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Arg-Gly-Asp (RGD) Peptides Alter Hepatic Killing of *Candida albicans* in the Isolated Perfused Mouse Liver Model

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The isolated perfused mouse liver model was used to study the effect of Arg-Gly-Asp (RGD)-containing peptides on hepatic trapping and killing of *Candida albicans*. After extensive washing, 10^6 *C. albicans* CFU were infused into mouse livers. At the time of recovery, 63% ± 2% (mean ± standard error of the mean) of the infused *C. albicans* CFU were recovered from the liver and 14% ± 1% were recovered from the effluent for a total recovery of 77% ± 2%. This indicates that 86% ± 9% of the original inoculum was trapped by the liver and that 23% ± 2% was killed within the liver. Prior to their infusion into livers, 10^7 CFU of *C. albicans* were incubated at 37°C for 30 min in the presence of various RGD peptides (0.1 mg/ml). Repeatedly, more than 90% of the infused RGD-treated *C. albicans* was trapped by the perfused liver. In comparison with the 23% killing rate observed in control livers, perfused livers killed approximately 40 to 50% of the infused *C. albicans* treated either with fibronectin, PepTite 2000, RGD, or RGDS. Hepatic killing of *C. albicans* treated with PepTite 2000 or fibronectin was dose dependent. Treatment of *C. albicans* with GRGDTP, GRGDSP, GRADSP, or GRGESP did not alter the ability of the perfused liver to kill *C. albicans*, suggesting that a degree of specificity for RGD peptides is associated with an increased ability of liver to kill RGD-treated *C. albicans*. Together, the data suggest that RGD peptides bind to a receptor on the surface of *C. albicans*, thereby increasing hepatic, and presumably Kupffer cell, killing of *C. albicans*. Natural or synthetic RGD peptides may serve as opsonins promoting *C. albicans* killing by Kupffer cells.

Bloodstream clearance of the opportunistic fungal pathogen *Candida albicans* results in the deposition of large numbers of yeasts in the liver (26, 27). The liver clears *C. albicans* from the bloodstream by two disparate mechanisms, both involving the adhesion of yeasts to liver cells. Endothelial trapping results from *C. albicans* adhesion to hepatic sinusoidal endothelial cells, whereas phagocytic trapping results from *C. albicans* adhesion to Kupffer cells (1, 26, 27). As a result of *C. albicans* adhesion, the liver subsequently eliminates trapped yeasts. Studies suggest that *C. albicans* adhesion is mediated in part by liver cell receptors combining with mannose-containing adhesions on the external cell wall of *C. albicans* (23, 25). Alteration of the yeast cell surface affects hepatic trapping and killing of *C. albicans* (25).

Cellular adhesion is necessary to maintain anatomical relationships within tissues and is mediated in part by a family of surface glycoproteins, the integrins. Integrins bind ligands within the basement membrane, collagens, fibronectin, or laminin by recognizing the amino acid sequence Arg-Gly-Asp (RGD). Integrins that specifically bind to the RGD sequence of fibronectin and other RGD-containing ligands are found on a variety of cells (7, 11, 20, 33). The molecular biology and structure of the various integrins and their ligands have been reviewed elsewhere (9, 22). The outer cell wall of *C. albicans* may contain three integrinlike proteins (8, 10, 16, 30). *C. albicans* binds RGD peptides such as fibronectin (12, 14, 29), laminin (3, 14), type I and type IV collagen (13, 14), fibrinogen (2), fibrin (16), iC3b (5, 8, 10), and synthetic RGD peptides (14). Klotz and Smith (14) suggested that the integrinlike RGD receptor on the surface of *C. albicans* plays a role in the adherence of *C. albicans* to fibronectin within the subendothelial extracellular matrix, and Gustafson et al. (8) suggested that an integrin analog structurally related to the alpha subunit of CD11b/CD18 plays a role in the adhesion of *C. albicans* to human endothelium. In the present study, we used the isolated perfused mouse liver model to test the hypothesis that hepatic trapping and killing of *C. albicans* are affected by RGD treatment of yeast cells.

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MATERIALS AND METHODS

The protocol used in this study was approved by the Mercer University Institutional Animal Care and Use Committee and conformed to National Institutes of Health guidelines for the humane use of experimental animals.

Animals. Female Hsd/ICR mice (Harlan Sprague-Dawley, Indianapolis, Ind.), 8 to 12 weeks old, were maintained under standard laboratory conditions with food and water available ad libitum.

Organism and culture conditions. *C. albicans* 20A was a gift of Judith E. Domer, Tulane University Medical School, New Orleans, La. Cultures were maintained on Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, Mich.) slants at 24°C. Fresh transfers were made into 100 ml of tryptic soy broth (Difco) plus 0.5 M galactose, and the pH was adjusted to 6.9. Liquid cultures were incubated at 37°C in an Orbit incubator-shaker (model 3526; Lab-Line Instruments, Inc., Melrose Park, Ill.) for 18 h. Cultures were harvested and washed three times in ice-cold sterile saline by centrifugation at 1,700 × *g* for 5 min at 4°C. Hemacytometer counts of 10-fold serial dilutions in ice-cold saline were used to adjust the concentration of inoculum to approximately 10^6 yeasts per ml of RPMI 1640 plus glutamine (Fischer Scientific Co., Atlanta, Ga.). Approximately 10^6 yeast cells per ml in 10 ml of RPMI 1640 were homogenized in a sterile ice-cold

tube with an ice-cold Teflon pestle, aseptically transferred to a sterile test tube, and held on ice. One milliliter of the stock inoculum was withdrawn with a 1-ml syringe and warmed to 37°C, providing the inoculum for infusion of yeast cells into perfused livers. Quantitative pour plates of 10-fold serial dilutions of the inoculum were made in SDA and incubated overnight at 37°C, and the number of CFU in the inoculum was counted on an model 880 colony counter (Artek Corp., New York, N.Y.). Less than 10% variability between hemacytometer counts and the number of CFU in the inoculum was noted throughout the study. In control studies, there was no decrease in yeast number or yeast viability due to killing of yeast cells by homogenization, aggregation of yeast cells within the inoculum (as determined by visual inspection), or adhesion of yeast cells to glassware or to cellular debris.

Mouse liver perfusion. The methods for mouse liver perfusion have been described in detail elsewhere (18). All perfusions were performed with buffers and with the inoculum prewarmed to 37°C. Mice were anesthetized by intraperitoneal injection of pentobarbital and then heparinized. A midline incision was made, and the intestines were reflected to expose the portal vein. Ligatures were placed around the portal vein, above and below the splenic vein, and above the renal veins. A cannula was inserted into the portal vein, the ligatures were secured, and perfusion medium was run out through a nick in the inferior vena cava to wash the liver free of blood. The rib cage was removed, and a ligature was placed around the inferior vena cava above the diaphragm. A cannula was inserted through a nick in the right atrium into the inferior vena cava and secured with the ligature. The ligature above the renal veins was closed. Perfusion medium was allowed to flow through the portal vein into the liver and was collected aseptically from the efferent cannula in a sterile bottle. The liver does not swell during perfusion.

Before *C. albicans* infusion, the liver was washed with approximately 20 to 30 ml of prewarmed RPMI 1640 (preperfusion washing). Approximately 10^6 CFU of *C. albicans* in 1 ml of RPMI 1640 was infused through a three-way valve placed on the afferent cannula. After infusion of *C. albicans*, the three-way valve was switched to the perfusion buffer (postperfusion buffer) reservoir, the liver was washed with 50 ml of RPMI 1640, and the perfusion buffer was collected aseptically from the efferent cannula in a sterile bottle. At the end of the perfusion, the liver was disconnected from the perfusion apparatus, excised, and transferred to 9 ml of sterile saline. The inoculum, the liver, and 10 ml of the effluent were individually homogenized in ice-cold tubes with an ice-cold Teflon pestle. Tenfold serial dilutions of the inoculum, liver homogenate, and effluent were made in ice-cold saline, and quantitative pour plates and SDA were used to determine the number of CFU. One hundred percent of the viable CFU were recovered from the inoculum control taken at the finish of the perfusion in comparison with the inoculum control taken before the infusion of 10^6 *C. albicans* CFU. This shows that there was no decrease in the number of CFU infused due to a loss in yeast viability, aggregation of yeast cells, or adhesion of yeast cells to glassware.

The percentage of *C. albicans* CFU killed was calculated as 100% minus the percentage recovered from the liver homogenate minus the percentage in the effluent. The percentage of *C. albicans* CFU trapped by the liver was calculated as the percentage recovered in the liver homogenate plus the percentage killed by the liver. On the basis of previous studies (18, 26, 27), we assumed that killing resulted from the phagocytic clearance and killing of microbes within Kupffer cells.

RGD peptides. Arg-Gly-Asp (RGD) was purchased from Sigma Chemical Co., St. Louis, Mo. The other synthetic peptides were purchased from Telios Pharmaceuticals, San Diego, Calif. RGD peptide purity was determined by the presence of a single peak by high-performance liquid chromatography. The following RGD peptides were used: RGD (Arg-Gly-Asp), RGDS (Arg-Gly-Asp-Ser), GRGDSP (Gly-Arg-Gly-Asp-Ser-Pro), GRGDTP (Gly-Arg-Gly-Asp-Thr-Pro), murine fibronectin, and PepTite 2000 (an RGD-containing synthetic peptide with an approximate molecular mass of 2,000 Da. The synthetic peptides GRADSP (Gly-Arg-Ala-Asp-Ser-Pro) and GRGESP (Gly-Arg-Gly-Glu-Ser-Pro) were used as controls.

RGD peptide treatment of *C. albicans*. *C. albicans* was incubated and harvested as described above, and the number of yeasts was adjusted to approximately 10^8/ml by hemacytometer counts. RGD peptides and control peptides were dissolved in 9 ml of saline at a concentration of 0.1 mg/ml and sterilized by filtration. A 1:10 dilution of the yeast cell stock into the saline plus peptide was made. *C. albicans* (10^7 CFU) was incubated in the presence of peptide at 37°C for 30 min on a tipping platform. After incubation, peptide-treated *C. albicans* was transferred to an ice-cold tube and homogenized with an ice-cold Teflon pestle. A 1:10 dilution into 9 ml of ice-cold RPMI 1640 was made in order to provide the inoculum, which contained approximately 10^6 peptide-treated *C. albicans* CFU/ml. Quantitative SDA pour plate counts of 10-fold serial dilutions of the inoculum were made after peptide treatment. Comparisons of the number of viable CFU made before and after peptide treatment were used to determine whether peptide treatment resulted in a reduction in *C. albicans* number or viability. As a result of peptide treatment, there was no reduction in yeast number or viability. Visual inspection of yeasts after peptide treatment indicated no aggregation of yeast cells. During the perfusion experiment, the treated inoculum was held on ice and homogenized as described above, and quantitative SDA pour plates were prepared at the end of the experiment. Comparison of the number of CFU recovered from the pretreatment inoculum and the number recovered from the treated inoculum before and after the perfusion experiment showed no reduction in yeast cell number or viability. This suggests that there was no artificial loss of yeast cells in the inoculum due to loss in viability from homogenization, aggregation of treated yeast cells, or adhesion of yeast cells to glassware or each other. At the end of the perfusion, the liver was excised and the numbers of CFU in the liver homogenate, inoculum, and effluent were determined as described above.

Hepatic clearance and killing of *C. albicans* treated with increasing concentrations of RGD peptides. *C. albicans* was incubated and harvested as described previously, and the number of yeasts was adjusted to approximately 10^8/ml by hemacytometer counts. PepTite 2000 and soluble murine fibronectin were dissolved in 9 ml of sterile saline at the desired concentrations and sterilized by filtration. A 1:10 dilution of the yeast cell suspension into the saline plus peptide was made. *C. albicans* (10^7 CFU) was incubated in the presence of increasing peptide concentrations at 37°C for 30 min on a tipping platform. After incubation, peptide-treated *C. albicans* was transferred to an ice-cold tube and homogenized with an ice-cold Teflon pestle. A 1:10 dilution into 9 ml of ice-cold RPMI 1640 was made so as to provide the inoculum containing approximately 10^6 peptide-treated

TABLE 1. Hepatic clearance and killing of *C. albicans* treated with peptides containing the Arg-Gly-Asp (RGD) sequence[a]

Expt/ treatment	% Recovery			% Killed	% Trapped
	Liver	Effluent	Total		
None[b]	63 (2)	14 (1)	77 (2)	23 (2)	86 (9)
RGD	56 (8)	4 (1)*	60 (5)*	40 (5)*	96 (1)
RGDS	54 (8)	4 (1)*	58 (8)*	42 (8)*	96 (1)
Fibronectin	46 (3)*	4 (1)*	50 (3)*	50 (2)*	96 (2)
PepTite 2000	49 (2)*	1 (1)*	50 (1)*	50 (2)*	99 (1)
GRGDTP	54 (4)	10 (1)	64 (4)	36 (4)	90 (1)
GRGDSP	58 (3)	9 (2)	67 (3)	33 (3)	91 (2)
GRGESP	64 (4)	7 (1)	71 (5)	29 (5)	93 (1)
GRADSP	59 (2)	11 (2)	70 (4)	30 (4)	89 (3)

[a] Each value is the mean of at least five separate experimental determinations, with the standard error of the mean given in parentheses. *, $P = 0.01$ (White rank order test).

[b] Control values represent the grand mean of 63 separate experimental determinations.

TABLE 2. Effect of PepTite 2000 on hepatic clearance and killing of *C. albicans*[a]

Use of PepTite 2000	% Recovery			% Killed	% Trapped
	Liver	Effluent	Total		
None[b]	63 (2)	14 (1)	77 (2)	23 (2)	86 (9)
Preperfusion	70 (7)	7 (1)	77 (7)	23 (6)	93 (1)
Treatment	49 (2)*	1 (1)*	50 (1)*	50 (2)*	99 (1)
Postperfusion	52 (6)	14 (4)	66 (7)	34 (7)	86 (4)

[a] Each value represents the mean of at least five separate experimental determinations, with the standard error of the mean given in parentheses. *, $P = 0.01$ (White rank order test).

[b] Control values represent the grand mean of 63 separate experimental determinations.

C. albicans CFU/ml. Liver perfusions and perfusion controls were performed as described previously. As a result of peptide treatment, there was no reduction in yeast number or viability and no aggregation of yeast cells in the inoculum. At the end of the perfusion, the liver was excised and the numbers of CFU in the liver homogenate, inoculum, and effluent were determined as above.

Statistics. Statistical analysis of data was performed by the White rank order test (32). Probability values equal to or less than 0.01 were considered significant.

Normally, for each perfusion experiment a group of five control perfusions with untreated yeast cells with no RGD peptide present was performed. A comparison of control data in the present study and similar perfusion data for mice in previous studies (23, 25–28) indicated that there was little, if any, significant variation in the hepatic clearance and killing of untreated *C. albicans* by normal mouse livers. For the sake of brevity, at the end of this study, we pooled the data from all of our control perfusions. In each table, the data presented in the experimental category of "none" represent a grand mean and standard error of the mean for 63 separate experimental determinations. Statistical comparisons were made by using the grand mean for control mice.

RESULTS

Hepatic trapping and killing of *C. albicans*. Hepatic tissue was washed with prewarmed RPMI 1640, and 10^6 CFU of untreated *C. albicans* were infused. Perfusions were performed in the absence of RGD peptide (Table 1). Approximately 63% of the inoculum was recovered from the liver homogenate, and 14% was recovered in the effluent, for a total recovery of 77%. This suggests that approximately 86% of the inoculum was trapped by the liver and that 23% of the total inoculum was killed by the liver.

Effect of RGD peptide treatment of *C. albicans* on hepatic killing of yeasts. Perfused livers trapped approximately 90% of the infused untreated or RGD-treated *C. albicans* (Table 1). In comparison with the 23% rate of killing of untreated *C. albicans*, significantly greater numbers of RGD-treated *C. albicans* cells were killed by perfused livers. Perfused livers killed 40 and 42% of the infused *C. albicans* treated with RGD and RGDS, respectively. Perfused livers killed 50% of the infused *C. albicans* treated either with murine fibronectin or with PepTite 2000. In contrast, hepatic killing of *C. albicans* was unchanged when the yeasts were treated with RGD peptides in which the RGD sequence was flanked by glycine on one side and either threonine-proline (GRGDTP) or serine-proline (GRGDSP) on the other side. Treatment with peptides containing amino acid substitutions in the RGD sequence (GRGESP or GRADSP) also failed to increase hepatic killing of *C. albicans*.

Effect of PepTite 2000 on hepatic trapping and killing of *C. albicans*. In order to more clearly define whether hepatic killing of RGD-treated *C. albicans* resulted from a direct effect on the liver or a direct effect on *C. albicans*, perfusion experiments in which PepTite 2000 was included in either the preperfusion or postperfusion buffer only were performed. With RGD peptide present only in the preperfusion buffer, we tested for the possibility that RGD peptide, by combining with hepatic integrins and not with *C. albicans*, would thereby affect hepatic trapping and killing of *C. albicans*. With RGD peptide present only in the postperfusion buffer, we tested for the possibility that RGD peptide would elute trapped, untreated *C. albicans*, suggesting that the affinity of hepatic integrins might be higher for the RGD peptide than for *C. albicans*. A comparison with hepatic killing of PepTite 2000-treated *C. albicans* was then made by testing the possibility that RGD peptides opsonize *C. albicans* for increased hepatic killing. In comparison with the controls (no RGD peptide), which killed approximately 23% of the infused untreated *C. albicans*, the inclusion of PepTite 2000 in either the preperfusion buffer alone or the postperfusion buffer alone had no effect on hepatic killing of untreated *C. albicans* (Table 2). Hepatic tissue killed approximately 50% of the infused PepTite 2000-treated *C. albicans*.

Hepatic trapping and killing of *C. albicans* treated with increasing concentrations of either PepTite 2000 or murine fibronectin. Since RGD peptides affect hepatic killing of *C. albicans* by exerting their effect on *C. albicans* and not directly on the liver, perfusion experiments were performed to determine whether treatment of *C. albicans* with increasing concentrations of either PepTite 2000 or murine fibronectin could increase killing of trapped yeasts. Hepatic killing of RGD peptide-treated *C. albicans* was dose dependent for both PepTite 2000 and murine fibronectin. The perfused liver killed approximately 50% of the infused *C. albicans* treated with 0.125 mg of PepTite 2000 per ml and approximately 37% of the *C. albicans* treated with 0.031 mg of PepTite 2000 per ml (Table 3). Approximately 39% of the infused *C. albicans* treated with 0.100 mg of fibronectin per ml was killed, whereas no significant change in hepatic killing was observed when yeasts were treated with 0.050 mg of fibronectin per ml (Table 4).

TABLE 3. Hepatic clearance and killing of *C. albicans* treated with increasing concentrations of PepTite 2000[a]

Treatment and concn (mg/ml)	% Recovery			% Killed	% Trapped
	Liver	Effluent	Total		
None[b]	63 (2)	14 (1)	77 (2)	23 (2)	86 (9)
PepTite 2000					
0.008	70 (3)	10 (2)	80 (3)	20 (3)	90 (2)
0.016	60 (3)	8 (2)	68 (3)	32 (3)	92 (2)
0.031	57 (3)	6 (2)*	63 (3)*	37 (3)*	94 (2)
0.063	56 (4)	5 (1)*	61 (5)*	39 (5)*	95 (1)
0.125	49 (2)*	1 (1)*	50 (1)*	50 (2)*	99 (1)

[a] Each value represents the mean of at least five separate experimental determinations, with the standard error of the mean given in parentheses. *, $P = 0.01$ (White rank order test).

[b] Control values represent the grand mean of 63 separate experimental determinations.

DISCUSSION

Normal mouse (23, 25, 28), rabbit (1), and rat (26, 27) livers trap greater than 90% of infused, untreated *C. albicans*. Trapping occurs by the adhesion of *C. albicans* to sinusoidal endothelial cells or to Kupffer cells, which presumably phagocytize and kill *C. albicans* after trapping the yeasts (26, 27). In part, trapping of *C. albicans* is the result of an interaction between external cell wall adhesins and receptors for those adhesins on sinusoidal endothelial cells or Kupffer cells. Cell wall mannans and glucomannoproteins mediate *C. albicans* adherence to the liver (23, 25). Less well characterized are the host cell receptors for *C. albicans*. In the present study, we used the perfused mouse liver model to explore the possibility that RGD peptides affect hepatic trapping and killing of *C. albicans*. When *C. albicans* was incubated in the presence of various RGD peptides and then infused into livers, there was no change in hepatic trapping of *C. albicans*. Perfused livers repeatedly trapped more than 90% of the inoculum. However, there was a dramatic increase in hepatic killing of trapped *C. albicans*.

Normal perfused mouse livers kill about 20% of infused *C. albicans* (23, 25, 28). Killing occurs when livers are perfused with buffer alone in the absence of antibody, complement, or other host plasma molecules. A previous study showed that rat perfused livers are unable to kill *C. albicans* either in the presence or in the absence of whole blood (26). In contrast, in the absence of blood or blood factors perfused mouse livers kill about 20% of infused *C. albicans* (25, 28). When *C. albicans* is opsonized by immune serum, killing is significantly increased to about 40% of the inoculum (28). An assumption central to these studies is the idea that the killing of *C. albicans* in the perfused liver model is due to the phagocytic uptake and killing of yeasts by Kupffer cells. This conclusion is supported by transmission (1) and scanning (26, 27) electron microscopic examination of livers infused with *C. albicans* and by studies in which Kupffer cell killing of microbes is blocked by silica treatment (6, 26, 27). However, it should be noted that differential cell counting of hepatic nonparenchymal cell populations isolated after enzymatic digestion of the liver suggests that as many as 2 to 3% of the nonparenchymal cells are neutrophils (unpublished observations). Highly candidacidal neutrophils marginated within hepatic sinusoids could account for some of the ability of perfused livers to kill *C. albicans*. Despite this pitfall, when taken together, these data suggest that Kupffer cells play a major role in the phagocytic killing of opsonized and unopsonized *C. albicans*. A study comparing the abilities of resident pulmonary alveolar macrophages to kill *C. albicans* demonstrated a disparity in the ability of resident pulmonary alveolar macrophages isolated from various inbred mouse strains to kill *C. albicans* in vitro (24). It is unknown whether Kupffer cell candidacidal activity in the perfused liver is related to the species or to the genetic strain of animal used.

TABLE 4. Hepatic clearance and killing of *C. albicans* treated with increasing concentrations of soluble murine fibronectin[a]

Treatment and concn (mg/ml)	% Recovery			% Killed	% Trapped
	Liver	Effluent	Total		
None[b]	63 (2)	14 (1)	77 (2)	23 (2)	86 (1)
Fibronectin					
0.025	64 (6)	10 (2)	74 (4)	26 (4)	90 (2)
0.050	79 (6)	16 (5)	95 (3)	5 (2)	84 (5)
0.100	45 (7)*	16 (3)	61 (5)*	39 (5)*	84 (3)

[a] Each value represents the mean of at least five separate experimental determinations, with the standard error of the mean given in parentheses. *, $P = 0.01$ (White rank order test).

[b] Control values represent the grand mean of 63 separate experimental determinations.

Perfused mouse livers killed 23% of infused untreated *C. albicans* and 40 to 50% of infused *C. albicans* treated with RGD, RGDS, murine fibronectin, or PepTite 2000. We found that PepTite 2000 enhanced hepatic killing of treated *C. albicans* and had no effect on hepatic killing of yeasts when it was included only in the preperfusion or postperfusion buffer. Killing of PepTite 2000-treated and fibronectin-treated *C. albicans* was dose dependent. Moreover, killing was not increased when *C. albicans* was treated with RGD peptides flanked by glycine on one side and either threonine-proline (GRGDTP) or serine-proline (GRGDSP) on the other side. Treatment of *C. albicans* with the non-RGD peptides GRADSP and GRGESP failed to increase hepatic killing of yeast cells. The data suggest that RGD, RGDS, fibronectin, and PepTite 2000 treatment opsonized *C. albicans* for increased killing within the perfused liver. PepTite 2000 is a proprietary synthetic peptide containing the amino acid sequence GRGDSPASSK surrounding the RGD binding sequence of native plasma fibronectin. It has been cited in preliminary work suggesting that RGD-containing peptides inhibit both the hematogenous dissemination of *C. albicans* (14a) and the adhesion of *C. albicans* to target substrates coated with extracellular matrix components (13a).

Candidacidal activity of the liver against RGD-treated *C. albicans* in perfusion buffer alone is curious. Ding and Nathan (4) show that ICR Kupffer cells lack the capacity for a respiratory burst in vitro. The defect is selective for the reduction of O_2 by NADPH, with no H_2O_2 being produced, and Kupffer cells recover the ability for oxidative metabolism upon prolonged culture. Lehrer and coworkers (15, 19) have demonstrated the importance of nonoxidative metabolism in the candidacidal activity of both neutrophils and mononuclear phagocytes. There may be a difference between the ability of Kupffer cells to generate nonoxidative killing mechanisms when encountering RGD-treated *C. albicans* in vitro and the ability of Kupffer cells which remain in a more unperturbed, natural setting such as the perfused liver to generate such mechanisms. It may be that RGD-treated *C. albicans* engage protein-based, nonoxidative candidacidal systems within Kupffer cells, similar to those described for other mononuclear phagocyte populations (19). Alternatively, RGD treatment might alter the cell wall

of *C. albicans*, rendering the yeast cells susceptible to candidacidal systems within Kupffer cells.

In the present study, fibronectin treatment of *C. albicans* enhanced the candidacidal activity of the perfused liver. Soluble fibronectin binds to *C. albicans* at a K_d of 1.1×10^{-7} M with approximately 8,000 binding sites per yeast cell (14). *C. albicans* adherence inhibition experiments suggest that the host cell receptor for *C. albicans* adhesion is an integrin-like molecule (14). There are at least three proteins on the surface of *C. albicans* with integrinlike binding activity. One protein binds antibody against the cytoplasmic domain of the B_1 integrin epitope (17), one protein reacts with antibody to the alpha subunit of the human integrin iC3b receptor (8, 10), and yet another protein may have broad binding activity for laminin, fibrinogen, and C3d (10, 30). When these observations are taken together, it is reasonable to conclude that Kupffer cell integrins act as receptors for RGD peptides which are bound to integrinlike molecules on the surface of *C. albicans*. We hypothesize that naturally occurring soluble RGD peptides such as soluble fibronectin function as an opsonic bridge for the phagocytic uptake and killing of *C. albicans* by Kupffer cells. The role of fibronectin as an opsonin is controversial, and its therapeutic use has not been effective (21). Van De Water et al. (31) found that while plasma fibronectin bound to a variety of gram-positive and gram-negative bacteria and to *Saccharomyces cerevisiae*, it was not needed for the uptake of microbes by a variety of phagocytic cell types. Whether the experimental results of Van De Water et al. (31) reflect differences between their in vitro phagocytic assay and the perfused liver model used in the present study is unknown. It may be the case, for example, that fibronectin does not enhance phagocytosis, although it might alter the ability of phagocytes to kill ingested microbes. Alternatively, phagocytic cells may recognize microbes by a variety of either specific (opsonization) or nonspecific adhesion mechanisms. Nevertheless, novel therapeutic agents such as PepTite 2000 or RGD may function as opsonins for *C. albicans*, promoting phagocytic clearance and killing of yeasts in certain life-threatening clinical settings such as *Candida* sepsis and hematogenously disseminated candidiasis.

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