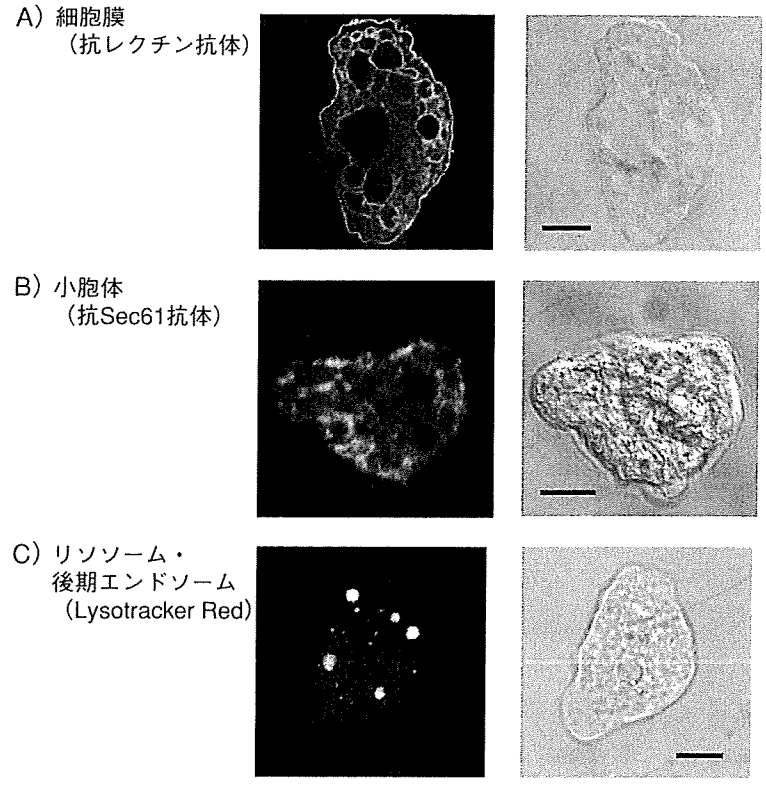

図4　赤痢アメーバのオルガネラ

赤痢アメーバを細胞表面レクチン（Igl）抗体（A），Sec61抗体（B），リソソーム集積性の色素（Lysotracker Red）（C）で染色し，共焦点レーザー顕微鏡で観察した．A）細胞表面一面にレクチン分子が分布する様子が観察される．また位相差像から細胞内部は大小の小胞からなることがわかる．これら小胞のいくつかがレクチン抗体で染色されることから，レクチン分子が細胞表面と内部小胞を行き来していることがわかる．B）Sec61はシグナル配列をもつタンパク質を小胞体内に転送する際の小孔をつくる，小胞体局在性タンパク質である．赤痢アメーバの小胞体は小胞の形態をとらず，ネットワークのように細胞全体に広がっている．これがより小さい小胞の集合なのか，他の形態をもつネットワークなのか，いまだ議論のあるところである．C）リソソーム・後期エンドソームは大小の小胞として観察される．赤痢アメーバでファゴサイトーシスやエンドサイトーシスの実験を行うとファゴソームやエンドソームが時間とともに酸性化されることが観察される（スケールバー：10 μm）

して存在していた．報告されたRab11Aの機能[15]は不明瞭であったので，Rab11Aに次いでmRNA発現量の多いRab11Bの解析を行ったところRab11Bの強発現によりCPの過剰な分泌が起こることが明らかになった[16]．Rab11Bは小胞体（図4）のようなネットワーク状に観察されたが，Sec61との共局在は観察されなかった．また一部エンドソームと共局在するものの，リソソームとの共局在は観察されなかった．よってRab11BはRab7A/7Bとは異なるメカニズムでCP輸送を制御していると考えられる．既知のRab11のエフェクター分子も赤痢アメーバには保存しておらず，どのような分子メカニズムが存在するのか，今後の解析が待たれる．

6）CPの内因性阻害タンパク質ICP

inhibitor of CP（ICP）はCPの活性中心に結合する内因性のCP阻害タンパク質である．ICPはもともと他の寄生性原虫である*Leishmania*や*Trypanosoma brucei*で発見された[17][18]が，赤痢アメーバでの存在はゲノム開示により明らかとなった．赤痢アメーバには，シグナル配列をもたない細胞質局在のICP1とシグナル配列をもつリソソーム局在のICP2の2分子が存在する．興味深いことに，2種類のICPどちらの過剰発現によってもCPの分泌が大きく抑制されること，細胞中のICPの分子数はCPの1/1000であること，約2倍のICPの強発現により70～90％のCP活性の減少が起きることから，この分泌抑制はCPの活性阻害

ではなくCP輸送の制御により起こったと考えられた[19]．CPの内因性阻害タンパク質がCP輸送を制御するとの報告はなく，きわめて重要な知見である．しかしながら，未だに細胞質内・小胞内それぞれにおけるICPのCP活性化抑制機構の分子メカニズムの詳細は明らかでなく，ICPの生理的役割とともに今後の解析が待たれる．

おわりに

赤痢アメーバは主に発展途上国で医学的インパクトの高い病原体であり，その病気の克服，例えばワクチンや薬剤の創成は重要な研究テーマである．しかし同時に，真核生物の進化の過程で初期に分化した生物としてユニークな生物現象や分子機構を備えている．本稿で紹介したように，真核生物によく保存されている小胞輸送，細胞骨格，オートファジー等のさまざまな細胞内メカニズムの進化を理解し，その普遍性と特殊性を解明するためにきわめて魅力的な研究材料である．紙面の都合上，本稿で紹介できたユニークな分子機構は，近年解明された重要な知見のごく一部に過ぎない．ここ1，2年ほどでも，遺伝子発現のエピジェネティック制御機構（gene silencing），microRNAによる転写制御，膜内プロテアーゼによるレクチン分子のプロセシング，免疫回避機構など生物学的にきわめて価値の高い新発見が次々と報告されている．また赤痢アメーバは嫌気的原虫であり，ミトコンドリア（マイトソームと呼ばれる）は高度に退化しているが，他種生物とは全く異なる代謝経路を区画化していることが徐々に明らかとなっている．赤痢アメーバ研究には研究者人口の少ないデメリットもあるが，顕微鏡下で盛んに動き回る様子は研究者の飽くなき探究心をくすぐり続ける．その魅力を若い研究者・学生や他分野の研究者にわかってもらえれば，原虫感染症学には常に新しい研究展開が生まれ続けると信じている．

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津久井久美子：東邦大学大学院理学研究科生物分子科学専攻修了，理学博士．バージニア州立大学（Dr. Kodi S. Ravichandran）にてポスドクの後，野崎智義博士のもとで赤痢アメーバの貪食，貪食胞成熟の分子機構の研究を開始．2005年10月群馬大学助教，'08年11月より国立感染症研究所寄生動物部主任研究官．研究者人口の少ない生物で研究を行うハンデをメリットに変え，一般生物学へインパクトを与えられるような研究を展開したい．

野崎智義：慶應義塾大学医学部卒．1987年慶應義塾大学医学部熱帯医学・寄生虫学助手．'88年JICA派遣専門家でブラジルレシフェで医療協力．'99年よりLPD, NIAID, NIH（James Dvorak博士）とロックフェラー大学（George Cross教授）で，トリパノソーマの薬剤耐性・ゲノム可塑性・遺伝子発現調節・鞭毛接着タンパク質の研究に従事する．'96年帰国後，赤痢アメーバの病原性・代謝・創薬の研究を開始し，'99年より国立感染症研究所第3室長，2005年より群馬大学大学院医学系研究科国際寄生虫病学教授，'08年7月より国立感染症研究所寄生動物部長．

Diphyllobothrium spp. (sparganosis)
Spirometra mansonoides (sparganosis)

TREMATODES

INTESTINAL

These organisms are uncommon within the United States except for four species of *Alaria*, which are endemic within North America.

Current Name

Fasciolopsis buski (giant intestinal fluke)
Echinostoma ilocanum
Eurytrema pancreaticum
Heterophyes heterophyes
Metagonimus yokogawai
Alaria spp.

LIVER AND LUNG

These organisms are not seen commonly within the United States; however, some Southeast Asian refugees do harbor some of these parasites.

Current Name

Clonorchis (Opisthorchis) sinensis (Chinese liver fluke)
Opisthorchis viverrini
Fasciola hepatica (sheep liver fluke)
Paragonimus westermani (lung fluke)
Paragonimus spp.
Metorchis conjunctus (North American liver fluke)

BLOOD

The schistosomes are acquired by penetration of the skin by the cercarial forms that are released from fresh-water snails. Although they are not endemic within the United States, occasionally patients are seen who may have these infections.

Current Name

Schistosoma mansoni
Schistosoma haematobium
Schistosoma japonicum
Schistosoma intercalatum
Schistosoma mekongi

ARTHROPODS

See Tables 220–1 and 220–2.

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SUBSECTION 1

Protozoa

A. Amebae

CHAPTER 221

AMEBIASIS

Shinjiro Hamano ✪ William A. Petri, Jr.

Diarrheal diseases continue to be major causes of morbidity and mortality in children in developing countries. In Bangladesh, 1 in 30 children dies of diarrhea or dysentery by age 5 years.[75] Amebiasis is an infection caused by the protozoan parasite *Entamoeba histolytica*. Infection occurs via ingestion of the parasite's cyst from fecally contaminated food, water, or hands. Approximately 50 million illnesses and 100,000 deaths occur annually from amebiasis, rendering it the third leading cause of death by parasitic disease in humans.[75] Long-term consequences of amebiasis in children include malnutrition and reduced cognitive

abilities.[69,93] Although amebiasis is present worldwide, it occurs most commonly in underdeveloped areas, especially Central and South America, Africa, and Asia. In the United States and other developed countries, cases of amebiasis are most likely to occur in immigrants from and travelers to endemic regions, but it can affect populations of the developed world, as shown by the epidemic that occurred in Tbilissi, Republic of Georgia, caused by contaminated municipal water.[13] Currently, there is no vaccine to prevent the childhood morbidity and mortality resulting from infection with *E. histolytica*.

ETIOLOGY

E. histolytica is named for the pathologic evidence of "lysis" of tissues. The first demonstration of the organism in human tissues was made by Lambl in 1859 in the postmortem examination of the colon of a child who died as a result of having excessive diarrhea.[16,71] No connection of the organism with the disease was made until 1875, when Losch, in St. Petersburg, Russia, found the organism at autopsy in the colon of a woodcutter. Losch induced diarrhea and ulcerations in a dog given feces from the patient.[59] He did not think, however, that a connection existed between the organism and the disease. The first patient described in the United States was a physician treated by Osler for an amebic liver abscess in 1890.[71] Councilman and Lafleur described the organism and the disease in 1891.[20,42] Further investigation of the disease was delayed until a better understanding of the life cycle of *E. histolytica* could be obtained.[26] In recent years, the application of modern molecular biology techniques to the study of *E. histolytica* and *Entamoeba dispar* has resulted in an explosion of information about the mechanisms of virulence, pathogenicity, and immune responses to these organisms.[85,87]

E. histolytica is the pathogenic species, having the capacity to invade tissue and cause symptomatic disease, whereas *E. dispar* (and *E. histolytica*) is associated with the asymptomatic carrier state.[6,85] More recently, a study revealed that all genotypes of *E. histolytica* are not equally capable of causing disease.[6] Morphologically distinct members of the genus *Entamoeba*, such as *Entamoeba coli* and *Entamoeba hartmanni*, also are nonpathogenic. *Dientamoeba fragilis* and *Entamoeba polecki* have been associated with diarrhea, and *Entamoeba gingivalis* has been associated with periodontal disease.

Members of the genus *Entamoeba*, which are protozoan organisms belonging to the subphylum Sarcodina and close to *Dictyostelium discoideum* on one of the lowest branches of the eukaryotic tree, have trophozoite and cyst forms.[37] The cysts of *E. histolytica* and *E. dispar* are almost spherical, being surrounded by a cell wall composed of chitin. The cysts may have one to four nuclei, although quadrinucleate cysts are most typical. This feature allows differentiation from *Escherichia coli*, which usually has 6 to 8 nuclei in the cysts and may have 32 nuclei.[72] Cysts of *E. histolytica* are 5 to 20 μm in diameter (average 12 μm) and have a greenish tint in the unstained condition.[60] Young cysts contain chromatoid bodies, which are composed of ribosome particles in crystalline arrays.[12] The cysts of *E. hartmanni* appear identical to those of *E. histolytica* except for being a smaller size (4 to 10 μm). *E. histolytica* cysts can survive for days in the dried state at 30° C or for months at 0° C to 4° C. They can be killed by temperatures greater than 50° C retained for 5 minutes.[42] They are completely resistant to the concentrations of chlorine used in water supplies, but may be killed with hyperchlorination or with iodine solutions.[60,72] They are filtered from water supplies that pass through a sand filtration phase. They resist acids well.

When these quadrinucleate cysts are ingested, they resist the acid pH of the stomach and ultimately excyst in the alkaline environment of the bowel. The process of excystation results in the release of four trophozoites that divide by binary fission to produce eight trophozoites. The usual trophozoites have a diameter of 25 μm (range 10 to 60 μm).[26,85] They have a single nucleus that is 3 to 5 μm in diameter and contains fine peripheral chromatin with a slightly eccentric karyosome. They have a granular endoplasm that typically contains vacuoles in which bacteria and debris can be seen. Some glycogen is present and can be stained with periodic acid–Schiff stain.

Although amebae were thought to lack organelles, such as mitochondria, endoplasmic reticulum, and Golgi apparatus, evidence to the contrary is coming to light. The existence of nuclear-encoded mitochondrial genes and a remnant mitochondrial organelle was reported more recently.[61,94] The presence of ingested erythrocytes is a characteristic feature of *E. histolytica*, but not *E. dispar*.[85] Movement is accomplished by extension of clear pseudopodia. Replication is by binary fission. These protozoa live in the colon of humans and other mammals. Trophozoites die quickly outside the body and are quite sensitive to acid—they generally are not considered to be infective.[31] When cooled (as when feces are expelled and gradually cooled from body temperature) or stimulated by as-yet-undefined luminal conditions, the trophozoites form cysts that can remain viable for weeks to months on excretion.[85]

Trophozoites of *E. coli* are 15 to 50 μm in diameter; have much more sluggish motility than the trophozoites of *E. histolytica*; and have blunt pseudopodia, rather than the sharp, finger-like pseudopodia of *E. histolytica*. Trophozoites of *E. hartmanni* are 4 to 14 μm in diameter and have much less glycogen than the trophozoites of *E. histolytica*.[26]

EPIDEMIOLOGY

Amebiasis is distributed throughout the world. The number of people infected with either *E. histolytica* or *E. dispar* per year is estimated to be 500 million. Although most individuals remain asymptomatic, perpetuating the natural cycle of the organism through fecal excretion of infective cysts, approximately 50 million people experience the severe morbidity associated with invasive disease, with an estimated 100,000 dying annually.[75,92] In the United States, 50 percent of amebiasis is observed in Hispanic/Asian/Pacific Islanders. Travelers from developing countries, men, and residents of institutions for the mentally retarded are considered to be at higher risk for amebiasis (Table 221–1).

During the 1990s, enough evidence had accumulated to support the formal separation of two morphologically identical species of ameba: the nonpathogenic *E. dispar* from the potentially pathogenic *E. histolytica*.[1,14,29,30,36,92] Morbidity and mortality data in absolute numbers that existed before this time pertaining to cases of invasive disease were not greatly affected by this reclassification because all invasive disease was known to be caused by *E. histolytica*.[92] Because most prevalence and incidence data previously collected pertained to asymptomatic individuals, however, and it was clear that most asymptomatic individuals with cysts detected in their stool were infected with nonpathogenic *E. dispar*; the true prevalence and incidence of *E. histolytica* became a matter of speculation.[92]

TABLE 221–1 Risk Factors for Amebiasis in the United States

Hispanic/Asian/Pacific Islanders—50% of U.S. cases reported to CDC
Travelers—0.3% incidence in one study
Institutions for mentally retarded
Men who have sex with men
Men—90% amebic liver abscesses in men, but rare in children

CDC, Centers for Disease Control and Prevention.

Estimates of *E. histolytica* infections have been based primarily on examinations of stool for cysts and parasites, but these tests are insensitive and cannot differentiate *E. histolytica* from morphologically identical species that are nonpathogenic, such as *E. dispar* and *Entamoeba moshkovskii*.[5,22] Specific and sensitive means to detect *E. histolytica* in stool are now available and include antigen detection and polymerase chain reaction (PCR).[34,41,56]

A prospective study of preschool children in a slum of Dhaka, Bangladesh, showed *E. histolytica*–associated diarrhea in 9 percent and *E. histolytica*–associated dysentery in 3 percent of the children annually.[39] Not all individuals are equally susceptible to amebiasis, with certain HLA-DR and HLA-DQ alleles associated with resistance to infection and disease.[23] The annual incidence of amebic liver abscess was reported to be 21 cases per 100,000 inhabitants in Hue City, Vietnam.[14] Carefully conducted serologic studies in Mexico, where amebiasis is endemic, showed antibody to *E. histolytica* in 8.4 percent of the population.[17] In the urban slum of Fortaleza, Brazil, 25 percent of all individuals tested carried antibody to *E. histolytica*; the prevalence of antiamebic antibodies in children 6 to 14 years old was 40 percent.[15]

PATHOGENESIS AND PATHOLOGY

The cysts are transported through the digestive tract to the intestine, where they release their mobile, disease-producing form, the trophozoite. *E. histolytica* trophozoites can live in the large intestine and form new cysts without causing disease. They also can invade the lining of the colon, killing host cells and causing diarrhea, amebic colitis, acute dysentery, or chronic diarrhea. The trophozoites also can be carried through the blood to other organs, most commonly the liver and occasionally the brain, where they form potentially life-threatening abscesses (Fig. 221–1). Important virulence factors include the trophozoite cell surface galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)–specific lectin that mediates adherence to colonic mucins and host cells,[74,86] cysteine proteinases that likely promote invasion by degrading extracellular matrix and serum components, and amoebapore pore-forming proteins involved in killing of bacteria and host cells.[57,88]

The interface of the Gal/GalNAc lectin with the host mucins lining the intestine is the defining moment of the infection.[19] If the parasite lectin attaches to the host mucin glycoproteins that line the intestinal lumen, a noninvasive gut infection ensues. The life cycle continues as the trophozoites reproduce by clonal expansion in the mucin layer. Subsequently, the Gal/GalNAc lectin, along with mucin glycoproteins or other gut bacteria, initiates the developmental pathway leading to encystment.[25,92]

Colitis is caused when the trophozoite penetrates the intestinal mucous layer, which otherwise acts as a barrier to invasion by inhibiting amebic adherence to the underlying epithelium and by slowing trophozoite motility.[19] Invasion is mediated by the killing of epithelial cells, neutrophils, and lymphocytes by trophozoites, which occurs only after the parasite lectin engages host GalNAc on O-linked cell surface oligosaccharides.[75] The interaction of the lectin with glycoconjugates is stereospecific and multivalent.[100] The identity of the high-affinity intestinal epithelial cell receptor is unknown. Secretion of amoebapore, a 5-kd pore-forming protein, by the ameba may contribute to killing.[55] Activation of human caspase 3, a distal effector molecule in the apoptotic pathway, occurs rapidly after amebic contact, and caspases are required for cell killing in vitro and for the formation of amebic liver abscesses in vivo.[45,99]

Interaction of the parasite with the intestinal epithelium causes an inflammatory response marked by the activation of nuclear factor κB and the secretion of cytokines.[24,89] The development of this epithelial response may depend on trophozoite virulence factors, such as cysteine proteinase, and leads to intestinal abnormalities through neutrophil-mediated damage. Neutrophils also can be protective, and activation of neutrophils or macrophages by tumor necrosis factor-α or interferon-γ kills amebae in vitro and limits the size of amebic liver abscesses.[7,21] In contrast to the intense inflammatory response, typical of early invasive amebiasis, inflammation surrounding well-established colonic ulcers and liver abscesses is minimal, given the degree of tissue damage.[16]

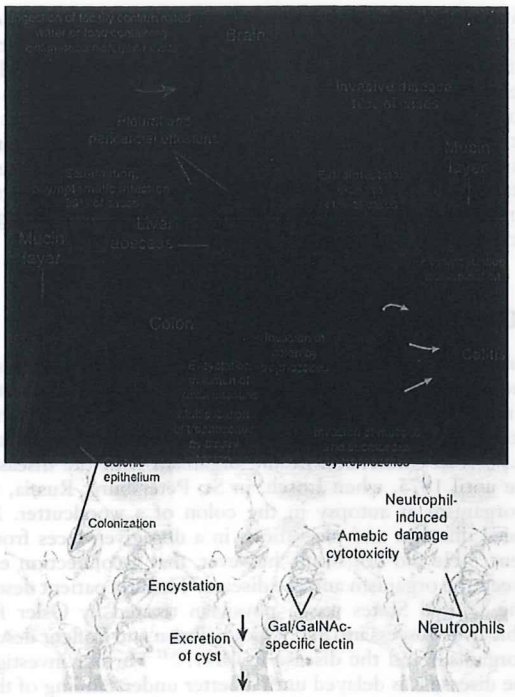

Figure 221–1 Life cycle of *Entamoeba histolytica*. Infection normally is initiated by the ingestion of fecally contaminated water or food containing *E. histolytica* cysts. The infective cyst form of the parasite survives passage through the stomach and small intestine. Excystation occurs in the bowel lumen, where motile and potentially invasive trophozoites are formed. In most infections, the trophozoites aggregate in the intestinal mucin layer and form new cysts, resulting in a self-limited and asymptomatic infection. In some cases, adherence to and lysis of the colonic epithelium, mediated by the galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)–specific lectin, initiates invasion of the colon by trophozoites. Neutrophils responding to the invasion contribute to cellular damage at the site of invasion. When the intestinal epithelium is invaded, extraintestinal spread to the peritoneum, liver, and other sites may follow. Factors controlling invasion, as opposed to encystation, most likely include parasite "quorum sensing" signaled by the Gal/GalNAc-specific lectin, interactions of amebae with the bacterial flora of the intestine, and innate and acquired immune responses of the host. (See companion Expert Consult web site for color version.) *(From Haque, R., Huston, C. D., Hughes, E., et al.: Amebiasis. N. Engl. J. Med. 348:1565-1573.)*

The initial lesions of clinical amebiasis often are small interglandular ulcers with a diameter of approximately 1 mm. They extend only to the muscularis mucosa.[16,64] The margins may be hyperemic, and slight edema of the surrounding mucosa is present. *E. histolytica* organisms seen in these ulcers stain well with periodic acid–Schiff stain.[76] Bleeding and friability are not prominent at this stage, although proctoscopic examination may find mucus coming from these ulcers, with an abundant number of amebae present.

The next stage of intestinal disease is the production of deeper ulcers. These "buttonhole" ulcers may be 1 cm in diameter and may extend into the submucosa.[16,76] The ulcer often extends laterally under normal-appearing mucosa, forming a characteristic flask shape. Occasional perforation through the serosa leads to peritonitis or pneumoperitoneum.[91] Extensive necrosis may be present, but usually only very little inflammation occurs. The edema is more intense, but the mucosa between ulcers is normal, in contrast to the marked inflammatory response seen in bacterial enteritis. When ulceration is more extensive, the edema surrounding the ulcers becomes confluent, and the mucosa appears gelatinous. In young children, this condition can progress to a fulminant necrotizing colitis associated with transmural necrosis. The pathologic events associated with this phenomenon are not understood. Rarely, an inflammatory response is present, resulting in granulation of the tissue with a fibrous outer wall.[72] It is given the name *ameboma*. Occasionally, an ameboma fills a significant portion of the lumen, which causes stricture or obstruction. Other complications of intestinal amebiasis result from direct extension of the ulcers. This extension may result in cutaneous involvement of the perianal area or lesions of the penis, vulva, vagina, or cervix.[2,72] Cutaneous and ophthalmologic amebiasis also is caused by fecal contamination of the face.[67]

Amebas disseminate to the liver in 50 percent of patients with fulminant amebiasis.[2,3] Dissemination to other organs directly from the intestine probably does not occur, but dissemination from the liver to lung, heart, brain, spleen, scapula, larynx, stomach, and aorta has been described.[16] Amebic abscess of the liver occurs more often in men than in women by a ratio of 16:1, but occurs equally often in prepubertal children of both sexes.[3,16] Abscesses occur more commonly in adults, but occur in children as young as 4 months of age.[70] These abscesses vary from microscopic lesions to massive necrosis of 90 percent of the liver. Fever, right upper quadrant pain, and the presence of serum antibodies to amebae point to hepatic amebic abscess.[84] Examination of the fluid from such an abscess frequently reveals a reddish, "anchovy paste" fluid that rarely may appear white or green. The fluid is acidic, with a pH ranging from 5.2 to 6.7.[82] Amebas are found in the walls of the abscess and only rarely in the fluid of the abscess. Many patients with amebic liver abscess also have anaerobic bacteria in the abscess fluid.[83] The walls are composed of a thin connective tissue capsule. The right lobe of the liver is involved with amebic liver abscess about six times as often as the left lobe. Abscesses in the right lobe can perforate and cause disease below the diaphragm or in the thoracic cavity. Abscesses in the left lobe can lead to pericardial effusions, which are less common than pleural effusions.[32,47]

Pleural effusions can remain loculated or lead to cutaneous fistulas or to bronchopleural fistulas. Drainage from these fistulas is acidic, in contrast to the neutral secretions in the normal lung. Seeding of the cardiac valves and of the brain has been described.[16] Cerebral abscesses have the same microscopic findings as do liver abscesses, with a thin capsule of connective tissue surrounding a fluid with little or no associated inflammatory response.

IMMUNITY

Protection from amebiasis, including acquired immunity to infection and invasion by *E. histolytica*, is associated with a mucosal IgA antibody response against the carbohydrate recognition domain of the parasite Gal/GalNAc lectin.[37,35,43,53] Cell-mediated immunity in protection from invasive amebiasis, but not infection per se, also has been shown. There is substantial evidence from in vitro, animal model, and most recently human studies of an important role for interferon-γ in protection from amebic colitis, acting in part by activating of macrophages to kill the parasite.[40,44] Invasive amebiasis rarely occurs in individuals with human immunodeficiency virus/acquired immunodeficiency syndrome, even in areas where amebiasis is common, suggesting an important role also exists for natural immunity or innate immune responses, or both, in protection from infection.[7,34]

CLINICAL MANIFESTATIONS

INTESTINAL AMEBIASIS

Asymptomatic Intraluminal Amebiasis

The most common type of amebic infestation is an asymptomatic cyst-passing carrier state. All *E. dispar* infections and 90 percent of *E. histolytica* infections are asymptomatic, manifesting with only *Entamoeba* cysts in the feces.[29,78] Some investigators have suggested that stools of these individuals generally are more liquid than stools of individuals without trophozoites.[26]

Entamoeba histolytica–Associated Diarrhea

Diarrhea is the most common manifestation of amebic disease, present in 9 percent of children in the Mirpur cohort each year, compared with only 3 percent of children having amebic colitis each year.[39] *E. histolytica*–associated diarrhea is defined as three or more unformed stools in a 24-hour period accompanied by a new episode of *E. histolytica* infection. This definition was validated previously in the cohort by (1) showing that diarrhea was approximately five times more common in the setting of a new infection (age-adjusted odds ratio for the association of new *E. histolytica* infection with diarrhea of 4.7; 95% confidence interval 2.9 to 7.6), and (2) showing by a complete bacteriologic, virologic, and parasitic work-up that only 32 percent of *E. histolytica*–associated diarrhea cases were co-infected with another pathogen compared with identification of an enteropathogen in 59 percent of all cases of diarrhea.[37]

Acute Amebic Colitis

Amebic dysentery was defined as a diarrheal stool sample containing occult or gross blood that was positive for *E. histolytica* antigen. Seventy percent of patients have a gradual onset of symptoms over 3 or 4 weeks after infestation, with increasingly severe diarrhea as the primary complaint, accompanied by general abdominal tenderness. Occasionally, the onset may be acute or may be delayed for several months after infestation. This onset differs from bacterial causes of dysentery, in which patients usually have only symptoms of 1 to 2 days' duration. The diarrhea is usually associated with pain in children. Pain may be of such severity that an acute abdomen is suspected.[2,10,48,76] The stools contain blood and mucus in virtually all cases.[2,76,77] Fever is present in only a few patients with amebic colitis. Abdominal distention and dehydration occur in less than 10 percent of patients. In young children, intussusception, perforation, peritonitis, or necrotizing colitis may develop rapidly.[10,48,91]

Ameboma

Unusual manifestations of amebic colitis include toxic megacolon (0.5% of cases, usually requires surgical intervention), ameboma (granulation tissue in colonic lumen mimicking colonic cancer in appearance), and a chronic nondysenteric form of infection that can manifest as years of waxing and waning diarrhea, abdominal pain, and weight loss (easily misdiagnosed as inflammatory bowel disease).

EXTRAINTESTINAL AMEBIASIS

Amebic Liver Abscess

The typical patient with an amebic liver abscess in the United States is an immigrant, usually a Hispanic/Asian/Pacific Islander; male; 20 to 40 years old; who presents with fever, right upper quadrant pain, leukocytosis, abnormal serum transaminases and alkaline phosphatase, and a defect on hepatic imaging study. Roughly 90 percent of patients with liver abscess are men. The abscess usually is single and is in the right lobe of the liver 80 percent of the time.[49] Most frequently, patients present with liver abscess without concurrent colitis. Amebae are seen infrequently in the stool at the time of diagnosis of liver abscess.[3] Liver abscess can manifest acutely with fever and right upper abdominal tenderness and pain, or subacutely, with prominent weight loss and less frequent fever and abdominal pain. The peripheral white blood cell count is elevated, as is the alkaline phosphatase level, in many patients.

Early evaluation of the hepatobiliary system with ultrasound or computed tomography (CT) is essential to show the abscess in the liver. The differential diagnosis of the lesion in the liver includes pyogenic abscess, hepatoma, and echinococcal cyst. Aspiration of the abscess occasionally is required to diagnose amebiasis (although amebae are visualized in the pus in only a few cases; if the abscess is pyogenic, the responsible bacteria are seen or cultured). Antibodies to *E. histolytica* are present in the serum of 92 to 97 percent of patients on acute presentation with amebic liver abscess and are very useful diagnostically. Unusual extraintestinal manifestations of amebiasis include direct extension of the liver abscess to pleura or pericardium and brain abscess. In a patient who presents with right upper quadrant pain, ultrasound, CT, or magnetic resonance imaging (MRI) should be performed to examine the liver and gallbladder.

If a space-filling defect in the liver is observed, the differential diagnosis includes (1) amebiasis (most common in men with a history of travel or residence in a developing country); (2) pyogenic or bacterial abscess (suspect in women, patients with cholecystitis, elderly individuals, individuals with diabetes, and patients presenting with jaundice); (3) echinococcal abscess (an incidental finding because echinococcal abscess should not cause pain or fever); and (4) cancer. Most patients with amebic liver abscess have detectable circulating antigen in serum and serum anti-amebic antibodies.[34]

In children, abdominal pain is reported infrequently with amebic liver abscess.[33,68] More commonly, high fever, abdominal distention, irritability, and tachypnea are noted. Some children are admitted to the hospital with a fever of unknown origin. Hepatomegaly occurs frequently, but elicitation of hepatic tenderness is not well documented. In one report, four of five children younger than 5 years died with amebic liver abscesses because the diagnosis was not suspected.[54] Death usually results from rupture of the liver abscess into the peritoneum, thorax, or pericardium, but may follow extensive hepatic damage and liver failure.[3,81]

Metastatic Amebiasis

Extra-abdominal amebiasis presumably follows direct extension from liver abscesses, rather than direct dissemination from the intestine.[3,16] Thoracic amebiasis is the most common type of extra-abdominal amebiasis and occurs in approximately 10 percent of patients with amebic liver abscess.[16,47] Symptoms depend on the type of involvement. Empyema, bronchohepatic fistulas, or extension of a pleuropulmonary abscess into the pericardium may occur.

Pericardial amebiasis is the next most common form of extraintestinal involvement and may result from rupture of a liver abscess in the left lobe of the liver into the pericardium or through extension of the right-sided pleural amebiasis.[16,27,28,32] It is estimated to occur in 3 percent of patients with hepatic abscesses.[28] It manifests as acute pericarditis with tamponade and, occasionally, as pneumopericardium.[27] Amebic liver abscess in the left lobe also may rupture directly into the left chest.[63]

Cerebral amebic abscesses were found in 8 percent of patients with amebic infections discovered at autopsy in one study.[58] In other studies, lower rates of 0.66 to 4.7 percent of patients with amebic liver abscess having brain abscesses were reported.[46] Patients with cerebral amebiasis frequently are so ill from the intestinal, liver, and possibly lung involvement that neurologic signs are not always assessed easily. In 18 patients with proven cerebral amebiasis, initial neurologic examination was normal in 13, and only 1 patient later developed seizures.

Other foci of infection are rare findings, but amebic rectovesical fistula formation and involvement of pharynx, heart, aorta, and scapula have been reported. Cutaneous extension after the adherence of perforated, inflamed bowel to the skin is an extremely painful and rare complication.[16,72] This situation also may arise after invasion of the skin by trophozoites emerging out from the rectum occurs.

DIAGNOSIS

A heightened suspicion of amebiasis should be present if the patient has been in a developing country as a resident or traveler. The diagnosis of amebiasis should be considered in any child who is passing diarrhea, bloody stools, or stools with mucus; any child with a hepatic abscess; and any febrile child with right upper quadrant pain, abdominal distention, or tachypnea.[54,68] In a patient with diarrhea, if blood is present in the stool (grossly bloody or occult blood positive), infectious (Shiga toxin–producing *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*; and *E. histolytica*) and noninfectious (inflammatory bowel disease, diverticulosis, arteriovenous malformations, cancer) causes should be considered.

IMMUNOLOGIC OR MOLECULAR EXAMINATION OF STOOL OR SERA

E. histolytica infection was defined as a positive test for antigen in stool. Antigen was detected using the TechLab (Blacksburg, VA) *E. histolytica* II stool antigen detection test, which specifically detects *E. histolytica* and does not cross-react with *E. dispar* or *E. moshkovskii*. The antigen test is 95 percent sensitive and specific compared with the "gold standard" of *E. histolytica* culture and zymodeme determination. It also is 80 percent sensitive compared with real-time PCR for *E. histolytica* DNA in stool. Although real-time PCR is excellent in sensitivity and specificity, real-time PCR still is not practical for the measurement of infection in the thousands of stool samples analyzed in this prospective study, so we usually use the less sensitive but highly specific antigen detection test, recognizing that in doing so we may underestimate the incidence of amebiasis.[41,56] In addition, immunohistochemical staining of amebae is useful in a difficult-to-diagnose case. Serologic tests for anti-amebic antibodies also are a very useful tool in diagnosis, with sensitivity of 70 to 80 percent early in disease and approaching 100 percent sensitivity on convalescence. The combined use of serology and stool antigen detection test offers the best diagnostic approach.

MICROSCOPIC EXAMINATION OF STOOL

Before the development of new antigen detection and PCR tests, amebiasis was diagnosed by examining a stool sample through a microscope to determine whether *E. histolytica* cysts were present. This method often requires more than one specimen, however, because the number of cysts in the stool varies greatly. In addition, stool microscopy has limited sensitivity and specificity. The body's own immune system produces macrophage cells that can look like the amebae. Three different amebae—*E. histolytica*, which causes amebiasis, and *E. dispar* and *E. moshkovskii*, which do not cause disease—look identical under a microscope.[22]

NONINVASIVE DIAGNOSIS OF EXTRAINTESTINAL AMEBIASIS

Amebiasis outside the intestine has been even more difficult to diagnose. Clinical manifestations of extraintestinal disease vary widely, and less than 10 percent of individuals with amebic liver abscesses have identifiable *E. histolytica* in their stools. The TechLab *E. histolytica* II test, which differentiates the true pathogen *E. histolytica* from *E. dispar*, was reported to detect Gal/GalNAc lectin in the sera of 22 of 23 (96%) patients with amebic liver abscess tested before treatment with the anti-amebic drug metronidazole and 0 of 70 (0%) controls. After 1 week of treatment with metronidazole, more than 80 percent of patients became serum lectin antigen–negative. Detection of *E. histolytica* Gal/GalNAc lectin in the sera using the TechLab *E. histolytica* II kit is sensitive to diagnose hepatic and intestinal amebiasis before the institution of metronidazole treatment.[38]

Noninvasive diagnostic procedures such as ultrasound, CT, and MRI can detect extracolonic amebiasis in the liver, paracecal masses, brain, and other sites, but they cannot distinguish between abscesses caused by amebae and those caused by bacteria, hampering proper treatment of the condition. Most patients with amebic liver abscess have a single abscess in the right lobe of the liver, although multiple lesions also can occur.[4] Chest radiographs show elevation of the right diaphragm in 56 percent of patients with hepatic abscess.[3] The diagnosis of cerebral amebiasis requires careful neurologic evaluation and radiographic evaluation with either CT or MRI.[16,46,58] Because of the risk for perforation, barium studies are relatively contraindicated in patients with amebic colitis.

BIOPSY STUDIES

The colonic and rectal mucosa in amebic colitis usually reveals ulcerations with a diameter of 1 to 10 mm. Amebic trophozoites often are at the periphery of these necrotic areas, which can be sampled through a biopsy specimen taken during sigmoidoscopy or colonoscopy.[42,49] Because of the potential for perforation, colonoscopy should be undertaken with caution.

In patients with amebic liver abscesses, amebic trophozoites are found near the capsule of the abscess. Until more recently, the most accurate diagnostic test involved the examination of a sample collected from the abscess tissue by needle aspiration, a procedure that is painful, potentially dangerous, and relatively insensitive, identifying amebic trophozoites only 20 percent of the time.

DIFFERENTIAL DIAGNOSIS

Invasive amebic colitis may resemble ulcerative colitis, Crohn disease of the colon (inflammatory bowel disease), bacillary dysentery, or tuberculous colitis.[11,18,42,89] Stool examinations, colonoscopic examination with biopsies, and serologic examination should be able to differentiate amebic colitis from these diseases. Histologic examination of involved colonic mucosa should differentiate amebic colitis, with its lack of inflammation and rare granulation tissue, from the inflammatory responses seen in ulcerative colitis, bacillary dysentery, and Crohn disease of the colon. Tuberculous colitis and Crohn disease are more likely to show granuloma formation than amebiasis. Ileocecal or small bowel involvement as seen on barium studies would suggest Crohn disease or tuberculosis of the gastrointestinal tract, rather than amebiasis. Tuberculous colitis usually is associated with pulmonary tuberculosis and with a strong reaction to tuberculin skin testing. In some cases, differentiating between invasive amebic colitis and inflammatory bowel disease may be impossible. If a patient with this differential diagnosis is placed on corticosteroids and deteriorates, the corticosteroids should be stopped, and repeat investigation for amebiasis should be performed.[18,68,72]

Amebic liver abscess must be differentiated from pyogenic abscesses and neoplastic lesions. Detection of *E. histolytica* Gal/GalNAc lectin in the sera using the TechLab *E. histolytica* II kit is quite helpful to diagnose hepatic and intestinal amebiasis before the institution of metronidazole treatment.[38] Total leukocyte counts and cultures of blood may help to differentiate pyogenic and amebic abscesses. Many children with pyogenic liver abscesses have negative blood cultures, however. Often, amebic and pyogenic liver abscesses show similar features on CT and MRI. Occasionally, nuclear imaging with gallium is helpful because, in contrast to a pyogenic abscess, very few neutrophils are contained within an amebic liver abscess.[85,87] Gallium scanning of an amebic liver abscess may reveal a cold spot, possibly with a bright rim. Several investigators recommend a trial using an appropriate drug for amebic abscess for 3 or 4 days while serologic and culture results are awaited.[68,98] Patients with amebic liver abscess should respond to treatment in this length of time by becoming afebrile. No change in size of the liver or size of the abscess should be noted at this time because resolution of the abscess usually takes 2 months to several years.[4,79,80,90,97]

COMPLICATIONS

Complications of amebiasis may be prevented by early establishment of diagnosis and initiation of treatment with appropriate agents.[46,68] When complications occur, the prognosis generally is poor.

Invasive intestinal amebiasis has been associated most commonly with perforation and peritonitis,[8,10,48,68,91,96] which apparently are an end result of "necrotizing" or "toxic" amebic colitis. In children, perforation may be heralded by the appearance of an acute abdomen or pneumoperitoneum, with rapid progression to death, presumably from sepsis.[8,68,96] Surgical resection and therapy for endotoxic shock improve the prognosis.[96] This complication is not rare and accounts for more than 30 percent of deaths from amebiasis in children.[11,50] Massive intestinal hemorrhage causes approximately 3 percent of deaths from amebiasis. Intussusception occasionally occurs and can be reduced with gentle barium enema. Multiple colonic strictures also can occur and cause obstructive symptoms. Fistulas to other organs or to the skin may develop.

Liver abscesses and their resultant complications account for approximately 40 percent of deaths from amebiasis.[50] Liver abscess also was found in 13 percent of patients with amebiasis who had postmortem examinations. Liver abscess with rupture into the abdomen was present in 8 percent of patients who died with amebiasis, and rupture of a liver abscess into the right pleural space was found in 12 percent.[50] Many patients with amebic liver abscess also have anaerobic bacteria in the abscess fluid.[83] In cases free of bacterial contamination, the fluid has few

inflammatory cells and an acidic pH. Amebic pericarditis or pneumopericardium occurs rarely and is found in only 1 percent of patients whose deaths were caused by amebiasis.[27,28,32,50] The fluid is similar to that found in the pleural space. A cerebral abscess was found in 4 percent of patients with amebiasis who died.[50] It has been reported in fewer than 10 children, only one of whom survived.[9,16,46,58] Other complications include infections of the retroperitoneal space, stomach, spleen, esophagus, and duodenum.[58]

TREATMENT

INTESTINAL AMEBIASIS

Asymptomatic Intraluminal Amebiasis

Therapy for asymptomatic and noninvasive infection differs from therapy for invasive infection. Asymptomatic infections may be treated with intraluminal agents, such as paromomycin or diloxanide furoate. Each agent has a high rate of success for eradication of cyst passage.[65,66] Paromomycin is a nonabsorbable aminoglycoside that is active against the cyst and trophozoite stages. High cure rates have been reported with a 7-day oral dose of paromomycin at 25 to 35 mg/kg/day in three divided doses (Table 221–2). Diloxanide furoate (Furamide) is a poorly absorbed agent that is quite active against only intraluminal amebiasis, but treats symptomatic and asymptomatic disease.[62,98] Cure rates have been greater than 90 percent with a 10-day oral course of diloxanide furoate at 20 mg/kg/day in three divided doses (maximum dose of 1500 mg/day).[65,66,73]

Acute Amebic Colitis

Nitroimidazoles, particularly metronidazole, are the mainstay of therapy for invasive amebiasis.[37] The oral dosage of metronidazole is 35 to 50 mg/kg/day (to a maximum of 2250 mg/day) in three divided doses for 7 to 10 days for severe intestinal or extraintestinal amebiasis. Metronidazole is concentrated in the ameba, probably via reduction of its nitro group by ferredoxin or flavodoxin-like electron transport proteins, which maintain a gradient for the entry of the unchanged drug. Metabolic intermediates of metronidazole damage DNA and possibly other macromolecules, and they deprive the organism of reducing equivalents by acting as an electron sink. Nitroimidazoles with longer half-lives (tinidazole, secnidazole, and ornidazole) are better tolerated and allow shorter periods of treatment.[38] The oral dosage of tinidazole is 60 mg/kg/day (to a maximum of 2000 mg/day) for 5 days for severe intestinal or extraintestinal amebiasis (see Table 221–2) (see http://www.medletter.com/).

Approximately 90 percent of patients who present with mild-to-moderate amebic dysentery have a response to nitroimidazole therapy. In the rare case of fulminant amebic colitis, adding broad-spectrum antibiotics to treat intestinal bacteria that may spill into the peritoneum is prudent; surgical intervention occasionally is required for acute abdomen, gastrointestinal bleeding, or toxic megacolon.[37] Agents such as metronidazole that are active against invasive and extraintestinal amebiasis are well absorbed and do not stay in the lumen long enough to have an effect on intestinal amebiasis. Parasites persist in the intestine in 40 to 60 percent of patients who receive nitroimidazole. Nitroimidazole treatment should be followed with paromomycin or the second-line agent diloxanide furoate to cure luminal infection.[38] Metronidazole and paromomycin should not be given at the same time because the diarrhea that is a common side effect of paromomycin may render assessing the patient's response to therapy difficult.[50-52]

EXTRAINTESTINAL AMEBIASIS

Amebic Liver Abscess and Metastatic Amebiasis

Extraintestinal and severe intestinal amebiasis must be treated with the tissue-active agents. Metronidazole (35 to 50 mg/kg/day in three divided doses for 7 to 10 days) is the preferred drug because it is effective and relatively free of serious side effects (see Table 221–2).[2,3,62,85,87,98] It is effective for extraintestinal amebiasis in any location, although amebic brain abscesses usually are not treated successfully with any medications. Most patients with amebic liver abscess respond to metronidazole within 72 hours. For amebic colitis, follow-up therapy with a luminal agent is very important because of the high rates of asymptomatic intestinal colonization in patients with amebic liver abscess.

Therapeutic aspiration of an amebic liver abscess occasionally is required as an adjunct to antiparasitic therapy. Drainage of the abscess should be considered in patients who have no clinical response to drug therapy within 5 to 7 days or patients with a high risk of experiencing rupture of the abscess, as defined by a cavity with a diameter of more than 5 cm or by the presence of lesions in the left lobe.[95] Because many patients with amebic liver abscess also have anaerobic bacteria in the abscess fluid,[83] addition of antibiotics, drainage, or both to the treatment regimen in the absence of a prompt response to nitroimidazole therapy is reasonable. Imaging-guided percutaneous treatment (needle aspiration or catheter drainage) has replaced surgical intervention as the procedure of choice for reducing the size of an abscess.[95]

PROGNOSIS

Invasive disease develops in 50 million people each year, and 50,000 to 100,000 deaths per year are caused by the invasive disease.[78,84,85] The case-fatality ratio is between 1 in 500 and 1 in 1000 diagnosed cases. Among patients with illness severe enough to require hospitalization, the case-fatality ratio is higher. One small study in children reported a 9 percent mortality rate and a 27 percent morbidity rate.[68]

Bowel necrosis or perforation is the cause of death from purely intestinal amebiasis, and early surgical intervention can reduce the mortality rate of these complications from 100 to 28 percent.[96] Amebic liver abscess has a case-fatality rate of 10 to 15 percent in combined figures of adults and children.[54,70,81] The mortality rate when pleural involvement is noted is 14 percent.[47,54] Amebic pericarditis has a case-fatality rate of 40 percent.[32] Cerebral amebiasis has a case-fatality rate of 96 percent.

TABLE 221–2 Pediatric Dosage of Drugs for Amebiasis

Symptomatic	Paromomycin	25-35 mg/kg/day in 3 doses × 7 days
Mild-to-moderate intestinal disease	Metronidazole	35-50 mg/kg/day in 3 doses × 7-10 days
	Trinidazole	50 mg/kg (maximum 2000 mg) qd × 3 days
Severe intestinal and extraintestinal disease	Metronidazole	35-50 mg/kg/day in 3 doses × 7-10 days
	Trinidazole	60 mg/kg/day (maximum 2000 mg) × 5 days

FUTURE

In a perfect world, amebiasis would be prevented by eradicating fecal contamination of food and water. Providing safe food and water for all children in developing countries would require massive societal changes and monetary investments. An effective vaccine would be much less costly, and for several reasons, a vaccine is a desirable and feasible goal. The high incidence of amebiasis in more recent community-based studies suggests that an effective vaccine would improve child health in developing countries.

The fact that humans naturally acquire partial immunity against intestinal infection indicates that barriers to stimulating an effective acquired immune response should not be insurmountable. Aiding vaccine design is the demonstration that several recombinant antigens, including the Gal/GalNAc-specific lectin, provide protection in animal models of amebiasis, and that human immunity is linked to intestinal IgA against the lectin.[35,37,43] The high degree of sequence conservation of the Gal/GalNAc-specific lectin suggests that a vaccine could be broadly protective. Finally, the absence of epidemiologically significant animal reservoirs suggests that herd immunity could interrupt fecal-oral transmission in humans. The challenges will be to design vaccines capable of eliciting durable mucosal immunity, to understand the correlates of acquired immunity, and, most important, to enlist the continued support of industrialized nations to combat diarrheal diseases of children in developing countries.

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CHAPTER 222

BLASTOCYSTIS HOMINIS INFECTION

Peter J. Hotez

Blastocystis hominis is one of the most common gastrointestinal protozoa of humans, with a worldwide prevalence that may be greater than 50 percent.[26,27] Other *Blastocystis* spp. have been described from numerous other vertebrates and insects.[27] Although new information about the molecular and cell biology of this organism has been acquired in recent years, considerable controversy regarding its true taxonomy and life cycle remains.[4,26,27,29,30] The pathogenicity of *B. hominis* and its ability to cause gastrointestinal illness in humans are equally controversial.[10,11,13,16,24]

Topics

感染性角膜炎サーベイランス（眼感染症学会）

鳥取大学医学部視覚病態学 井上幸次

■感染性角膜炎サーベイランスの重要性

日本眼感染症学会による感染性角膜炎サーベイランスは角膜感染症診療の中心となっている全国24施設を対象として行われ，その結果が二つの論文として日本眼科学会雑誌に掲載されている[1,2]．施行後，5年を経過しているが，その後，同じく日本眼科学会雑誌に掲載された「感染性角膜炎診療ガイドライン」[3]や，現在全国224施設において行われている「コンタクトレンズ関連角膜感染症全国調査」の礎になるものとして重要な位置を占めている．

■感染性角膜炎サーベイランスのポイント

サーベイランスの結果の要約は以下の通りである．

① 計261例が集積．内訳は男性128例，女性133例，両眼例が11例含まれている．

② 角膜病巣よりの分離菌は113例で陽性であり，全133株の内訳はグラム陽性球菌63株，グラム陰性桿菌42株，グラム陽性桿菌10株，嫌気性菌4株，真菌12株（うち酵母菌9株，糸状菌3株），アカントアメーバ2株であった．

③ 年齢分布は20歳代と60歳代にピークを有する2峰性を示し，20歳代のピークの方が高かった．また，一方のピークである20歳代のコンタクトレンズ（CL）使用率が89.8%であり，10歳代のCL使用率はそれよりさらに高く94.1%であった．CLが，かつて60歳代にピークを一つ認める一峰性であった感染性角膜炎の年齢分布を，2峰性にしていると考えられた．

④ 特にグラム陰性桿菌と真菌・アメーバについてはCL装用者の割合が高かった．また，従来型のsoft CL（SCL），frequent replacement SCLではグラム陰性桿菌が多いのに比べて，disposable SCL，治療用SCLではグラム陽性球菌が多かった．また，CL装用者中44.0%で誤使用が認められた．

⑤ 糖尿病・アトピー性皮膚炎合併例では起炎菌として黄色ブドウ球菌が多かった．

⑥ 角膜移植後では起炎菌としてグラム陽性球菌と真菌（特にカンジダ）が多かった．

⑦ CL以外の感染誘因として外傷が17.2%，ステロイド点眼薬使用が15.7%に認められた．

⑧ 前医にて治療のなされていた例では菌の検出率が低かった．

⑨ 治療薬として特にレボフロキサシンとセフメノキシムがよく使用されており，特に両者併用が多かった．一方，アミノグリコシド系点眼薬の使用率は低かった．

⑩ 抗真菌薬使用例で真菌・アメーバの感染と考えられる例は半数に満たなかった．

⑪ 治療に日数を要する症例ほど，菌検出率は高く，また，真菌・アメーバの割合が高くなる傾向を認めた．

⑫ 分離菌に関してminimum inhibitory concentration（MIC）と市販抗菌点眼薬による4分間接触後のpostantibiotic bactericidal effect（PABE）の測定を行った．MICとPABEの間に相関はなく，アミノグリコシド系で優れたPABEが確認された[2]．

■感染性角膜炎サーベイランスが示したもの，示せなかったもの

このサーベイランスは現在の感染性角膜炎診療の現状を把握するうえで重要で，特にCL関連角膜感染症の増加と低年齢化を明確に示した点が重要である．ただ，その後にアカントアメーバ角膜炎症例が全国的に急激に増加して，そのため，特に重症角膜感染症の様相に大きな変化が生じている．その辺の動向は後述（356頁）の「コンタクトレンズ関連角膜感染症全国調査」で今後明らかにされていくであろう．

文献

1）感染性角膜炎全国サーベイランス・スタディグループ：感染性角膜炎全国サーベイランス―分離菌・患者背景・治療の現況―．日眼会誌 110：961-972, 2006

2）砂田淳子ほか：感染性角膜炎全国サーベイランス分離菌における薬剤感受性と市販点眼薬のpostantibiotic effectの比較．日眼会誌 110：973-983, 2006

3）井上幸次ほか：感染性角膜炎診療ガイドライン．日眼会誌 111：769-809, 2007

Topics

CL関連角膜感染症全国調査

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■目的

日本コンタクトレンズ(CL)学会と日本眼感染症学会はCL関連角膜感染症の実態を全国規模で明らかにするため「CL関連角膜感染症全国調査」を実施している．今回のデータを元に国民を啓発し，CL診療の重要性をアピールすることを目標とする．

■調査方法

平成19年2月14日付けで，すべての日本眼科学会専門医制度認定施設に対して調査協力の可否について問い合わせを行い，各施設の担当者に症例入力画面(調査内容と患者用アンケート)を送付した．回答方法はホームページに書き込みとした．

■対象

CL装用が原因と考えられる角膜感染症で入院治療をした症例．検討期間は平成19年4月から平成20年8月中旬であった．年齢は9歳から90歳(平均28歳)で女性104例，男性129例であった．

■結果

初診時視力を表1に示した．

塗抹検鏡，分離培養と3ヵ月後の矯正視力を表2～4に示した．

■おわりに

初診時視力は重症例が多く，微生物学検査では緑膿菌やアカントアメーバが多く，検出部位は角膜病巣部とCLケースが多かった．患者のアンケート結果ではCLの取扱，定期検査に関する意識は非常に低いことが判明した．

CLは医師の定期検査が必要な医療機器であり，危険を伴うものであることの益々の啓発が必要であると考えられる．

[表1] 初診時視力(0.1未満の症例は47%であった)

sl～mm	43(18%)	47%
nd～0.03	51(22%)	
0.04～0.06	9(4%)	
0.07～0.09	6(3%)	
0.1～0.3	35(15%)	
0.4～0.6	27(12%)	
0.7以上	40(17%)	
記載なし	22(9%)	

[表2] 3ヵ月後の矯正視力

1.0以上	85(36%)
0.7～0.9	37(16%)
0.4～0.6	30(13%)
0.1～0.3	16(7%)
0.07～0.09	26(11%)
0.04～0.06	4(2%)
nd～0.03	12(5%)
sl～mm	3(1%)
0	1(0.4%)

[表3] 塗抹検鏡(特に多く，注意を要するものを青字で示した)

菌種	G+球菌 24	G+桿菌 16	G−球菌 9	G−桿菌 44	糸状菌 3	アカントアメーバ 44
角膜病巣	14	13	4	25	1	40
結膜嚢	2	1	0	1	0	0
眼脂	1	0	0	4	0	0
CL	2	0	1	3	0	5
CLケース	8	6	4	22	2	7
その他	0	0	0	0	0	0

(181/233, 78%)

[表4] 主要な菌のまとめ(特に多く，注意を要するものを青字で示した)

菌種	黄色ブ菌 7	表皮ブ菌 11	コリネバクテリウム 13	緑膿菌 58	セラチア 18	その他のG−桿菌 24	アスペルギルス 1	アカントアメーバ 35
角膜病巣	3	4	6	47	3	4	0	32
結膜嚢	1	2	4	1	1	0	0	0
眼脂	0	1	1	7	1	0	0	0
CL	2	2	1	8	2	6	0	0
CLケース	1	2	4	26	12	21	1	17
その他	0	1	0	2	0	0	0	1

分離培養(218/233, 94%)，検出率(144/218, 66%)

Molecular & Biochemical Parasitology 163 (2009) 39–47

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Isoform-dependent feedback regulation of serine *O*-acetyltransferase isoenzymes involved in L-cysteine biosynthesis of *Entamoeba histolytica*☆

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ARTICLE INFO

Article history:
Received 24 April 2008
Received in revised form 28 August 2008
Accepted 12 September 2008
Available online 23 September 2008

Keywords:
Cysteine biosynthesis
Sulfur assimilation
Sulfur-containing amino acids
Feedback inhibition
E. histolytica
Amoebiasis

ABSTRACT

Serine acetyltransferase (SAT; EC 2.3.1.30) catalyzes the CoA-dependent acetylation of the side chain hydroxyl group of L-serine to form *O*-acetyl serine, in the first step of the L-cysteine biosynthetic pathway. Since this pathway is selectively present in a few parasitic protists and absent in mammals, it represents a reasonable target to develop new chemotherapeutics. *Entamoeba histolytica* apparently possesses three SAT isotypes (EhSAT1-3) showing 48–73% mutual identity, a calculated molecular mass of 34.4–37.7 kDa, and an isoelectric point of 5.70–6.63. To better understand the role of individual SAT isotypes, we determined kinetic and inhibitory parameters of recombinant SAT isotypes. While the three SAT isotypes showed comparable K_m and k_{cat} for L-serine and acetyl-CoA, they showed remarkable differences in their sensitivity to inhibition by L-cysteine. The K_i values for L-cysteine varied by 100-fold (4.7–460 μM) among SAT isotypes (EhSAT1 < EhSAT2 < EhSAT3). Consequently, these EhSAT isotypes revealed remarkable differences in activity in the presence of physiological L-serine and L-cysteine concentrations. We propose that multiple SAT isotypes with different properties may play complementary roles in the regulation of the cysteine biosynthetic pathway in *E. histolytica* under different conditions, e.g. during colonization of the intestine and tissue invasion.

1. Introduction

The L-cysteine biosynthetic pathway plays a key role in the sulfur assimilatory cycle in nature. Inorganic sulfur is incorporated from the extracellular milieu or the soil by microorganisms or plants, respectively, reduced, and fixed into L-cysteine, the first reduced sulfur-containing organic compound [1]. L-Cysteine is used as a sulfur donor for synthesis of methionine and sulfur-containing secondary metabolites, or, alternatively, incorporated into proteins, glutathione, and iron–sulfur clusters. The cysteine biosynthetic pathway consists of several enzymatic reactions [1–4]. In the first committed reaction of the two last steps, serine acetyltransferase (SAT, EC 2.3.1.30) catalyzes the CoA-dependent acetylation of the side chain hydroxyl group of L-serine to form *O*-acetylserine (OAS) [5]. Cysteine synthase [CS; OAS (thiol) lyase; EC 4.2.99.8] subsequently catalyzes β-replacement of the acetyl moiety on OAS with sulfide to form L-cysteine. In plants, these two enzymes form a heteromeric complex ("cysteine synthase complex"), and play a key role in cross-talk, via the generation of OAS, between sulfur assimilation and carbon and nitrogen metabolism [6]. L-Cysteine potentially inhibits its own synthesis by negative feedback of SAT. In plants, the nature of SATs varies in cellular compartmentalization and sensitivity to L-cysteine inhibition [7,8]. For instance, cytosolic SAT from *Citrullus vulgaris* (watermelon) [7], and *Arabidopsis thaliana* (SAT-c) [8,9] are highly sensitive to feedback-inhibition by L-cysteine at the physiological concentrations (3 μM). In contrast, the plastid SAT (SAT-p) [10,11], and mitochondrial SAT (SAT-m) [12–14] isoforms from *A. thaliana* are insensitive to L-cysteine inhibition [8].

Other than bacteria and plants, where cysteine biosynthesis has been well conserved, only a limited lineages of parasitic protists such as *Entamoeba histolytica*, *Trichomonas vaginalis*, and

Abbreviations: SAT, serine *O*-acetyltransferase; EhSAT, *Entamoeba histolytica* serine *O*-acetyltransferase; IPTG, isopropyl β-D-thio galactopyranoside; E64, trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane; PCR, polymerase chain reaction.

☆ *Note:* The nucleotide sequence data of *E. histolytica* SAT1, SAT2 and SAT3 reported in this paper has been submitted to the DDBJ data bank with accession numbers, AB023954, AB232374, and AB232375, respectively.

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0166-6851/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbiopara.2008.09.006