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Mouse models of amoebiasis and culture methods of amoeba

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ABSTRACT

Entamoeba histolytica is the third leading parasitic cause of man mortality in the world. Infection occurs *via* ingestion of food or water contaminated with cysts of *E. histolytica*. Amoebae primarily colonize the intestine. The majority of amoebic infections are asymptomatic, but under some conditions, approximately 4–10% of infections progress to the invasive form of the disease. To better understand the pathogenesis of amoebiasis and the interaction between amoebae and their hosts, the development of suitable animal models is crucial. Pigs, gerbils, cats and mice are used as animal models for the study of amoebiasis in the laboratory. Among these, the most commonly used model is the mouse. In addition to intestinal amoebiasis, we developed a mouse model of liver abscess by inoculating amoeba through portal vein. However, the frequency of successful infection remains low, which is dependent on the conditions of amoebae in the laboratory. As the maintenance of virulent amoebae in the laboratory is unstable, it needs further refinement. This review summarizes mouse models of amoebiasis and the current state of laboratory culture method of amoebae.

1. Introduction

Entamoeba histolytica is the third leading parasitic cause of man mortality in the world [1–3]. Annually, more than 50 million cases of diseases and 40,000–100,000 deaths related to amoebiasis are reported [4–8]. Amoebic infections are mostly reported among children in developing countries [9–13], but an increasing number of amoebic cases are also observed among adults who have sex with men [14–20]. Infection occurs *via* ingestion of food or water contaminated with cysts of *E. histolytica* as a result of poor hygiene and sanitation. Other unusual modes of transmission include oral and anal sex, usage of contaminated enema apparatus, *etc.* [21,22]. Excystation that produces trophozoites occurs in the intestinal lumen [23]. Amoebae primarily colonize the intestine. The majority of amoebic infections are asymptomatic, and under some conditions, approximately 4–10% of infections progress to the intestinal amoebiasis, an invasive form of the disease [24,25]. The most common extra-intestinal features are amoebic liver, lung or brain abscesses [8,26–31], although less than 3% of cases develop these complications [25,32–35] as 90% of cases are self-limiting [36]. To better understand the pathogenesis of amoebiasis and the interaction between amoebae and their hosts, the development of suitable animal models is crucial.

Pigs [37], gerbils [38], cats [39] and mice are used as animal models for the study of amoebiasis in the laboratory. Among these, the most commonly used model is the mouse [40–45]. However, the frequency of successful infection remains low, which is dependent on the conditions of amoebae in the laboratory. As the maintenance of virulent amoebae in the laboratory is unstable, it needs further refinement.

This review summarizes mouse models of amoebiasis and the current state of laboratory culture methods of amoebae.

Cultivation of *E. histolytica* for diagnostic purposes is not easy, time-consuming, and the vast majority of which is for research only. Boeck and Drbohlav first established the laboratory culture of amoeba in 1925 [46,47]. Cutler (1918) also reported the cultivation of *E. histolytica* isolated from a patient, but he did not describe his methodology in detail [46]. Diamond LS modified and refined this technique to produce the protocol that is widely used today [48]. Details of this process are summarized below.

2. Laboratory culture

To enable amoebae to be cultured in the laboratory, the parasite needs to pass through several different processes of culturing. These processes are of xenic, monoxenic and axenic cultures. Xenic culture is defined as a parasite culture with an undefined flora while monoxenic

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culture is with known single species of organisms. Axenic culture indicates a condition where parasites grow in the absence of any other organisms [48]. For *in vitro* culture, amoebae collected as a clinical sample are necessary to be cultivated in the xenic condition, at first, then in a monoxenic condition, and finally, to adapt to an axenic culture. The development of axenic culture is time-consuming, and amoebae often become less infectious to hosts [49,50]. Axenic culture-adapted amoebae can often not infect to laboratory animals [51,52].

2.1. Amoebae change food uptake processes depending on culture medium

Amoeba uses different processes for the uptake of nutrients from the environment. The most common procedures are phagocytosis [53–58], pinocytosis [59–62] and trogocytosis [63]. Phagocytosis is defined as the taking up of large particles (>0.5 μm) into cells while pinocytosis is as the uptake of fluid droplets by cells. *E. histolytica* trophozoites ingest pieces of intact living cells *via* trogocytosis [63]. This process is essentially similar to phagocytosis, except killing of the cells before ingestion.

In the xenic culture, amoebae present with bacteria and phagocytize them [48,64,65]. When trophozoites are transferred into a monoxenic or an axenic culture from the xenic, due to the drastic change of the bacterial load, the amoebae need to change their food uptake process from phagocytosis to pinocytosis to ingest solutes or nutrients directly from media [66,67]. The radical change in conditions from xenic to axenic culture results in atrophy of large numbers of trophozoites and adaptation takes an extended period. Once adapted to axenic condition, amoebae may remain in successful culture for long periods of time.

2.2. Culture materials

2.2.1. Media

Boeck and Drbohlav (1925) first used a diphasic egg slant medium for culturing this parasite, called LES medium, which had been used for isolation of intestinal flagellates [46]. Later on, Dobell and Laidlaw used rice starch as a carbohydrate source in the medium that is still used as a component for xenic culture media [68]. Soluble sugar is not used in xenic media as this would result in overgrowth of bacteria. The current most commonly used media for xenic culture are the diaphasic LE and Robinson's media [69]. Axenic culture medium Trypticase-Yeast extract-Iron-Serum (TYI-S-33), which is supplemented with heat-inactivated 10% adult bovine serum, was introduced by Diamond LS in 1978 [70]. Biosate-Iron-Serum (BI-S-33) medium has a similar composition to TYI-S-33 and can also be used to maintain axenic culture [71] (Tables 1 and 2).

2.2.2. Hemoflagellates

Diamond (2002) also used hemoflagellates such as *Crithidia* sp., *Trypanosoma lewisi*, *Leishmania enrietii*, or *Endotrypanum schaudinni* [49,51,72] to develop monoxenic amoebae culture [73]. These flagellates help to maintain the conditions of monoxenic cultures through regulation of bacteria.

2.3. Culturing of patient-derived amoebae

Culture methods for *E. histolytica* derived from clinical samples were described and reviewed by Diamond LS [48]. Trophozoites are collected from the stool, rectal biopsy specimens or liver abscess aspirates [50].

As liver aspirates are sterile, the addition of specific bacteria is necessary for the establishment of xenic cultures. In such cases, either specific bacteria or trypanosomatids can be used for establishment of monoxenic cultures. In samples collected from the stool, unwanted bacteria or other organisms may overgrow and suppress amoebic growth. The most prominent problem occurs when cultures are contaminated with *Blastocystis hominis*, a common human parasite, although this can be cleared by acid treatment with 0.1 N hydrochloric acid [68].

Smedley (1956) described a method to remove unwanted organism during culture *of E. histolytica* from stool samples. This involves pelleting the sample before suspension in distilled water for 15 min at room temperature [74]. Through this process, trophozoites can survive, but other unwanted organisms are removed. Trophozoites are then placed in xenic culture media. This process can then be repeated several times until cultures are free of contaminating organisms [74].

For the isolation of trophozoites from the stool, an emulsion of the sample in saline proceeds a mesh based filtering step. Following the removal of large particles, trophozoites are placed into the xenic medium. Cultures should always be performed in duplicate, with one culture containing antibiotics. Penicillin–streptomycin or erythromycin can be used as they have little direct effect on amoeba. Ceftazidime, ofloxacin or amikacin sulfate can also be used [51]. *E. histolytica* culture should be incubated at 37 °C for 48 h in a slanted tube. If attached amoeba cannot be observed in the glass tube, then the procedure needs to be repeated. After two or three repetitions in the absence of amoeba, the culture is considered negative. If the culture becomes positive, then serial passages are required for maintenance of amoebae.

Once amoeba was adapted to the xenic condition, trophozoites can be cultivated in axenic media. TYI-S-33 or BI-S-33 media are widely used for this purpose. Initial inoculum sizes need to be high, for example, 1×10^6 or higher, and antibiotics are required. Rifampin, amikacin, oxytetracycline and cefotaxime are good antibiotics of this stage [48].

Table 1
Composition of BI-S-33 media.

Reagent	Amount (g/880 ml)
Mixture A	
Biosate	30.0
D-glucose	10.0
Sodium chloride (NaCl)	2.0
Potassium phosphate (KH_2PO_4)	0.6
Dipotassium hydrogen phosphate (K_2HPO_4)	1.0
L-Cysteine	1.3
L-Ascorbic acid	0.2
Ferric ammonium citrate	0.0228
ddH_2O	X ml[a]
[a]Subtotal (Mixture A)	880 ml
[#]Mixture A	880 ml
[#]ABS (adult bovine serum)	150 ml
[#]Vitamin mixture (see Table 2)	20 ml
[#]Penicillin–Streptomycin (each 10,000 U/ml)	14 ml
Total	1064 ml

#After cooling the media (Mixture A), heat inactivated adult bovine serum, a vitamin mixture, and antibiotics are added. Penicillin–streptomycin is generally used. Fetal bovine serum is not acceptable because fetuin is toxic to the parasite.

[a] Firstly 600 mL ddH_2O should be taken in a beaker. Then all ingredients of mixture A should be added to the beaker. Next, add water up to 880 mL and adjust pH 6.8. This mixture needs to be autoclaved at 121 °C for 15 min.

Table 2
Composition of vitamin mixture.

	Reagent	Amount (mg)
A 25 ml ddH_2O	Niacinamide	45
	Pyridoxal HCl	4
	Calcium pantothenate	23
	Thiamine hydrochloride	5
	Vitamin B12	1.2
B 45 ml ddH_2O (#1)	Riboflavin	7
C 45 ml ddH_2O (#1)	Folic acid	5.5
D 45 ml ddH_2O	d-Biotin	2
Solution 1 (A + B + C + D)		160 ml
E 5 ml 95% ethanol	Oxidized DL-6, 8-thioctic acid	1 mg
F 30 ml ddH_2O	Tween 80	500 mg
Solution 2 (E + F)		35 ml
ddH_2O		X ml
Total (Solution 1 + 2 + ddH2O)		200 ml

#1 0.1 N NaOH is used to dissolve Riboflavin (B) and Folic acid (C) in ddH_2O (10 mL) and the final volume of them is 45 ml each.