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NEW INSIGHTS INTO HOST–PATHOGEN INTERACTIONS DURING *ENTAMOEBA HISTOLYTICA* LIVER INFECTION

D. M. Faust, J. Marquay Markiewicz[#], J. Santi-Rocca[##] and N. Guillen*

Institut Pasteur, Cell Biology of Parasitism Unit, Paris, France
Inserm U786, Paris, France

Amoebiasis is the third worldwide disease due to a parasite. The causative agent of this disease, the unicellular eukaryote *Entamoeba histolytica,* causes dysentery and liver abscesses associated with inflammation and human cell death. During liver invasion, before entering the parenchyma, *E. histolytica* trophozoites are in contact with liver sinusoidal endothelial cells (LSEC). We present data characterizing human LSEC responses to interaction with *E. histolytica* and identifying amoebic factors involved in the process of cell death in this cell culture model potentially relevant for early steps of hepatic amoebiasis. *E. histolytica* interferes with host cell adhesion signalling and leads to diminished adhesion and target cell death. Contact with parasites induces disruption of actin stress fibers and focal adhesion complexes. We conclude that interference with LSEC signalling may result from amoeba-triggered changes in the mechanical forces in the vicinity of cells in contact with parasites, sensed and transmitted by focal adhesion complexes. The study highlights for the first time the potential role in the onset of hepatic amoebiasis of the loss of liver endothelium integrity by disturbance of focal adhesion function and adhesion signalling. Among the amoebic factors required for changed LSEC adherence properties we identified the Gal/GalNAC lectin, cysteine proteases and KERP1.

Keywords: *Entamoeba,* amoebiasis, KERP1, liver sinusoidal endothelial cells, cell death, integrins, focal adhesions

The amoeba parasite *Entamoeba histolytica* causes amoebiasis in humans. Invasive trophozoites resident in the colon target the intestine, eventually generating dysentery. By haematogenous spread, amoebae may reach the liver where they form abscesses [1]. Multiple parasite factors are associated with pathogenicity and include markers for: adhesion, motility, extracellular matrix (ECM) degradation, cytotoxicity for and phagocytosis of human cells, induction of host cell death and inflammation. During intestinal invasive infection, *E. histolytica* degrades the colonic mucosa with amoebic proteolytic enzymes like Cysteine Proteinase (CP) A5 [2]. Trophozoites then interact with the intestinal epithelium, cross the basal lamina and disrupt the ECM. Invasion induces an acute inflammatory response characterised by the increase of Interleukin (IL)-1 and -8, Interferon (IFN)-γ and Tumour Necrosis Factor (TNF) [2, 3], which is chemo-attractant for amoebae *in vitro* [4]. Crossing the intestinal barrier allows subsequent *E. histolytica* dissemination, particularly to the liver via the portal vein. Hepatic sinusoids irrigate the organ and are the sites where *E. histolytica* interacts with endothelial cells and liver-resident macrophages (Kupffer cells), and crosses the endothelial barrier, prior to the penetration into the parenchyma. This leads to the formation of inflammatory loci by neutrophils and macrophages, and the establishment of abscesses (see [5] for review).

Tissue modifications during abscess establishment

Liver invasion by *E. histolytica* with production of abscesses is the most common extra-intestinal manifestation of amoebiasis. The hamster is a powerful model for hepatic amoebiasis. After intra-portal inoculation of trophozoites, histological features of infected livers are similar to those found in humans and allow to study amoebic liver abscess (ALA) development *(Fig. 1).* The ALA in humans and hamsters have a common characteristic structure: a central necrotic region containing inflammatory cells and lysed hepatocytes surrounded by a ring of motile trophozoites and few inflammatory cells that delimit the abscess from the apparently healthy hepatic tissue [6, 7]. *E. histolytica* infection of the liver has a fast temporal program during which parasites cross the liver sinusoidal endothelium, penetrate into the tissue and adapt to the new environment before starting division and successful establishment of the infection. Histological analysis revealed that at four hours post-inoculation small foci have already formed in the liver parenchyma containing *E. histolytica*, scattered inflammatory cells and some dead hepatocytes. Between six and twelve hours, parasites are massively destroyed by the innate immune system, as well as some host cells, a phenomenon that may be at least partially responsible for organ damage. Thus, early infection is critical for amoeba sur-

*Corresponding author: Nancy Guillen; Phone: 00331-45688675; Fax: 00331-45688674; E-mail: nguillen@pasteur.fr
[#] Present address: Laboratoire de Biologie et de Pharmacologie Appliquée, CNRS UMR 8113, Ecole Normale Supérieure de Cachan, Cachan, France
[##] Present address: Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, Madrid, Spain

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vival, host responses and consequently for subsequent ALA development [7]. Infected livers of severe combined immune deficiency (SCID) mice harbor immune cells and hepatocytes with positive TUNEL labelling, indicating that they undergo apoptosis [8]. Gene expression profiles of SCID mouse liver regions infested with virulent *E. histolytica* trophozoites for 4 h, 12 h and 24 h have been reported [9]. Profiles are supposedly composed of the response of several cell types of hepatic resident (mainly hepatocytes, but also Kupffer, stellate and endothelial cells) and circulating cells attracted to the sites of infection (neutrophils, macrophages, natural killer T (NKT) cells) and reflect the cross-talks between these cells. The gene expression changes indicate simultaneous activation of inflammatory, regenerative and apoptotic pathways with a bias towards cell death induction.

Trophozoites from ALA can be purified and adapted to *in vitro* culture, during which their ability to form abscesses in animals decreases. Recurrent passage into animals maintains parasite virulence [10]. These observations underline either adaptation of *E. histolytica* to the environment they encounter upon invasion, or selection of invasion-prone parasites during the pathology development.

Cell activation during abscess development

Liver presents a specific environment characterized by immunological tolerance to resident intestinal flora and innate and acquired immune responses against enteric pathogens. The first line of liver defence against *E. histolytica* invasion is composed of cells of the innate immunity that lead, upon recognition of pathogen-associated molecular patterns (PAMP) to the triggering of an inflammatory response. Integration of the immune response into the hepatic microenvironment can be achieved by interaction of antigen-presenting cells (APC) with various T lymphocyte subtypes. Among them, NKT cells are of major interest for their particular functions and for their abundance in the liver (approximately 30% of the lymphocytes). NKT cells have the ability to secrete various cytokines upon direct binding of PAMPs, cytokine stimulation or recognition of glycolipidic antigens presented by antigen-presenting glycoprotein CD1 family member d (CD1d) molecules on APC. The different NKT subtypes, which produce cytokines as diverse as IFN-γ, IL-4 or IL-17, determine diametrically opposite immune polarizations (see [11] for a complete review). Thus, NKT cells play crucial roles in the orientation of the hepatic immune response.

Differences in the early response to *E. histolytica* liver invasion were observed between female and male mice [12]. Females rapidly cleared the parasites, recruited higher numbers of NKTs to the site of infection, and IFN-γ was produced at higher levels. NKT-deficiency in females or IFN-γ neutralization led to increased parasite survival. IFN-γ is a key regulator of inflammation initiation, by activating macrophage TNF production, which in turn promotes nitric oxide (NO) synthesis by macrophages themselves and by polymorphonuclear neutrophils (PMN). Early in the host defence response, NKT cells are among the IFN-γ producing cell types, the so-called "innate" lymphocytes, which reside in tissues, and IFN-γ may also originate from macrophages. The IFN-γ effect can be bypassed *in vitro* by the recognition of *E. histolytica* surface proteophosphoglycan (LPPG) by Toll-like receptors 2 and 4, which results in direct production of cytokines such as TNF, IL-12p40 and IL-8 [13]. However, this model does not fully account for the interactions occurring in the hepatic microenvironment, since the cells used are non-immune Human Embryonic Kidney cells, transfected to overexpress either Toll-like receptor 2 or 4 (TLR2 or TLR4), and hepatic cell types that may modulate immune responses were not included in the system.

It has been proposed that either the inflammatory response or the death of immune cells recruited to inflammatory foci are responsible for liver damage, since leukopenic animals did not develop liver abscesses [14]. To produce ALA, *E. histolytica* may thus need leukocyte recruitment, their activation and eventually their death, triggering a delicate balance to create an environment in which host tissues are destroyed but not all the amoebae. This controlled state of inflammation may result from the integration of different pro- and anti-inflammatory as well as chemokine signals, starting from early times of infection. In this context, NKT cells may play an important role, since they can produce the different types of cytokines. Further characterization of the NKT subtypes during ALA will help to decipher their complementary, synergistic or eventually opposite roles in the host defence response to invading *E. histolytica*.

In hepatic sinusoids, amoebae enter in contact with liver sinusoidal endothelial cells (LSEC) which comprise 50% of the non-parenchymal cells of the liver [15]. The particular LSEC phenotype is adapted to the high metabolic and immuno-modulatory activity of the organ [16]. LSEC have open transcytoplasmic pores (fenestrations of ~100 nm diameter; [17]) clustered into sieve plates, are devoid of a basement membrane and of tight junctions and form a discontinuous sinusoid lining. The Disse's space between LSEC and hepatocytes contains an attenuated ECM consisting mostly of fibronectin, tenascin and some collagen I and VII. This fenestrated sinusoidal endothelium allows direct exchange between the blood and the parenchymal compartment, and proteoglycans on the hepatocyte surface may reach through the Disse's space into the sinusoid lumen.

LSEC potentially play a dual role in the establishment of liver infection by *E. histolytica*, as a barrier and as modulators of host defence responses. In particular, LSEC function as APC. They are characterized by a high endocytic activity, express scavenger, mannose and Fc-γ IIb2 receptor involved in the uptake of circulating proteins and they process and present antigens via major histocompatibility complex MHC I and II molecules. LSEC also participate in the modulation of the immune response by expressing surface receptors for leukocyte adhesion and their subsequent transendothelial migration, and by secreting cytokines and modulators upon stimulation (e.g. IL-1β, IL-6, IL-10, NO) [16].

It was found that in humans suffering from amoebiasis liver endothelial cells produce pro-inflammatory factors following contact with *E. histolytica* [18]. At early stages post-infection, the pro-inflammatory factors intercellular adhesion molecules ICAM-1 and -2 lead to recruitment and activation of immune cells that are responsible for parasite killing. At early stages of ALA development in hamsters, amoeba-secreted material is deposited on the surface of LSEC [7] suggesting that these cells are rapidly aggressed by elements of the cytotoxic system of the parasites. This might be the initial trigger for the inflammatory response. Hamster sinusoidal endothelial cells undergo apoptosis in the presence of trophozoites [19]. The resulting disturbance of the endothelial barrier likely facilitates passage of trophozoites from the blood circulation into the hepatic tissue. *E. histolytica* then divides and induces abscess formation [7], dependent upon the expression of virulence factors like Gal/GalNAc lectin [20], amoebapore A [21], CP-A5 [22] and the highly positively-charged lysine- and glutamic acid-rich protein KERP1 [23].

Interaction between E. histolytica *and human liver sinusoidal endothelial cells*

Assuming that the initial stage of liver infection, when parasites arrive in the hepatic sinusoids, is crucial for the subsequent establishment of abscesses, the characterization of the interactions between amoebae and human hepatic target cells is relevant to understand the host defence and, in particular, the inflammatory response, as well as the changes in parasite phenotype required for adaptation and survival in the hepatic environment. To gain insight into early hepatic infection we have recently used a LSEC line established from immortalized cells of human liver endothelial cell primary cultures [24]. Cells are not tumorigenic, express phenotypic markers of the primary cultures [25] and respond to TNF [26]. Notably, the cells are sensitive to hypothermia/hypoxia-reoxygenation treatment inducing cell death by necrosis and apoptosis, causally related to an increase in matrix metalloprotease 2 release and NO production [27].

Using cultures of the human LSEC line incubated with virulent or virulence-attenuated (i.e. having lost their capacity to form ALA in the hamster model) *E. histolytica*, we documented cellular changes by confocal microscopy and video-microscopy *(Figs 2 and 3),* and cell death by FACS analysis [28]. We observed that *E. histolytica* induced retraction, apoptosis and death of the human cells. LSEC retraction was detected earlier than cell death suggesting that reduced spreading of LSEC could account *in vivo* for the physical changes in tissue architecture allowing *E. histolytica* to pass the endothelial barrier and to penetrate into the liver parenchyma, a process possibly facilitated by the absence of tight junctions and by inflammation-induced gap formation between neighbouring cells [29]. In the presence of virulent amoebae, the LSEC network of actin stress fibers was disrupted, and the subcellular localisation of paxillin and phosphorylated focal adhesion kinase (FAK), key components of focal adhesion complexes formed upon activation of integrin receptors, was altered. These changes in the LSEC adhesion state could be a key event in the cascade leading to cell death induced by *E. histolytica*.

Endothelial cells are among the most sensitive cell types for apoptosis triggered by loss of adhesion, named anoikis and associated with decreased protein tyrosine phosphorylation levels [30]. By analysis of the LSEC transcriptome, we have shown that the integrin signalling pathway was modulated specifically by virulent trophozoites [28], suggesting that in addition to the cell retraction observed, virulent parasites rapidly trigger a signal transduction pathway not only determining cell adhesion properties, but also sensing the adhesion state and responding to its changes. Integrins are a family of cell surface receptor proteins mediating cell-to-cell and cell-to-ECM adhesion signalling. Integrin receptors are α/β subunit heterodimers, with ligand specificity dependent on the subunit combinations. Activation of the receptors occurs upon conformational conversion into the high affinity state for ligand binding and is regulated by intracellular signals (inside-out signalling), such as the concentration of free magnesium and calcium. Ligand binding promotes clustering of integrins, which do not have direct signalling functions, but serve as an anchoring point for many scaffold proteins and recruitment of numerous signalling molecules (outside-in-signalling). The resulting multiprotein complexes formed are named focal adhesions (FA). Key steps in their assembly are the initial association with talin, paxillin and FAK, leading to the activation of FAK by autophosphorylation (likely due to the increase in local concentration above critical threshold) and phosphorylation of paxillin. These tyrosine phosphorylations create binding sites for adaptor proteins like SHC1, signalling molecules such as Src kinase and for proteins involved in the organization of actin filaments (stress fibers). SHC1 is a substrate of FAK and is localized to FAs involved in mitogenic and survival signalling. Activation by tyrosine phosphorylation leads to downstream activity of Ras and MAPK pathways and SHC contributes also to cytoskeleton organization (see [31] for review). Furthermore, FAs and stress fibers play important roles in mechanotransduction and in line with this, endothelial cells which *in vivo* are exposed to mechanical forces induced by the blood flow, respond to fluid shear stress by changes in stress fiber and FA organization [32].

Most functions involved in the LSEC integrin/FA signalling pathway modified by *E. histolytica* were up-regulated and participate either in signalling (integrins, FAK and SHC1) or in actin cytoskeleton organization (β- and γ-actin, adducin 1, plectin 1, spectrin-α, talin1, vinexin, zyxin and regulators of Rho activity).

Amoebic factors involved in LSEC death

Amoebic factors responsible for LSEC retraction and death have been studied according to their abundance in lipid-protein clusters present in amoebic uropod-released frac-

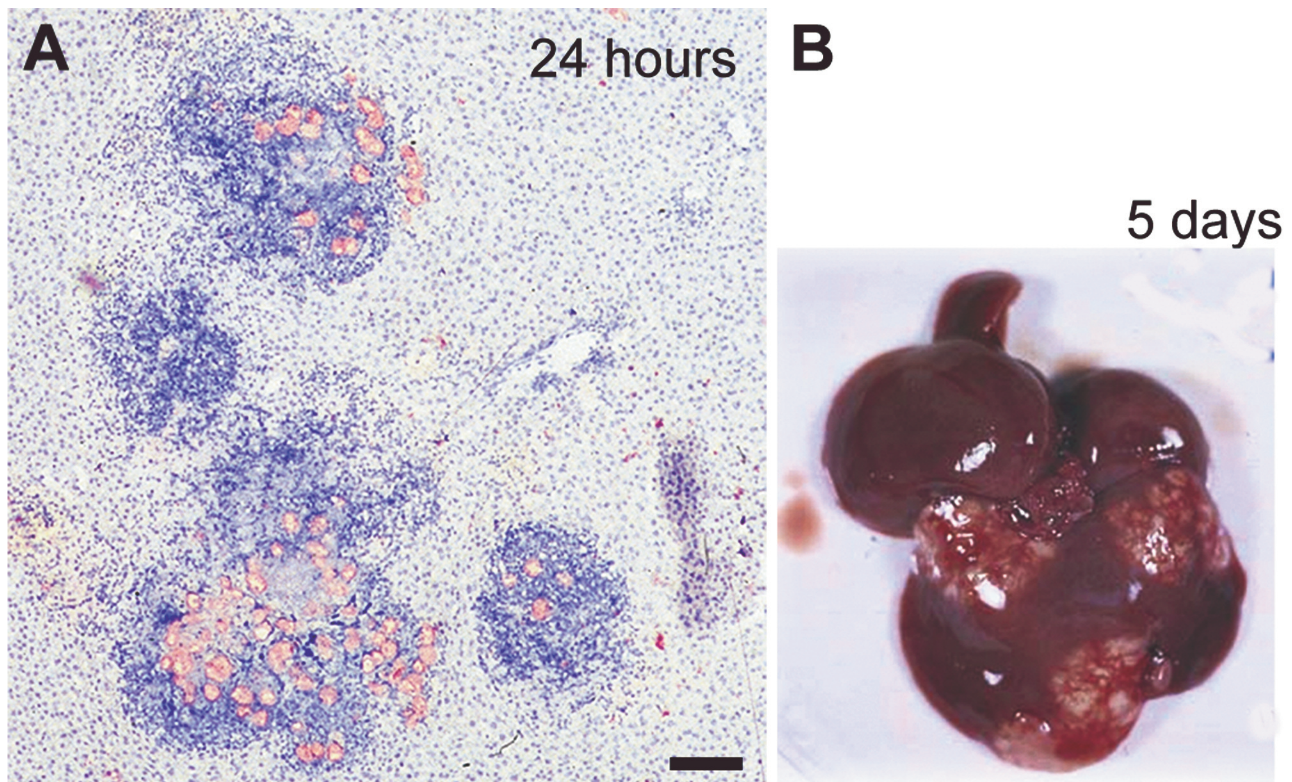

Fig. 1. Amoebic liver abscess formation in the hamster model of hepatic amoebiasis

Male Syrian golden hamsters *(Mesocricetus auratus)* were infected intraportally with virulent parasites (8×10^5 trophozoites per animal) according to our published protocol [7]. Livers were excised and fixed immediately after necropsy at 24 hours post-inoculation (A). Shown is a paraffin-embedded section of infected liver stained with hematoxylin to visualize the mammalian cells (in blue) and immuno-labelled with a human anti-*E. histolytica* antibody (in pink). Note the presence of massive infiltrates containing inflammatory cells. Over time, the inflammatory foci coalesce and form macroscopic abscesses (B). Scale bar, 100 μm

tions, which allow *E. histolytica* to escape from the immune response and are thus relevant for parasite virulence. A proteomic analysis of uropod components in fact showed the abundance of factors such as the Gal/GalNAc lectin and several CPs [33]. Using amoebic strains silenced for CP-A5 gene expression and parasites blocked for Gal/GalNAc lectin activity, we have identified these functions as important factors involved in parasite adhesion, LSEC retraction and death. In contrast, amoebapores are not required for LSEC killing [28].

The pro-form of CP-A5 contains an RGD motif for which a role in target cell adherence and triggering of a pro-inflammatory host cell response has been described [34]. Thus, CP-A5 could play a role as a protease and as a RGD-motif ligand for integrins modifying target cell signalling. By flow cytometry analysis we observed that pre-treatment of trophozoites with an RGD-containing peptide reduced the fraction of dead LSEC, suggesting an involvement of amoebic RGD-binding proteins, such as the β1 integrin-like FN receptor (β1*Eh*FNR), which shares a high degree of homology with the intermediate Gal/GalNAc lectin subunits Igl1 and Igl2 ([35] and references therein). Amoebic RGD receptors could be involved in adherence to ECM components like fibronectin, produced by and present on LSEC, and serve as additional adhesion molecules.

Conclusion

Amoebiasis can be viewed as resulting from the balance between the "fitness" of the parasites (i.e. to express the phenotype required to resist host defence, to rapidly adapt to changes in the environment, to invade and to survive) and the "adequate" host defence response (e.g. the control of the parasite burden, of the degree of inflammation and immune responses, the type of cell death). Both are conditioned by the interactions between parasites, environmental factors and host target cells. The *in vivo* LSEC response to *E. histolytica* is likely composed of a reaction to contact with amoebae and to parasite-induced alterations of the microenvironment. For example, LSEC receive pro-inflammatory signals produced by Kupffer cells. A direct cytotoxic effect on LSEC of elevated NO levels has been demonstrated as well as a protective role of prostaglandin E2 [27]. Trophozoites of 10–50 μm in diameter with highly versatile morphology cause hindrance in sinusoids (diameter of 5–7 μm; [36]) particularly narrow and tortuous in periportal regions [15]. Obstruction may be reinforced by the recruitment of activated immune cells during the acute inflammatory response [29]. Amoebae may thus exert mechanical forces on the endothelium and the underlying Disse's space and reduce the blood flow, and consequently

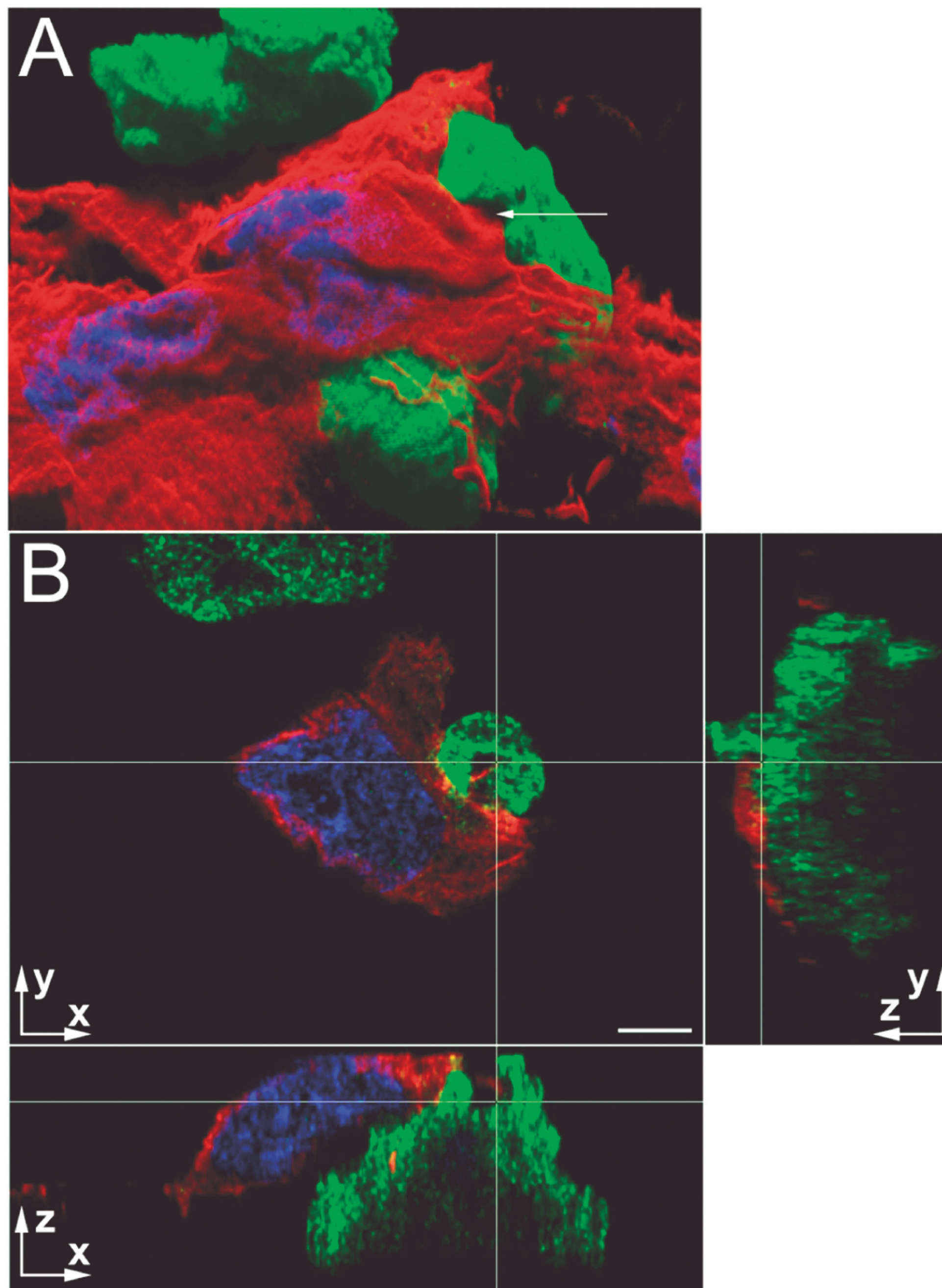

Fig. 2. Immunolocalization of amoebic protein KERP1 during interaction of virulent trophozoites with LSEC

Confocal microscopy acquisitions showing immunolocalization of KERP1 (green) delimiting the parasite and detection of F-actin (red) the LSEC. Nuclei are stained by DAPI (blue). Scale bars (on axis labels), 5 µm.

A. Virtual three-dimensional reconstruction of the stack. Blending and shadowing reveal the volumes on this two-dimensional representation obtained with Imaris software. The nuclei of two LSEC are detected. A trophozoite is seen in the upper part of the image and in the centre an amoeba is partially covered by a LSEC, which has retracted from the substrate and the neighbouring cells. A membrane protrusion emitted by the trophozoite engulfs a portion of the LSEC (arrow and intersection of the white lines in Panel B).

B. The micrograph shows three orthogonal planar sections. The white lines correspond to the orthogonal projection of the planes presented aside and their intersection represents the point indicated by the arrow in Panel A. The arrows indicate the orientation of the plane and z=0 corresponds to the focal plane acquired closest to substratum. The retracted LSEC is detected with its nucleus. The trophozoite surface in contact with the LSEC is not homogeneously enriched in KERP1, as detected at the top of the trophozoite in the xz section. The three orthogonal sections show actin from LSEC (at the intersection of the white lines) surrounded by membrane protrusions from the parasite that are strongly enriched in KERP1. Note that, in the yz section, the membrane invagination is also detected and corresponds to the area with the highest z coordinates (i.e. the most distant from the z=0) and the greatest KERP1 density. The three-dimensional reconstruction allowed detection of this topological link

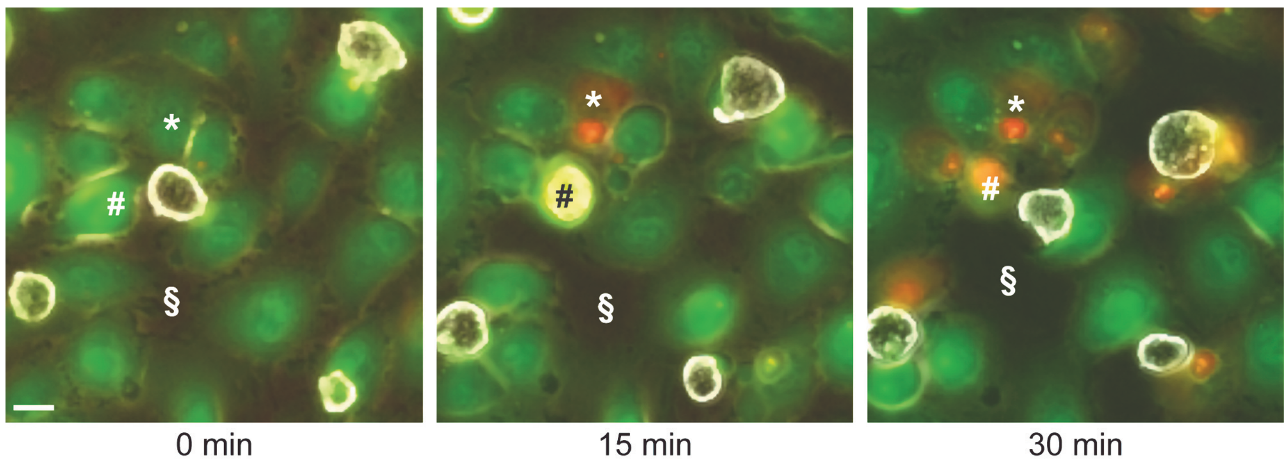

Fig. 3. Retraction and cell death in LSEC cultures during incubation with *E. histolytica*

Micrographs of video-microscopy sequences show frames taken at 0, 15 and 30 min of incubation.
LSEC were labelled with fluorescent CMFDA cell tracker (green). Trophozoites were then added (parasite to LSEC ratio 1:10) and observed by phase contrast microscopy, as non-fluorescent cells (white) with brightly reflecting plasma membranes.
Dead cells were detected by incorporation of propidium iodide (red). Note that human cells (i.e. * and #) were in contact with an amoeba before dying. The space not occupied by cells (i.e. §) is increasing over time, indicating LSEC retraction. Scale bar, 10 μm

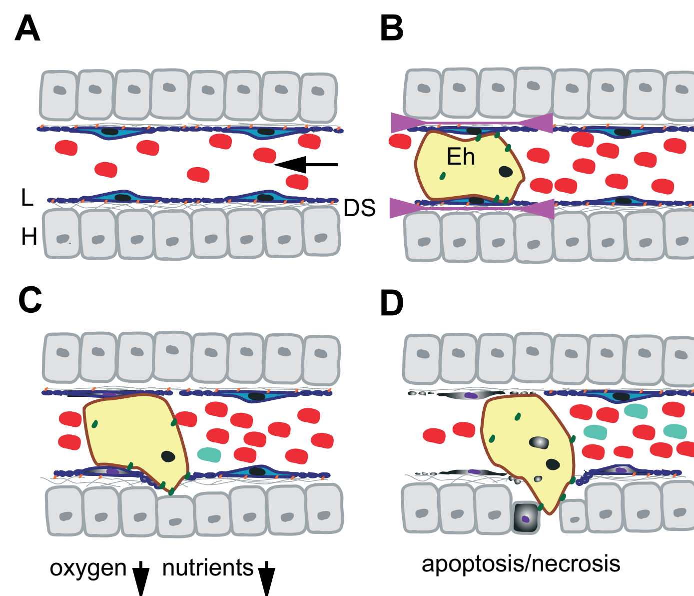

Fig. 4. Model for the passage of *E. histolytica* through the liver sinusoidal barrier

A. Schematic representation of a liver sinusoid. Red blood cells circulate in the sinusoidal lumen lined by fenestrated LSEC (L; blue). LSEC adhere at FA plates (orange) to the ECM (gray lines) present in the Disse's space (DS) and in contact which the hepatocytes (H). Stellate and Kupffer cells are not represented.
The arrow indicates the direction of the blood flow. Note that the drawing is not in scale.
B. During early stages of hepatic amoebiasis, *E. histolytica* trophozoites (yellow) obstruct hepatic sinusoid capillaries and induce LSEC retraction (indicated by arrowheads). The amoeba (10–50 μm) being bigger than the sinusoid diameter (5–7 μm), trophozoites exert mechanical forces on the endothelium. In addition, several virulence factors and adhesion molecules (green) facilitate the loss of FA complexes accelerating LSEC retraction.
C. Obstruction caused by amoebae reduces the blood flow, locally creating ischemia and decreasing concentrations of oxygen and nutrients. As a consequence, the oxidative stress for the amoebae is diminished, LSEC death by apoptosis and necrosis (purple nucleus, grey cytoplasm) is induced and the inflammatory response initiated by immune cells (light blue).
D. Retraction and cell death allow the amoeba to penetrate into the liver parenchyma in which it induces hepatocyte death. Phagocytosis of red blood cells, apoptotic bodies and necrotic debris provides nutrients and energy to the trophozoites.
The immune response is developing

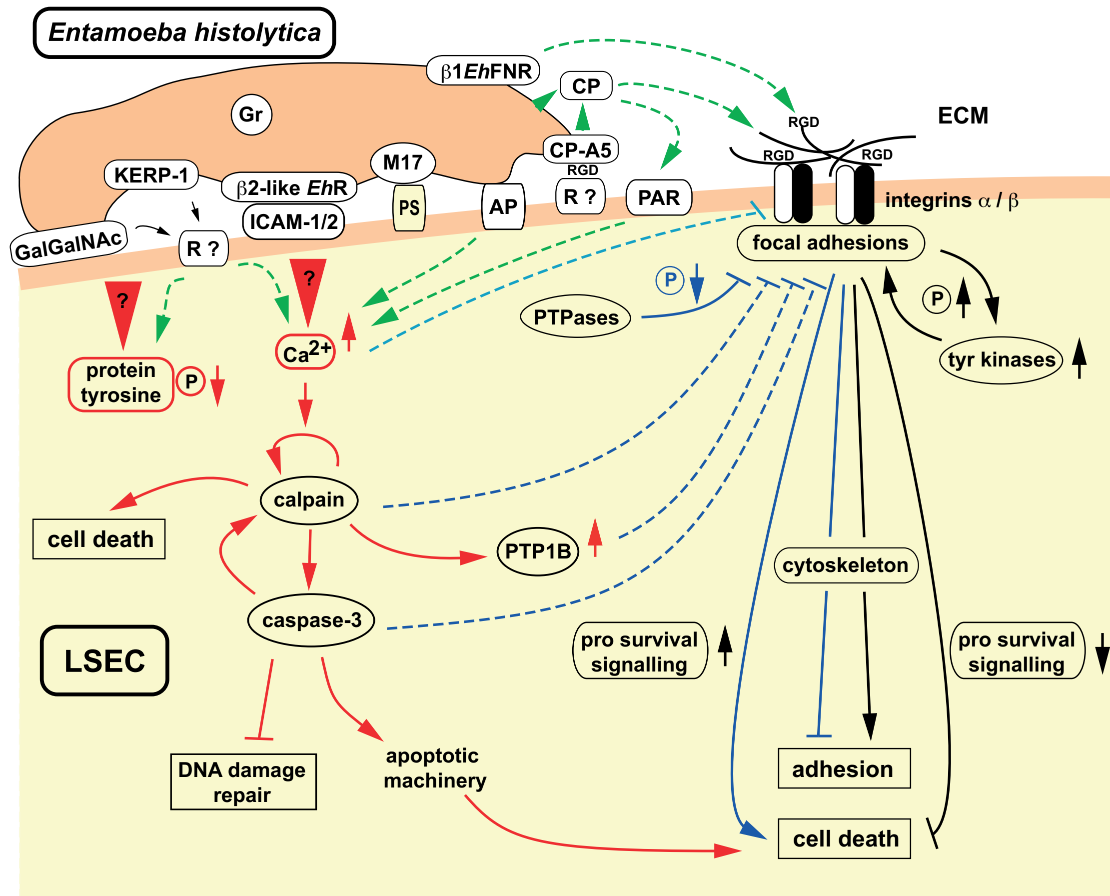

Fig. 5. *E. histolytica* interference with cell adhesion signalling may induce cell death

The scheme summarizes the levels at which *E. histolytica* may interfere with target cell integrin-mediated cell adhesion signalling, here LSEC. Several relevant virulence factors are presented: Gal/GalNAc lectin and KERP-1 [44].
Host cell receptors for both amoebic molecules remain to be identified. Amoebic β2-integrin like molecule (β2-like *Eh*R) shown to bind to intercellular adhesion molecules ICAM-1 and -2 [45] whose expression is induced in activated endothelial cells.
RGD motif containing proteins, like the ECM component fibronectin expressed by LSEC, may serve as binding sites for β1 integrin-like fibronectin receptor β1*Eh*FNR which shares a high degree of homology with the intermediate Gal/GalNAc lectin subunits Igl1 and Igl2 ([35] and references therein).
Interaction may reinforce trophozoite adherence and eventually compete with integrins for RGD ligands, thus interfering with LSEC adhesion signalling. The immuno-dominant amoeba protein M17 [46] may be involved in recognition of phosphatidyl-serine (PS) exposed at the surface of apoptotic cells, which are preferentially phagocytosed by amoebae.
Pore-forming amoebapore proteins (AP) though involved in lysis of other host cells and ALA, are not essential for LSEC apoptosis and death. The calcium-binding proteins Grainin-1 and -2 (Gr) are found in secreted granules, but their potential role in LSEC killing has not been elucidated so far. Cysteine proteases, among which CP-A5, present at the trophozoite surface or released into the extracellular medium, are implicated in ECM degradation. The pro-form of CP-A5, localized in secreted and surface-bound fractions, contains a RGD sequence that confers to this protein a protease activity-independent additional function in adherence and host cell inflammatory response induction; binding to integrin αvβ3 from colonic enterocyte cells has been found [34], potential receptors on LSEC are not characterized. Activation of vascular endothelial cell G-protein coupled receptors, like the protease-activated receptors (PAR), by pathogen-encoded CPs have been described for several microorganisms, including African trypanosome parasites.
Anchorage-dependent survival of adherent cells relies on the activity of the integrin-mediated signal transduction pathway, regulating cell survival/death signalling activities and cytoskeleton organization. LSEC death results from abrogation of adhesion signalling leading to diminished adhesion, to inactivation of survival and to activity of cell death pathways.
Loss of adhesion may in turn trigger apoptosis (anoikis).
Elements intervening in *E. histolytica* contact-dependent apoptosis of LSEC were inferred from data obtained with the human lymphoma line Jurkat [47–49]. Upon contact with trophozoites, the protein tyrosine phosphorylation level rapidly decreases and the concentration of free intracellular calcium drastically rises. The initial trigger for both events remain yet unknown.
Calcium-dependent activation of calpain in turn activates caspase-3 responsible for the execution of the apoptotic program.
Calpain activity may also account for necrotic cell death and diminished protein phosphorylation levels, by cleavage-dependent activation of protein tyrosine phosphatase PTP1B. Amoeba-induced activity of calpain, caspase-3 and PTP1B and several other protein tyrosine phosphatases as well as increased calcium levels may negatively modulate integrin/FA pathway activity

the oxygen and nutrient supply of surrounding areas *(Fig. 4).* These changes probably create conditions similar to hypoxia/ischemia. Hypoxic conditions may reduce the oxidative stress for the trophozoites. Microcirculatory dysfunction has been proposed recently as the major cause of tissue necrosis during ALA formation [37]. LSEC respond to ischemia/reoxygenation stress by detaching from the sinusoids and undergo cell death by apoptosis, dependent on caspase as well as calpain protease activity [38, 39].

We propose that at early stages of liver infection and upon contact with the sinusoidal endothelium *E. histolytica* interferes with host cell adhesion signalling leading to diminished adhesion and induction of target cell death *(Fig. 5).* Disruption of FA complexes has been described as an early event in endothelial cell apoptosis, preceding caspase-dependent proteolysis of complex components and cell death [40]. For a variety of pathogens (bacterial, fungal, viral and parasitic) disseminating through the vascular system, interference with endothelial cell integrity/function is known as an important mechanism sustaining infection, immune response escape, propagation and dissemination to the underlying tissues. Interference results in changes in vascular permeability and/or in endothelial cell death, mainly suppression/delay or induction of apoptosis (e.g. [41–43]).

Altogether, the *in vivo* and *in vitro* approaches to study hepatic amoebiasis and the molecular tools used to interfere with key factor functions will help to define the molecular and physical bases of ALA. These advances in the understanding of this neglected parasitic disease, used as a model, will help to understand better the physiology and the immunology of the liver in situations of health and in the context of other diseases.

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