3. For the isolation of $HBLV_{dp}$ DNA, supernatants from HBLV-infected umbilical-cord blood cells were layered onto 20% glycerol cushions and centrifuged at 30,000 rev/min for 3 hours in a Beckman SW41 rotor at 4°C. The pellets were suspended in TNE buffer (10 m*M* tris-HCl, *p*H 9; 100 m*M* NaCl; 1 m*M* EDTA), and extracted with PCI9 (phenol:chloroform:isoamyl alcohol:tris-HCl, *p*H 9::100:100:1 by volume) followed by extraction with chloroform–isoamyl alcohol (24:1 by volume). Nucleic acids were precipitated by addition of two volumes of 95% ethanol ($HBLV_{dp}$ DNA). $HBLV_{dp}$ DNA was digested with Hind III and cloned into the Bluescribe vector (Vector Cloning Systems). Several clones obtained after screening with nick-translated (^{32}P-labeled) $HBLV_{dp}$ DNA as a probe were examined for specificity of hybridization to infected human umbilical-cord blood cell DNA and by in situ hybridization to cytoplasmic RNA.
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5. For the isolation of $HBLV_{bv}$ DNA, the gradient-banded virions were lysed by addition of SDS to 1% and proteinase K to 100 μg/ml and incubated at 37°C for 30 minutes and then at 65°C for 5 minutes. One volume of PCI9 was added and the mixture gently shaken for 5 minutes and then centrifuged in an Eppendorf centrifuge at 4°C for 15 minutes. The supernatant was removed, and extracted with one volume of chloroform–isoamyl alcohol (24:1) and centrifuged for 5 minutes. The aqueous layer was removed and the DNA precipitated by addition of 2 volumes of ethanol. The DNA was dissolved in 50 μl of water. The DNA recovered amounted to approximately 1 μg.
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9. The HSV-1 probe pSG18F-B50 was a gift of R. Robinson of the University of Texas Health Science Center, Dallas, TX. The VZV Bam HI–E fragment probe and VZV genomic DNA were gifts of S. Strauss of NIAID, NIH, Bethesda, MD. The HVS probe pT7.4 and HVS genomic DNA were gifts of R. Desrosier [R. C. Desrosier, R. L. Burghoff, A. Bakker, J. Kamine, *ibid.* **49**, 343 (1984)]. The CMV Eco RI–A fragment (pHD-1) probe was a gift of E. S. Huang [M. G. Davis, E. C. Mar, Y.-M. Wu, E.-S. Huang, *ibid.* **52**, 129 (1984)]. The EBV Bam HI–K fragment (strain B 95-8) was a gift of M. Nonayamo [G. Miller, T. Shope, H. Lisco, P. Stitt, M. Kipman, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 383 (1972)]. The EBV Bam HI–C fragment was provided by N. T. Chang of Baylor School of Medicine, Houston, TX. EBV genomic DNA was purchased from Showa University Research Institute for Biomedicine in Florida, St. Petersburg, FL.
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Trypanosoma cruzi Infection Inhibited by Peptides Modeled from a Fibronectin Cell Attachment Domain

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The mechanism by which *Trypanosoma cruzi*, the protozoan parasite that causes Chagas' disease, becomes attached to mammalian cells is not well understood. Fibronectin is thought to participate in the attachment, and in this study the region of fibronectin that interacts with the surface receptors of *T. cruzi* trypomastigotes was investigated by testing the binding of the amino acid sequence Arg-Gly-Asp-Ser, corresponding to the cell attachment site of fibronectin to *T. cruzi* trypomastigotes. Peptides with the sequence Arg-Gly-Asp-Ser, but not Arg-Phe-Asp-Ser, Arg-Phe-Asp-Ser-Ala-Ala-Arg-Phe-Asp, Ser-Lys-Pro, Glu-Ser-Gly, or Ala-Lys-Thr-Lys-Pro, bound to the parasite surface and inhibited cell invasion by the pathogen. Monoclonal antibodies to the cell attachment domain of fibronectin also inhibited cell infection by the parasite. The immunization of BALB/c mice with tetanus toxoid–conjugated peptide induced a significant protection against *T. cruzi*. The data support the notion that the sequence Arg-Gly-Asp-Ser of cell surface fibronectin acts as a recognition site for attachment of the parasites.

Attachment of *Trypanosoma cruzi*, the causative agent of Chagas' disease, to mammalian cells involves the interaction of the plasma membranes of the pathogen and the host cell. This interaction is believed to be of fundamental importance in the host cell invasion by the pathogen. Investigators searching for molecules that might mediate parasite-cell interactions believe that the receptor or receptors on host cells for parasite binding are glycoproteins (*1*). However, little is known about their nature.

Fibronectin (Fn), which is a high molecular weight glycoprotein composed of 220-kD subunits linked into dimers and polymers by disulfide bonds, is present in blood and connective tissue and at cell surfaces (*2*). It participates in a number of cell surface interactions with the local extracellular microenvironment (*3, 4*). Fibronectin can mediate the attachment of pathogens such as *Treponema pallidum* (*5*) and *Leishmania* species (*6*) to the host cell surface. Recently, we demonstrated that Fn interacts with *T. cruzi* trypomastigote surface receptor (*7*). In addition, involvement of this Fn receptor in the interaction between *T. cruzi* and vertebrate cells has been reported (*8*).

The Fn molecule consists of highly structured domains containing the binding sites for the macromolecules that interact with it (*9*). The site of its attachment to mammalian cells has been characterized (*10*), and the analysis of small synthetic peptides has shown that this recognition site is carried by the sequence Arg-Gly-Asp-Ser (*11*). The region of Fn that interacts with the surface of the trypomastigote, the infective stage of the parasite, is unknown. We used monoclonal antibodies to the cell-attachment site in Fn (*10*), as well as synthetic peptides modeled from the sequence of this domain (Table 1), to show that this dimeric protein acts as a bridge between the cell surfaces of host cell and *T. cruzi* with the same hydrophilic sequence Arg-Gly-Asp-Ser of Fn participating in these interactions.

Analysis of the data in Fig. 1 indicates that the infectivity of 3T3 fibroblasts by *T. cruzi* can be inhibited by monoclonal antibodies to Fn. The antibodies M1205 and 3E3 inhibited cell invasion by the parasite at antibody concentrations greater than 0.1 mg/ml, whereas 4B2 was inhibitory only at high concentrations.

Both 3E3 and 4B2 reacted with the 120-kD chymotryptic fragment of Fn. However, fractionation of a pepsin digest of this fragment allowed the identification of a large fragment of 50 kD that binds to a 4B2-adsorbed Sepharose column and a small one (15 kD) that binds to 3E3-adsorbed Sepharose. Biological activity that promoted cell attachment was found in the 15-kD peptide (*10*). On account of the inhibitory effect of the monoclonal antibody 3E3 on cell inva-

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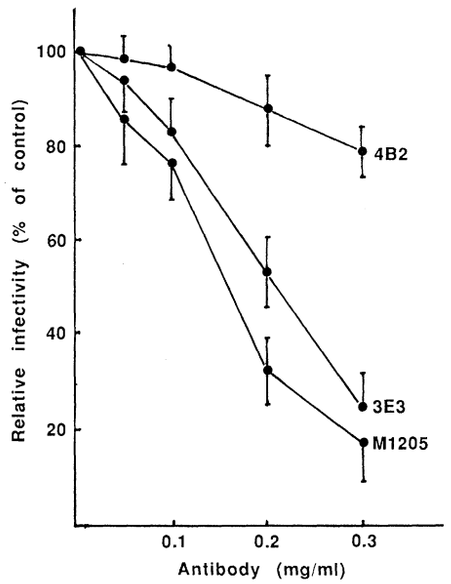

Fig. 1. Relative infectivity of fibroblasts in the presence of monoclonal antibodies to Fn. Monolayers of 3T3 cloned fibroblasts were maintained as described (*7*), and stock cultures were grown as monolayers at 37°C in RPMI 1640 supplemented with 5% (v/v) heat-inactivated fetal calf serum (FCS), penicillin (100 U/ml), and streptomycin (100 μg/ml). The Y strain of *T. cruzi* (*20*) was used throughout. Trypomastigotes were maintained in tissue culture by weekly infection of 3T3 fibroblasts as described (*21*). Parasite suspensions consisted of 90% trypomastigotes and 10% amastigotes. For the infection assay, 3T3 fibroblasts were dispersed with versene-trypsin and washed three times with RPMI 1640 then x-irradiated (10^4 rads). Aliquots (0.2 ml) of cell suspension containing 10^5 cells were placed in 24-well microplates; each well contained a glass cover slip (12 mm in diameter). After cells attachment, the cover slips were washed twice with RPMI 1640 supplemented with 1% (w/v) bovine serum albumin (BSA, Sigma) to remove FCS. The cells were then incubated with 0.2 ml of RPMI 1640 containing various amounts of monoclonal antibodies. Controls consisted of medium alone or medium supplemented with normal mouse immunoglobulin G. Viable trypomastigotes were added in RPMI 1640 at 10^5 parasites per well. After 3 hours at 37°C, each well was emptied and washed twice to remove free-swimming parasites, refilled with 0.5 ml of culture medium, and incubated (48 hours at 37°C). Each well was then emptied and washed twice, fixed, and stained. The proportion of infected cells was counted by two observers. A cell was considered positive if it contained more than four cytoplasmic amastigotes. Each value represents the mean ± SEM of three experiments carried out in triplicate.

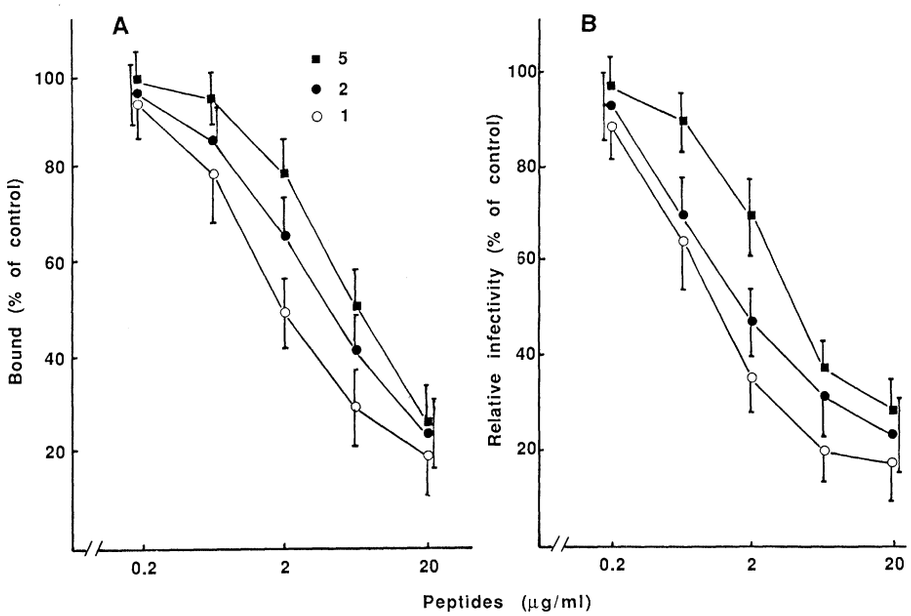

Fig. 2. (A) Inhibition of binding of ^{125}I-labeled Fn to *T. cruzi* trypomastigotes. Parasites (10^9) in 25 μl of RPMI 1640 were incubated with 25 ng of ^{125}I-labeled Fn (50 μl) with each of the following peptides: **5, 2**, or **1** in a total volume of 150 μl for 1 hour at 37°C, in polystyrene tubes previously saturated with phosphate-buffered saline (PBS) containing 2% BSA to prevent binding of Fn to the tube surfaces. Next, 50 μl of the mixture was applied to a cushion of 200 μl of dibutyl phthalate (Aldrich) in a 300-μl Eppendorf tube and centrifuged at room temperature. After incubation pellets and supernatants pooled from replicate tubes were directly assayed for radioactivity. Nonspecific binding (determined in the presence of 100-fold excess of unlabeled Fn) represented 12 to 18% of total binding. The percentage of ^{125}I-labeled Fn specifically bound was calculated by subtracting from the total binding the nonspecific binding. The results were normalized to the counts incorporated in the absence of unlabeled Fn. Each point represents the mean ± SEM of three independent experiments carried out in triplicate. (B) Cloned 3T3 fibroblasts were x-irradiated (10^4 rads) and distributed into 24-well microplates (10^5 cells per well). Before cell distribution, a cover slip was deposited into each well. After overnight incubation (37°C; humidified 5% CO_2, 95% air), the cover slips were washed twice with RPMI 1640. Viable trypomastigotes that had been incubated with various amounts of peptides (**5, 2**, or **1**) at 37°C for 1 hour were added in 0.3 ml of culture medium (10^5 trypomastigotes per well) to 3T3 fibroblast cultures. Contact was for 3 hours at 37°C. The cover slips were then treated as described in Fig. 1. Each value represents the mean count ± SEM of three experiments carried out in triplicate.

sion by the parasite, we thought that the domain of Fn that interacts with the parasite surface receptors would probably be localized within the 15-kD peptide of the Fn molecule. In this case, synthetic peptides modeled from the sequence of the cell-attachment site would be expected to be capable of modulating parasite-cell interaction. Therefore, we examined the possible inhibitory effect of synthetic peptides on cell invasion by the parasites.

Fibronectin and its larger fragments may form microaggregates (*8*). In contrast, the smaller peptides are highly soluble, which makes it possible to test them at low concentrations for inhibition of cell invasion. For these tests, we chose three peptides [where (Acm) is the acetamidomethyl group]: Arg-Gly-Asp-Ser (**5**), Cys(Acm)-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Cys(Acm) (**2**), and Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Cys (Acm) (**1**). The three peptides did interfere with the invasion of 3T3 fibroblasts by trypomastigotes (Fig. 2B). The inhibition occurred in a dose-dependent manner, at peptide concentrations greater than $10^{-6}M$.

The inhibitory effect of synthetic peptides on the infection of 3T3 fibroblasts by *T. cruzi* may indicate that the sequence Arg-Gly-Asp-Ser binds to parasite Fn receptors, thus preventing the trypomastigotes from attaching to the cell surface. To examine this possibility, we conducted competition experiments. When trypomastigotes were incubated with peptides containing the sequence Arg-Gly-Asp-Ser, a decrease of the binding of iodinated Fn to the parasites could be observed, and this phenomenon was related to the concentration of peptides used (Fig. 2A). Peptides differing by one amino acid of the Arg-Gly-Asp-Ser sequence (**6** or **7**) as well as unrelated peptides (Ser-Lys-Pro, Glu-Ser-Gly, and Ala-Lys-Thr-Lys-Pro) had no significant effect on the binding of ^{125}I-labeled Fn to the parasite surface and showed no significant inhibition of cell invasion by the parasites (Table 2).

To further define the binding capacity of the peptide, we used an iodine-labeled peptide. Incubation of *T. cruzi* trypomastigotes with increasing amounts of ^{125}I-labeled Tyr-Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Cys(Acm) (^{125}I-labeled **3**) showed that the binding is a saturable process (Fig. 3); about 4 nmol of radioligand bound per 10^9 trypomastigotes was sufficient to achieve apparent saturation. Half-maximal saturation, at 1.5 nmol of radioligand, provided an estimate of the equilibrium dissociation constant, K_d = 0.7 nmol (SEM = 0.15 nmol) of the radioligand for binding sites on trypomastigotes. There are about 5×10^4 peptide-binding sites per trypomastigote (Fig. 3) (*12*).

Even nanomolar concentrations of ^{125}I-

Table 1. Sequences of the synthetic peptides tested. Details of the synthesis are described (*13*). (Acm) is the acetamidomethyl group.

Structure	Sequence
1	Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Cys(Acm)
2	Cys(Acm)-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Cys(Acm)
3	Tyr-Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Cys(Acm)
4	Fluorescenyl-Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Cys(Acm)
5	Arg-Gly-Asp-Ser
6	Arg-Phe-Asp-Ser
7	Arg-Phe-Asp-Ser-Ala-Ala-Arg-Phe-Asp-Ser
8	Fluorescenyl-Arg-Phe-Asp-Ser-Ala-Ala-Arg-Phe-Asp-Ser

labeled **3** were sufficient to achieve apparent saturation. However, we were unable to inhibit the binding of ^{125}I-labeled Fn to trypomastigotes or the attachment of parasites to the Fn of fibroblast cell surfaces using nanomolar concentrations of peptides **1**, **2**, or **5**. Micromolar quantities of peptides were needed to overcome the cooperative interaction of many Fn molecules with the parasite surface. Indeed, Fn exists as a dimer, and a portion may aggregate spontaneously. This would be a possible explanation for this apparent discrepancy.

The nature of the binding of iodine-labeled peptide **3** was further investigated by competition experiments (Table 3). Peptides containing the sequence Arg-Gly-Asp-Ser competed efficiently with the radiolabeled ligand for binding to the parasite surface. High concentrations of sugars had no significant effect on the binding of iodine-labeled peptide to *T. cruzi* trypomastigotes. Lysine and arginine slightly inhibited binding at high concentrations.

The binding of Fn peptide **1** to *T. cruzi* trypomastigotes was confirmed by fluorescence-activated cell-sorting analysis with the use of fluorescein-labeled peptide **4**. No significant binding was observed when we used fluorescenyl-peptide **8**. All these experiments were performed with the *T. cruzi* Y strain; the results were similar when we used the Tehuantepec strain.

We further examined whether the peptides could modify the development of *T. cruzi* in BALB/c mice. We conducted two types of experiments. (i) Mice were infected with *T. cruzi* and once every day for 3 weeks were injected intraperitoneally either with the Fn peptide **1** diluted in RPMI 1640 or with medium alone. (ii) Mice were immunized with Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Cys conjugated through the carboxyl-terminal cysteine to tetanus toxoid (*13*) or with tetanus toxoid alone, and then infected with trypomastigotes. The infection progressed gradually in the two groups of animals (Table 4). Although the peptide had a slow inhibitory effect, the number of blood parasites in the peptide-treated mice reached the control values after 19 days of infection. There was a high variation within and between groups after they had been infected for 12 days. No animals died before the 22nd day of infection. A rapid proteolytic cleavage and elimination of the peptide in vivo might explain the failure of peptide **1** to effectively inhibit parasite development.

Representative kinetics of blood parasitemias in mice immunized with tetanus toxoid–conjugated peptide **1** is shown in Table 3. The conjugated peptide induced a significant level of protection among mice. Four mice in this group survived until the end of the experiments (26 days after they were infected). In contrast, animals in the group receiving tetanus toxoid showed high levels of parasitemia and died between 15 and 19 days after infection. We have demonstrated that polyclonal antibodies to Fn inhibit cell invasion by *T. cruzi* (*7, 8*). Here we have shown that monoclonal antibodies to Fn inhibit cell infection by the parasites. The constant presence of antibodies to Fn in the circulation of Fn peptide–immunized mice might bind to cell surface Fn, thus preventing the trypomastigotes from attaching to the host cell surface. This would explain the inhibitory effect on parasite development.

Our data show that the Arg-Gly-Asp-Ser sequence of the cell-binding domain of Fn is the cell recognition site for the *T. cruzi* trypomastigote. Moreover, the experiments in vivo show the modulatory effect of the peptide on parasite development. Since the Fn molecule consists of two subunits each containing the sequence Arg-Gly-Asp-Ser in a hydrophilic β-turn (*11, 14, 15*), it might be that as in the case of the syphilis spirochete (*16*), the parasite may interact with an

Table 2. (A) Inhibition of binding of ^{125}I-labeled Fn to *T. cruzi* trypomastigotes. Parasites (10^9) in 25 μl of RPMI 1640 were incubated with 25 ng of ^{125}I-labeled Fn (50 μl) and one of the peptides in various amounts as listed in a total volume of 150 μl for 1 hour at 37°C and were treated according to the procedure described in the legend to Fig. 2A. Each value is the mean ± SEM of three experiments carried out in triplicate. (B) Relative infectivity. Viable trypomastigotes that had been incubated with various amounts of a peptide at 37°C for 1 hour were added in 0.3 ml of culture medium (10^5 trypomastigotes per well) to 3T3 fibroblast cultures and then cultured in the same conditions as reported in the legend to Fig. 2B. Each value is the mean count ± SEM of three experiments carried out in triplicate.

Inhibitor	Final concentration of inhibitor (μg/ml)		
	0.2	2	20
(A) *Binding of ^{125}I-labeled Fn (% of control)*			
Peptide **1**	95 ± 11	49 ± 8	19 ± 10
Peptide **6**	97 ± 5	92 ± 4	91 ± 6
Peptide **7**	98 ± 12	88 ± 16	89 ± 5
Ser-Lys-Pro	96 ± 12	83 ± 15	78 ± 19
Glu-Ser-Gly	97 ± 3	85 ± 6	95 ± 4
Ala-Lys-Thr-Lys-Pro	91 ± 7	82 ± 13	86 ± 23
(B) *Relative infectivity (% of control)*			
Peptide **1**	88 ± 7	35 ± 8	17 ± 9
Peptide **6**	96 ± 3	89 ± 10	90 ± 5
Peptide **7**	92 ± 8	83 ± 11	85 ± 7
Ser-Lys-Pro	92 ± 12	85 ± 10	91 ± 19
Glu-Ser-Gly	81 ± 12	98 ± 5	85 ± 16
Ala-Lys-Thr-Lys-Pro	99 ± 5	91 ± 18	86 ± 11

Table 3. Inhibition of binding of ^{125}I-labeled Tyr-Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Cys(Acm) (^{125}I-labeled **3**) to *T. cruzi* trypomastigote culture forms. Trypomastigotes (10^9) in 25 μl of RPMI 1640 were incubated with 2 μg of ^{125}I-labeled **3** (50 μl) with each of the reagents listed in a total volume of 150 μl (all the reagents except peptides were purchased from Sigma). The binding assay was as described in the legend to Fig. 2A. Percentage inhibition was calculated as follows: $\{1 - [(cpm + i)/(cpm - i)]\} \times 100$, where $(cpm + i)$ is the radioactivity bound to trypomastigotes in the presence of inhibitor minus the radioactivity bound in the presence of a 100-fold excess of unlabeled peptide **3** and $(cpm - i)$ is the radioactivity bound to trypomastigotes without inhibitor minus the radioactivity bound in the presence of a 100-fold excess of unlabeled peptide **3**. The results are the mean of three experiments ± SEM.

Inhibitor	Final concentration of inhibitor (M)	% Inhibition of binding
	$\times 10^{-9}$	
Peptide **3**	10	35 ± 7
Peptide **1**	10	42 ± 11
Peptide **2**	10	29 ± 9
Peptide **5**	1	12 ± 5
	10	23 ± 7
	20	68 ± 9
Peptide **6**	10	0
Peptide **7**	10	12 ± 5
	20	10 ± 7
	40	13 ± 9
Ser-Lys-Pro	20	16 ± 5
Glu-Ser-Gly	20	9 ± 2
Ala-Lys-Thr-Lys-Pro	20	10 ± 5
	$\times 10^{-1}$	
Lysine	2	28 ± 5
Arginine	2	15 ± 4
Glutamine	5	7 ± 2
Glucose	5	0
N-Acetylglucosamine	2	10 ± 3

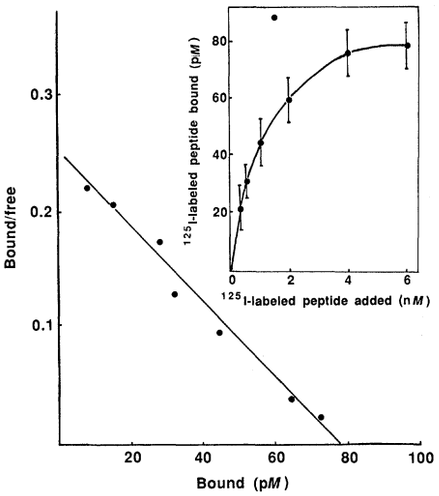

Fig. 3. Binding of iodine-labeled peptide **3** (^{125}I-labeled **3**) to viable *T. cruzi* trypomastigote culture forms. Trypomastigotes (10^9) in 25 μl of RPMI 1640 were incubated with increasing amounts of labeled peptide (specific activity, 1.6×10^4 cpm/μg) in a total volume of 150 μl for 1 hour at 37°C. The binding assay was as described in Fig. 2A. Nonspecific binding was determined in the presence of a 100-fold excess of unlabeled peptide. The plotted points represent the mean ± SEM of two experiments carried out in triplicate. (Inset) Binding of ^{125}I-labeled **3** to *T. cruzi* trypomastigote culture forms as a function of ^{125}I-labeled **3** input. (Left) Scatchard (*12*) analysis of the binding of ^{125}I-labeled peptide to trypomastigotes.

Table 4. Effects of peptide on the development of *T. cruzi* in BALB/c mice. The Y strain of *T. cruzi* was maintained by serial passages of infected blood in BALB/c mice as described (*17*). Bloodstream forms of trypomastigotes were obtained by cardiac puncture from infected animals. The blood was mixed with calciparine (25 IU/ml, Laboratorie Choay) and diluted in RMPI 1640. (A) Trypomastigotes (10^5) were incubated with 1 mg of peptide **1** in 0.3 ml of RPMI 1640 (1 hour at 4°C) and then diluted in Alsever's solution to 3×10^4 parasites per milliliter (controls were incubated in medium alone). Mice received intraperitoneal injections of 0.3 ml of the dilution. Injections of 0.3 ml of RPMI 1640 containing 100 μg of peptide or RPMI 1640 alone were continued in both groups (ten mice each) for 3 weeks after infection. The number of parasites in 30 microscopic fields (10× ocular, 40× objective) was counted on a thin smear of tail blood. (B) Immunization studies. Each mouse (n = 10) received intraperitoneally, three times per month, 50 μg of peptide conjugated to tetanus toxoid in 0.1 ml of saline and 0.1 ml of Freund's complete adjuvant (FCA), mixed before injection. Controls received tetanus toxoid and FCA. Two weeks after the last injection, blood was withdrawn through the retro-orbital sinus. Sera were assayed by an enzyme-linked immunosorbent assay (*18*) in which Fn or peptide **1** were adsorbed to untreated polystyrene microtiter wells. Sera were diluted in PBS, *p*H 7.2, and incubated at 4°C overnight. Bound antibody was then detected with peroxidase-labeled rabbit antibodies to mouse immunoglobulins. Mice immunized with peptide conjugated to tetanus toxoid developed antibody that reacted with the immobilized peptide as well as with Fn molecule (antibody titer, 1/1000 to 1/10,000). A rabbit polyclonal antibody to Fn (*7*) was used as control. None of the tetanus toxoid–immunized animals showed positive reactions against the peptide or Fn. Plasma Fn levels were also measured by a competitive radioimmunoassay (*19*) in peptide-immunized mice. The mean concentration decreased from 250 ± 22 μg/ml before treatment to 123 ± 12 μg/ml after immunization. However, no bleeding diathesis was observed in peptide-immunized group. Mice were then inoculated intraperitoneally with 10^4 parasites. Blood smears from tails of all mice were examined as described above. The mean parasitemia (±SEM) was computed for each group.

Treatments	Days after infection	Parasitemia	Proportion of survivors (%)
		Experiment A	
Peptide **1**	6	2.75 ± 1.49	100
Control		4.22 ± 1.56	100
Peptide **1**	10	22.25 ± 12.12	100
Control		27.56 ± 14.24	100
Peptide **1**	12	22.30 ± 12.96	100
Control		43.25 ± 31.60	90
Peptide **1**	14	48.67 ± 30.06	80
Control		84.50 ± 83.77	90
Peptide **1**	18	77.50 ± 60.27	50
Control		94.50 ± 89.64	40
Peptide **1**	21	56.70 ± 73.54	40
Control		103.67 ± 131.17	10
		Experiment B	
Peptide **1**	6	7.75 ± 3.77	100
Control		5.44 ± 5.36	100
Peptide **1**	10	8.88 ± 4.39	100
Control		32.89 ± 21.89	100
Peptide **1**	12	3.75 ± 1.67	100
Control		34.00 ± 24.72	100
Peptide **1**	14	12.25 ± 6.45	100
Control		87.78 ± 64.93	100
Peptide **1**	18	10.38 ± 9.86	100
Control		95.00 ± 63.78	60
Peptide **1**	21	19.14 ± 19.86	90
Control		220.00 ± 0.0	10

accessible cell-binding domain of Fn not occupied by the cell surface receptor. The sequence Arg-Gly-Asp-Ser is also found in at least six other proteins including the phage receptor on the surface of *Escherichia coli* and the Sindbis virus coat protein, and it has been proposed that some microorganisms might use this sequence to interact with eukaryotic cells (*11*). Our study adds the protozoan parasite, *T. cruzi*, to the list of pathogens that bind the Arg-Gly-Asp-Ser sequence of Fn and may utilize it for association with host cells.

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    After gel filtration followed by preparation on a system of C-18 reversed-phase high-performance liquid chromatography (HPLC) (peptides **1, 2, 3, 5, 6**, and 7) and lyophilization, we found that each peptide had the expected amino acid composition by amino acid analysis and eluted as a major peak (>95%) on C-18 reversed-phase HPLC in a linear gradient of acetonitrile:water:sodium phosphate, *p*H 2.5. The fluorescenyl-peptides **6** and **8** were quantitatively obtained from peptides **1** and 7, respectively, by coupling of fluorescenylisothiocyanate (tenfold excess) overnight, in pyridine:water (2:1), at 4°C. Excess fluorescenylisothiocyanate was then extracted from reaction mixture with ethyl acetate. The aqueous phase was lyophilized, and the fluorescenyl-peptides were checked by HPLC and were more than 95% pure.

    The decapeptide **1** was conjugated to tetanus toxoid through the deprotected sulfhydril [S. Moore, A. Felix, J. Meienhofer, C. W. Smith, R. Walter, *J. Med. Chem.* **20**, 495 (1977)] group with maleimidobenzoyl-*N*-hydroxysuccinimide ester according to the procedure of Lee *et al.* [A. C. Lee, J. E. Powell, G. W. Tregear, H. D. Niall, V. C. Stevens, *Mol.*

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Genetic Selection of a *Plasmodium*-Refractory Strain of the Malaria Vector *Anopheles gambiae*

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The anopheline mosquito is the target in most malaria control programs, primarily through the use of residual insecticides. A mosquito was studied that is refractory to most species of malaria through a genetically controlled mechanism. A strain of *Anopheles gambiae*, which was selected for complete refractoriness to the simian malaria parasite *Plasmodium cynomolgi*, also has varying degrees of refractoriness to most other malaria species examined, including the human parasites *P. falciparum, P. ovale*, and *P. vivax* for which this mosquito is the principal African vector. Furthermore, the refractoriness extends to other subhuman primate malarias, to rodent malaria, and to avian malaria. Refractoriness is manifested by encapsulation of the malaria ookinete after it completes its passage through the mosquito midgut, approximately 16 to 24 hours after ingestion of an infective blood meal. Fully encapsulated ookinetes show no abnormalities in parasite organelles, suggesting that refractoriness is due to an enhanced ability of the host to recognize the living parasite rather than to a passive encapsulation of a dead or dying parasite. Production of fully refractory and fully susceptible mosquito strains was achieved through a short series of selective breeding steps. This result indicates a relatively simple genetic basis for refractoriness. In addition to the value these strains may serve in general studies of insect immune mechanisms, this finding encourages consideration of genetic manipulation of natural vector populations as a malaria control strategy.

MALARIA PERSISTS TODAY AS THE most important infectious disease in sub-Saharan Africa despite repeated attempts to control the vector mosquitoes with insecticides and massive programs for the distribution of antimalarial drugs (*1*). The rapid spread of multiple drug–resistant strains of *Plasmodium falciparum* now seriously threatens the population with increased sickness and death (*2*); alternatives to drugs as the major approach to malaria control in Africa must be found. Research on the construction of malaria vaccines is under way, but concerns about the delivery of vaccine and about parasite evasion mechanisms persist. Vector control is an attractive alternative, but conventional strategies have failed in Africa and new methods must be developed. The production of genetically defined lines of vector mosquitoes refractory to the development of the parasite and thus incapable of transmitting the infection is one possible method for controlling malaria.

Genetic variation in mosquito susceptibility has been recorded, and vector strains refractory to various avian and rodent malarias have been selected and studied (*3*). We have now established a defined form of refractoriness to the human *Plasmodium* species in a major vector of malaria. Study of the susceptibility of the G3 strain of *Anopheles gambiae* (colonized in Gambia in 1975) revealed reduced susceptibility to the simian malaria *P. cynomolgi* in a portion of the mosquitoes examined. In addition to a few or no normal oocysts, the midguts of these rare mosquitoes were covered with large numbers of small melanized structures (Fig. 1). The oocyst is the stage of the parasite that begins to form about 24 hours after the mosquito ingests an infected blood meal. After fertilization in the lumen of the midgut, the newly formed ookinetes invade the midgut epithelium and develop into oocysts wherein sporozoites, the infectious stage of the malaria parasite, are formed. Encapsulation leads to death of the parasite in the mosquito and thus inability to transmit the infection.

Selection against *P. cynomolgi* resulted in the production of two mosquito lines, one that is fully refractory and one that is fully susceptible to this parasite (*4*). Ultrastructural studies of the encapsulated bodies on the midgut of the mosquitoes revealed both heme pigment granules and subcellular features typical of normal *Plasmodium* parasites. The encapsulated parasites, which are physically located between the midgut epithelial cells and the surrounding noncellular basal lamina, are enclosed by an electron-dense matrix probably composed of the protein-melanin complex typical of the immunological encapsulation reaction described in other Diptera (*5, 6*). Neither hemocytes, often involved in capsule formation, nor their fragments are evident (Fig. 1). These two mosquito lines have continued to manifest these patterns of refractoriness and susceptibility for more than 40 generations without additional selection.

The encapsulation reaction is first detected 16 hours after the refractory mosquito has ingested an infective blood meal. Electron microscopy shows that a melanin-like substance usually begins to appear on the ookinete after it has traversed the gut wall.

Assessment of the refractoriness and susceptibility of the two lines to other strains and species of *Plasmodium* indicates that the mechanism of refractoriness is a general response to most malaria parasites. Refractoriness is manifested by encapsulation. The percentage of infected mosquitoes and the number of oocysts per infected mosquito were similar in the refractory and susceptible lines (Table 1). The refractory line is almost

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