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HUMAN AMEBIC LIVER ABSCESS: EXPRESSION OF INTERCELLULAR ADHESIÓN MOLECUES1 AND 2 AND VON WILLEBRAND FACTOR IN ENDOTELIAL CELLS.

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SHORT COMMUNICATION

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Human amebic liver abscess: expression of intercellular adhesion molecules 1 and 2 and of von Willebrand factor in endothelial cells

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Abstract Liver invasion by amebas with production of amebic liver abscess (ALA) is the most common extraintestinal lesion produced by the protozoan parasite *Entamoeba histolytica*. This hepatic damage is characterized by the presence of extensive tissue necrosis. However, little is known about the parasite and host factors involved in the process of tissue damage. During the early establishment of amebas in the liver parenchyma as well as during the extension of the tissue necrosis, parasites interact with sinusoidal endothelial cells. As a consequence of ameba-endothelial cell interactions, the latter can be activated and express proinflammatory factors that could be related to tissue destruction. We studied by immunohistochemistry the localization of antigenic molecules of *E. histolytica* trophozoites and of molecules such as intercellular adhesion molecule 1 (ICAM-1), ICAM-2, and von willebrand factor in activated endothelial cells of human ALA, which could be related to the pathophysiological mechanisms of tissue destruction in amebiasis.

Introduction

Hepatic lesions produced by *Entamoeba histolytica* trophozoites in humans are characterized by extensive tissue necrosis. Multiple factors concerned with both parasite and host have been related to the mechanisms of pathogenicity during the production of amebic liver abscess (ALA). In experimentally induced liver damage in hamsters and gerbils we have stressed the major role of acute inflammatory cells during the amebic infection (Tsutsumi et al. 1984; Shibayama-Salas et al. 1992). However, factors associated with attraction, recruitment, and subsequent lysis of inflammatory cells at the site of parasite invasion are unknown; moreover, information is not available regarding the role of amebic components as activators of host cells that could express proinflammatory molecules. Other histopathological change observed in amebic liver lesions is the presence of parenchymal ischemia in areas adjacent to tissue necrosis (Aikat et al. 1979; Pérez-Tamayo et al. 1992). The role of altered circulatory and blood-coagulation processes as a part of the physiopathological mechanisms involved in liver damage in amebiasis is also poorly understood.

Endothelial cells are involved in multiple local and systemic physiological processes. In vitro activated endothelial cells exhibit molecules capable of attracting and adhering to inflammatory cells at their surfaces (Pober and Cotran 1990; Turunen et al. 1993; Imhof and Dunon 1995). Whether these factors are also manifested by liver endothelial cells when stimulated by the amebic infection is not known. These molecules include, among others, intercellular adhesion molecule 1 (ICAM-1) and ICAM-2. The former has been related to the attraction and adhesion of acute inflammatory cells and the latter, to the recruitment of chronic mononuclear cells. Endothelial cells are also known to secrete substances associated with the process of blood clotting, such as von Willebrand factor (vWf) (Ewenstein et al. 1987; Wagner 1990; Wu 1992). Although activation of these cells has been associated with the production of cytokines, the possible role of amebic components as activators of endothelial cells has not been elucidated.

Using autopsy samples obtained from human ALA cases we studied by immunohistochemistry the

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localization of amebic antigens in various hepatic cells and detected the adhesion molecules ICAM-1 and ICAM-2 and the blood-clotting vWf.

Materials and methods

Human specimens

Selected liver fragments taken from 17 autopsy cases diagnosed as ALA by histopathology were supplied by the Unidad de Patología, Hospital General de México, and Facultad de Medicina, UNAM. Patients were of both sexes and their ages ranged from 30 to 73 years. The clinical evolution at the hospital varied from 4 days to 9 months (Table 1). For control, we used liver specimens obtained from four autopsy cases of polytraumatized patients who had been admitted to an emergency hospital in Mexico City and had no previous history of amebiasis or ALA. Liver histology of these control cases was normal.

Immunohistochemistry

Paraffin sections measuring 7 μm in thickness were mounted onto 3-aminopropyltriethoxysilane-coated slides. After the sections had been subjected to dewaxing in xylene and rehydration, endogenous peroxidase activity was blocked by incubation in 3% H_2O_2 in methanol for 30 min, after which the specimens were washed with distilled water and with phosphate-buffered saline (PBS). Sections were also stained with hematoxylin and eosin (H&E) for histology. Pretreated sections were further incubated with (a) rabbit polyclonal anti-*Entamoeba histolytica* serum (prepared in our laboratory) diluted 1:500 in PBS-Tween 0.025%, (b) mouse monoclonal IgG anti-human ICAM-1 (Bender MedSystem) diluted 1:50 in PBS-Tween, and (c) rat IgG2a anti-mouse ICAM-2 (Pharmingen) diluted 1:50 in PBS-Tween for 1 h in a humidified chamber at room temperature (RT). Slides were washed for 5 min in PBS-Tween and then incubated (a) with goat anti-rabbit IgG-PO serum (HyClone) diluted 1:100 in PBS-Tween, (b) with goat anti-mouse IgG-PO serum (HyClone) diluted 1:100 in PBS-Tween, or (c) with goat anti-rat IgG2a-biotin serum (Serotec) diluted 1:50 in PBS-Tween for 1 h in a humidified chamber at RT. Slides were washed for 5 min in PBS-Tween. For detection of ICAM-2 (c), sections were incubated with Avidin-PO (HyClone) diluted 1:200 in PBS-Tween for 40 min. All slides were washed for 5 min in PBS-Tween. Reactions were developed with ImmunoPure metal-enhanced DAB (Pierce) for 10 min and washed with PBS-Tween. Finally, sections were counterstained with hematoxylin diluted 1:50 in distilled water for 5 min, dehydrated through graded series of alcohol, cleared in xylene, and mounted in synthetic resin. Detection of vWf (d) was done according to the procedure described in the PAP KIT (Dako) for human monoclonal vWf.

Results

Histology

The histology study of paraffin sections stained with H&E showed large liver areas of coagulative eosinophilic

Table 1 Clinical and immunohistochemical data gathered from human cases of amebic liver abscess (*a* amikacin, *am* ampicillin, *an* antipyrin, *cl* chloramphenicol, *e* emetine, *g* gentamycin, *hy* hydroxyquinoline, *ka* kanamycin, *m* metronidazole, *n* neomycin, *pe* penicillin, *p* prednisolone, *ND* not documented, – none, + weak, + + moderate, + + + intense)

Patient number	File number	Age (years)	Sex	Evol. time (days after admission)	Treatment[a]	Liver weight (g)	Number of abscesses	Average size (cm)	Staining		
									vWf	ICAM-1	ICAM-2
1	A-86-126	40	M	21	e,g,m, cl,a,p,	3,750	11	2–8	+ +	+ –	–
2	A-87-11	65	M	20	m,am,an	2,400	1	10	+ + +	+ +	–
3	A-87-29	43	M	34	g,am,an	3,100	2	3–7	+ + +	+ –	–
4	A-87-365	45	M	244	oral proteins	1,100	1	12	+ + +	+	–
5	A-87-417	42	M	64	an,m	2,200	1	12	+ +	+ –	–
6	A-86-30	48	M	20	m,n,p,an	ND	1	17	+ +	–	–
7	A-86-211	51	M	7	Hartman sol	2,700	Multiple	2–5	+ +	–	–
8	A-72-760	54	M	4	Hartman sol	750	1	10	+ +	+ + +	+
9	A-74-526	33	M	15	e,am,p	1,700	Multiple	2–10	+ +	+	–
10	A-75-476	73	F	30	a,am,ka, hy,p,g	2,300	Multiple	ND	+ + +	+	+
11	A-76-635	62	F	39	an,e,ka	ND	1	ND	+ + +	+	–
12	A-76-849	72	M	24	Hartman sol,ka	1,600	1	ND	+ +	+ +	+
13	A-78-74	56	M	18	e,am,m, ka,p,an	3,000	Multiple	1–3	+ +	–	–
14	A-78-355	60	M	272	am,ka,m, an	2,475	2	ND	+ + +	–	–
15	A-78-604	37	F	228	radio-therapy, colostomy	1,830	ND	ND	–	–	+
16	A-78-635	39	F	13	ND	4,500	Multiple	ND	+ + +	–	–
17	A-87-633	30	F	20	p,a,am	1,500	Multiple	0.5–1	– +	–	–

[a] In all, 14 patients received antiamebic and antimicrobial treatment and 9 received antiinflammatory treatment with prednisone or antypyrin

necrosis with hemorrhages and fibrin deposits outlined by loose connective tissue infiltrated with chronic inflammatory cells and cell debris (Fig. 1). In most cases, variable numbers of *Entamoeba histolytica* trophozoites were detected at the periphery of necrotic areas (Fig. 2). The parasites were seen intermixed with irregular strands of connective tissue with chronic inflammatory reaction or directly in contact with liver parenchymal cells. A few cases also displayed an acute inflammatory infiltrate.

Immunodetection of amebic antigen, ICAM-1, ICAM-2, and vWf

Amebic antigens

E. histolytica trophozoites were intensively labeled with the rabbit antiamebic antibodies. The plasma membrane and the cytoplasmic vacuole membranes of the trophozoites were strongly labeled (Fig. 3). Undamaged or slightly compressed hepatocytes in close contact with trophozoites were also labeled to varying degrees of intensity (Fig. 3). The inner surface of medium-caliber blood vessels located near the amebic lesions also had significant deposits of amebic (Eh) antigen.

Detection of ICAM-2

Four cases of human ALA were positive for ICAM-2. Although of less intensity than that seen for ICAM-1, label was irregularly detected in the inner surface of small-caliber blood vessels and sinusoids (Fig. 4).

Detection of ICAM-1

No reactivity was detected in non-ALA control specimens incubated with the anti-ICAM-1 antibody (Fig. 5). Specimens from 10 of 17 cases of ALA were positive for ICAM-1. The label was detected as dark irregular strands in the inner surface of sinusoids bordering hepatocytes (Fig. 6), localized mainly peripherally to necrosis. Medium-caliber blood vessels were also labeled when closely associated with abscess areas. One case of ALA associated with biliar duct hyperplasia showed additional intensive positivity for ICAM-1 in the proliferative epithelium.

Detection of vWf

Of 17 cases of ALA studied, 16 presented positive reactions to anti-vWf. Endothelial cells from small- and medium-caliber blood vessels and sinusoids near the necrotic tissue were mainly labeled (Fig. 8). Control specimens showed the absence of a reaction in sinusoidal cells. Labeling was seen only in endothelial cells from large-caliber blood vessels (Fig. 7).

Discussion

The presence and the role of inflammatory infiltrate in cases of invasive amebiasis have long been neglected by pathologists. However, in reports on experimental hepatic amebiasis studies using hamsters and gerbils (Tsutsumi et al. 1984; Tsutsumi and Martinez-Palomo 1988; Shibayama-Salas et al. 1992) it has been suggested that the presence of inflammatory cells is associated with *Entamoeba histolytica*, and this infiltrate seems to be important in the physiopathology of tissue damage. Accordingly, in vivo interaction of inflammatory cells with *E. histolytica* trophozoites leads to the lysis and release of potent enzymes by the former cells, and this could in turn destroy the liver parenchymal cells (Tsutsumi et al. 1984). To correlate evidence obtained in experimental models with human cases of ALA and to obtain some clues on the mechanisms of liver damage, especially those associated with the inflammatory reaction, in the present work we studied human specimens diagnosed as ALA. All the material studied exhibited different degrees of acute and chronic inflammatory reaction, which cannot simply be disregarded as a consequence of secondary infection. Furthermore, we consider that the observed extensive liver necrosis together with the abundant cell debris, including lysed leukocytes, could in some cases partly mask the actual presence of inflammation in invasive amebiasis.

The presence of specific ameba components that activate host cells in vivo, whether by contact or by diffusion, and the question as to whether these activated cells could in turn express or secrete molecules with proinflammatory properties are also aspects that have been poorly investigated. In this work, immunohistochemical detection of ameba antigens in human specimens revealed that well-preserved trophozoites were strongly labeled. Moreover, not only was the presence of ameba components limited to the parasite itself, but host cells such as endothelial, inflammatory, or hepatic parenchymal cells were also labeled in varying densities as a continuous gradient, depending on their closeness to the ameba. This feature suggests that besides via contact, some ameba components can also be secreted in the medium and captured by the neighboring host cells, which could be consecutively activated. Recent in vitro studies of *E. histolytica* interaction with human enterocyte cell lines have shown that a 170-kDa Gal/GalNAc-specific amebic lectin can be transferred to the host cells as one of the first steps in cell damage (Leroy et al. 1995).

On the basis of the observation that in the ameba-infected liver these parasites regularly interact directly or indirectly with endothelial cells, and in addition to the current knowledge on the role of activated endothelial cells as promoters of inflammatory reaction in other systems (Jonjic et al. 1992; Turunen et al. 1993; Imhof and Dunon 1995), we investigated by immunohistochemistry techniques the expression of endothelial molecules associated with inflammatory recruitment, such as

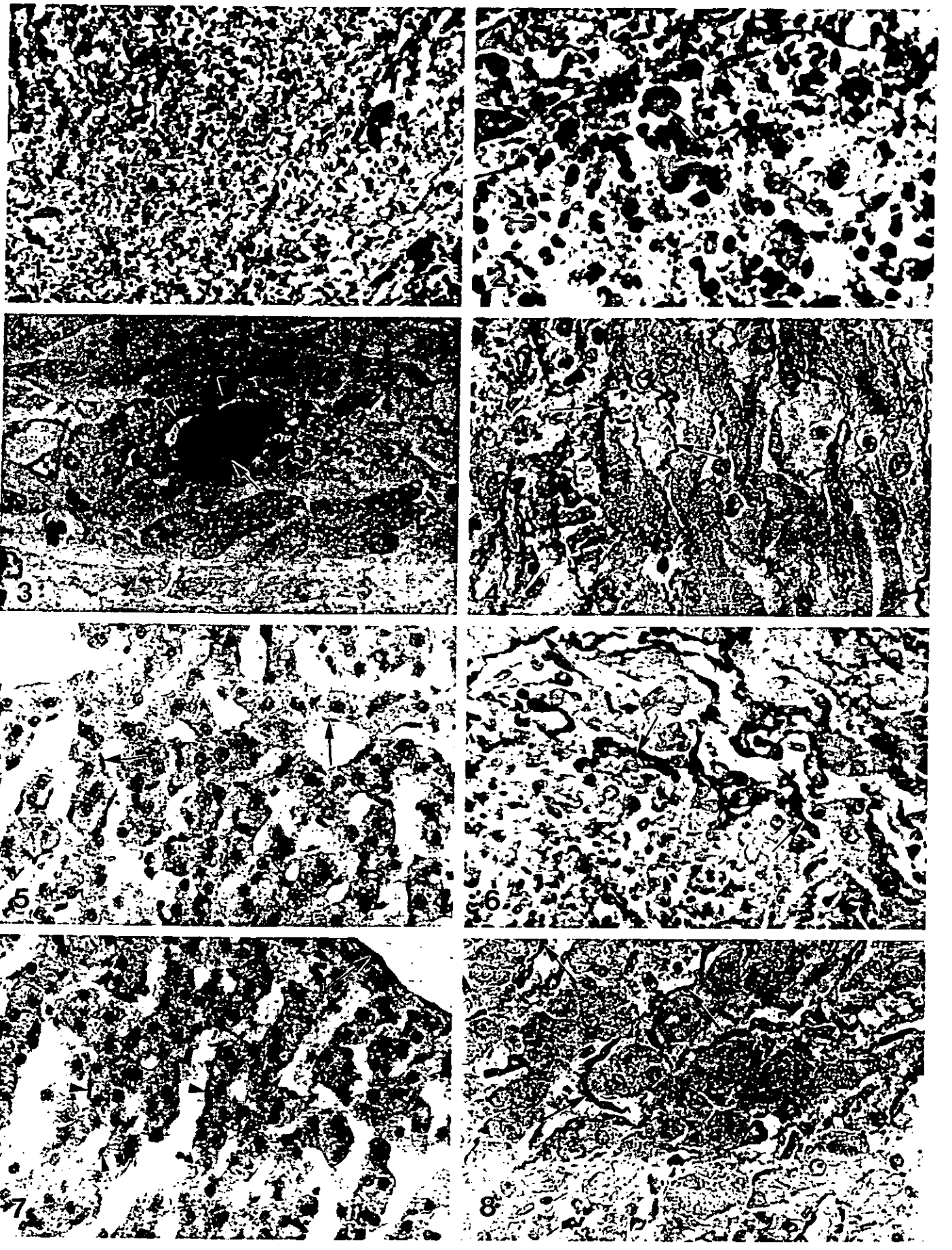
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Fig. 1 A large area of hepatic necrosis with cell debris and inflammatory infiltrate. The normal hepatic architecture has been lost. H&E. × 160. **Fig. 2** An *Entamoeba histolytica* trophozoite (*arrow*) surrounded by necrotic tissue and chronic inflammatory cells. H&E. × 330. **Fig. 3** A trophozoite of *E. histolytica* (*arrow*) with high reactivity to a polyclonal antibody against Eh antigens is localized in a sinusoid. Surrounding hepatocytes are also partially labeled (*arrowheads*). Peroxidase. × 330. **Fig. 4** Area of hepatic tissue close to necrosis. ICAM-2-positive endothelial cells (*arrows*) are limiting the inner surface of sinusoids. Peroxidase. × 330. **Fig. 5** Normal hepatic tissues were stained with anti-ICAM-1 human monoclonal antibody (Bender MedSystem). The endothelial cells were not stained (*arrows*). Peroxidase. × 330. **Fig. 6** Hepatic parenchyma neighboring amebic necrotic tissue shows ICAM-1-positive endothelial cells (*arrows*). Peroxidase. × 330. **Fig. 7** Normal hepatic tissues were reacted with the anti-human vWf antibody. Endothelial cells of some large-caliber vessels were stained (*arrow*), but the sinusoidal endothelial cells were negative (*arrowheads*). Peroxidase. × 330. **Fig. 8** vWf-positive sinusoidal endothelial cells (*arrows*) in an area similar to the one shown in Fig. 6. Peroxidase. × 330.

ICAM-1 and ICAM-2. The strong labeling by anti-ICAM-1 of endothelial cells from hepatic sinusoids localized close to amebic necrotic areas suggests that these endothelial cells are activated. The absence of a reaction to ICAM-1 observed in control non-ALA samples as compared with the positive labeling seen in 70% of ALA cases implies that amebas can induce an inflammatory reaction during the invasion.

Indirect stimulation of endothelial cells by cytokines produced by macrophages that have been activated by amebic antigens constitutes an alternative explanation of the observed inflammatory attraction. Eckmann et al. (1995) have recently reported that several human cell lines cocultured with *E. histolytica* trophozoites secrete several chemoattractant and proinflammatory cytokines, including interleukin 8 (IL-8) and IL-6. Although the cases that were negative for ICAM-1 in the present study could have been due to the antiinflammatory treatment of patients, this statement cannot be strongly supported, since a couple of untreated patients displayed moderately positive reactivity to ICAM-1 (Table 1).

The few cases of weak labeling for ICAM-2 shown in human ALA may have been due to technical difficulties rather than being an actual event since due to the lack of a commercial anti-human ICAM-2 antibody, the primary antibody we used was directed against mice in expectations of a cross-reactivity with human antibody, a result that was not clear.

Another molecule associated with activated endothelial cells is vWf (Wagner et al. 1982; Wagner 1990; Wu 1992). This factor, which is related to blood clotting, may enhance thrombus formation, which can in turn produce ischemia. In most of the human ALA cases of ALA studied histochemically, this factor was positive on the inner surface and lumen of sinusoids localized in the neighborhood of necrotic tissue, suggesting an activation of sinusoidal cells, however, the relevance of the high level of expression of this molecule found in ALA remains to be determined. In experimental models we have shown the presence of ischemic parenchymal areas, although this phenomenon has been ascribed more to physical occlusion of blood vessels by the parasites than to thrombus formation (Tsutsumi et al. 1984).

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