The N-Terminal 513 Amino Acids of the Envelope Glycoprotein gB of Human Cytomegalovirus Stimulates both B- and T-Cell Immune Responses in Humans

YUNG-NAN C. LIU,^{1*} AMY KLAUS,¹ BRUCE KARI,¹ MARK F. STINSKI,² JUNE ECKHARDT,¹
AND RICHARD C. GEHRZ¹

Immunobiology Division, Sutton Immunobiology Research Laboratory, Children's Biomedical Research Institute, St. Paul, Minnesota 55102,¹ and Department of Microbiology, University of Iowa, Iowa City, Iowa 52242²

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Host defense against human cytomegalovirus (HCMV) involves both humoral and cell-mediated immunity. In this report, human immune responses to glycoproteins encoded by the HCMV gB homolog gene have been examined by using glycoproteins purified by immunoaffinity from HCMV virions and recombinant proteins expressed by vaccinia viruses containing either the entire gB open reading frame or a C-terminal deletion mutant, gBm165, coding for the N-terminal 513 amino acids of gB. Neutralizing antibodies, helper T cells, and cytotoxic T cells reactive with epitopes on the N-terminal portion of gB were detected in some seropositive individuals, suggesting that this region of gB may be important in eliciting protective immunity during natural infection for some individuals.

Human cytomegalovirus (HCMV) is an important cause of serious illness in infants infected in utero and in immunocompromised individuals, including cancer patients receiving immunosuppressive drugs, organ and bone marrow transplant recipients, and patients with AIDS (8). Both humoral and cell-mediated immunity appear to be important in host defense against HCMV, since patients with deficits in these immune responses are susceptible to dissemination of HCMV and progression to symptomatic disease. Although it is likely that neutralizing antibodies inactivate extracellular virions, helper T cells (T_H) and cytotoxic T cells (T_C) may control the spread of intracellular virus and prevent reactivation of latent virus. Therefore, an ideal subunit vaccine should contain epitopes that induce both antibody and T-cell immune responses.

Viral glycoproteins are important targets of the immune response. They are found in the envelopes of virions as well as in the membranes of infected cells. In the viral envelope, HCMV contains at least three families of glycoprotein complexes designated gC1, gC2, and gC3, each containing two or more constituent glycoproteins associated by disulfide bonds (9, 12). The gC1 complexes contain two mature envelope glycoproteins with molecular weights of 93,000 to 130,000 (gp93) and 52,000 to 58,000 (gp55), derived by proteolytic cleavage of a precursor glycoprotein with a molecular weight of 138,000 (gp138) (4, 10, 19). The polypeptide backbone of these glycoproteins is encoded by a gene identified as a gB homolog by Cranage et al. (7) and Mach et al. (14) because of its sequence homology with the gene encoding glycoprotein B (gB) of herpes simplex virus. Spaete et al. demonstrated that gp55 originates from the C-terminal region of gB (19), and our recent results indicate that gp93 originates from the N-terminal region of gB (11).

In this report, we have analyzed the human immune response to glycoproteins expressed in recombinant vaccinia viruses containing the entire gB open reading frame (vac-gB)

or a C-terminal deletion mutant, gBm165 encoding the N-terminal 513 amino acids of gB (vac-gBm165).

The *Xma*III fragment (3,125 bp) containing the gB gene from the Towne strain of HCMV, which codes for a 907-amino-acid polypeptide, was cloned into the *Sma*I site of the pSC11 vaccinia virus vector (a generous gift from B. Moss, National Institutes of Health) as described previously (15). The HCMV gB gene was introduced into vaccinia virus by homologous recombination after transfection with plasmid pSC11-gB and coinfection with wild-type vaccinia virus strain WR (from the American Type Culture Collection). Thymidine kinase-negative viruses were selected in HuTK-143 cells in the presence of 25 μ g of bromodeoxyuridine (BUDR) per ml. Viruses expressing β -galactosidase were detected by staining with 320 μ g of 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside per ml. Blue plaques were picked and purified three times on HuTK-143 cells in the presence of BUDR. Purified vac-gB recombinant viruses were expanded in HuTK-143 cells at a multiplicity of infection (moi) of 0.01.

C-terminal deletion mutants of gB were generated by Tn5 mutagenesis (6, 21) by infecting pSC11-gB recombinants carrying *Escherichia coli* DH1 cells with λ 467::Tn5 phage. The plasmids carrying the Tn5 transposon were selected by kanamycin and screened as previously described (21). The physical location of Tn5 insertion was obtained by restriction endonuclease analysis, which was shown to map the insertion site within 50 bp of the actual nucleotide. The precise site of insertion was determined by double-stranded DNA sequencing, using a primer approximately 50 to 100 nucleotides from the insertion site (18). The gB mutant gBm165 had Tn5 inserted 1,539 nucleotides downstream of the initiation codon. Therefore, it coded for the N-terminal 513 amino acids of gB.

The neutralizing activity of serial dilutions of three human serum samples containing HCMV-specific antibodies was determined in an agarose plaque reduction assay using Towne HCMV (Fig. 1A). Serum from donors WRC, HW, and SR exhibited 50% plaque reduction at dilutions of 1:400, 1:300, and 1:300, respectively. Sera at dilutions exhibiting

* Corresponding author.

FIG. 1. gB-specific neutralizing activity. Human sera were analyzed for neutralizing activity in an agarose plaque reduction assay using Towne strain HCMV. (A) Serum-neutralizing activity of untreated sera. (B) Sera were diluted to exhibit 50% plaque reduction prior to adsorption. Residual neutralizing activity of sera after adsorption with cells infected with vac-gB or vac-gBm165 was compared with nonspecific adsorption with vac-pSC11 and determined according to the formula shown in the text.

50% plaque reduction were adsorbed three times with methanol-acetone (1:1)-fixed CV-1 cells preinfected with either vac-gB or vac-gBm165 to remove antibodies reactive with epitopes on glycoproteins encoded by gB (amino acids 1 to 907) or the truncated glycoprotein encoded by gBm165 (amino acids 1 to 513), respectively. Sera were also adsorbed with cells infected with vaccinia virus containing the transfer vector pSC111 (vac-pSC11) without any foreign gene insert as a negative control. Percent residual neutralizing activity after adsorption with vac-gB or vac-gBm165 was compared with that after nonspecific adsorption with vac-pSC11 according to the following formula: % residual neutralizing activity = $[(100 - \text{percent plaque reduction postadsorption with vac-gB/vac-gBm165}) / (100 - \text{percent plaque reduction postadsorption with vac-pSC11})] \times 100$.

Sera from donors WRC, HW, and SR adsorbed with vac-pSC11 retained 99, 94, and 100% neutralizing activity,

respectively, compared with unadsorbed sera. For WRC and HW (Fig. 1B), approximately 50% of total serum-neutralizing activity was adsorbed by vac-gB glycoproteins, whereas approximately 25 to 30% was adsorbed by vac-gBm165. These data indicate that these human sera contain neutralizing antibodies reactive with epitopes on both the N-terminal portion of gB spanning amino acids 1 to 513 and the C-terminal portion spanning amino acids 514 to 907. For SR serum, approximately 30% of neutralization activity was due to antibodies specific for gB glycoproteins, whereas an insignificant amount of neutralizing antibodies reacted with epitopes on the N-terminal portion of gB spanning amino acids 1 to 513 (Fig. 1B).

Monoclonal antibodies (MAbs) obtained from mice immunized with lysates of HCMV-infected cells or HCMV virions are predominantly reactive with gp55, suggesting that this glycoprotein may be immunodominant when presented in its native configuration in gC1 complexes (3, 12, 16, 17). Utz et al. localized B-cell epitopes recognized by gp55-specific MAbs to a region spanning amino acids 608 to 625 on gB (20), and Banks et al. mapped two B-cell antigenic domains to amino acids 461 to 619 and 620 to 680, respectively (1). A number of these MAbs neutralize HCMV in the presence or absence of complement. Recently, we have obtained three gp93-specific complement-independent neutralizing MAbs by immunizing mice with biochemically purified gp93 (11). Human antibodies reactive with both gp93 and gp55 can be detected in sera from most immune adults and congenitally infected infants by Western immunoblot, and Britt et al. (5) have shown that 40 to 70% of HCMV-specific neutralizing activity can be adsorbed by fibroblasts infected with vac-gB recombinant virus. Our results suggest that epitopes on both the N-terminal 513 amino acids and the C-terminal 394 amino acids elicit a neutralizing antibody response in vivo in some individuals.

The reactivity of HCMV-specific T_h was first determined by using biochemically purified gC1 complexes and gB glycoproteins. Glycoprotein gC1 complexes were first purified by the immunoaffinity method using biotinylated gp55-specific MAbs 41C2 and 9B7 (12). These affinity-purified gC1 complexes were then reduced with β -mercaptoethanol and fractionated by 9% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Individual proteins (gp55, gp93, and gp138) were cut out and electroeluted into SDS-PAGE buffer. Bovine serum albumin was also eluted from the gel as a negative control. The electroeluted proteins were dialyzed against phosphate-buffered saline (pH 7.4) before use. The lymphocyte proliferation assays were set up as previously described (13). The polyclonal gC1-specific T-cell line from donor KM (13) exhibited proliferative responses to whole virus; gC1 complexes; and gp138, gp93, and gp55 purified by electroelution after separation by SDS-PAGE (Table 1). This T-cell line, therefore, contains T_h precursors reactive with epitopes on both gp93 and gp55. The gB-specific T_h clone WRC-T3-3 reacted with gp93 and its precursor glycoprotein, gp138, but not with gp55; this clone, therefore, recognizes an epitope uniquely expressed on gp93. In contrast, the HCMV-specific T_h clone WRC-L26 did not respond to any of the gC1 complexes or glycoproteins even though it did respond to HCMV.

Proliferative responses of peripheral blood mononuclear cells (PBMC) from five HCMV-seropositive donors (WRC, KM, DC, SK, and SR) and one HCMV-seronegative donor (AK) were then examined, using recombinant proteins immunoaffinity purified from CV-1 cells infected with vac-gB or vac-gBm165 (Table 2, experiment 1) using a biotinylated

TABLE 1. Lymphocyte proliferative responses to biochemically purified gC complexes and gB glycoproteins^a

Cell line ^b	Antigen						
	Medium	HCMV ^c	gC complex ^d	gp55 ^e	gp93 ^e	gp138 ^e	BSA ^f
KM anti-gC	3,808	36,610	20,128	10,643	18,293	13,340	2,337
WRC-T3-3	142	12,835	13,997	193	9,518	9,960	118
WRC-L26	61	3,785	74	135	126	128	57

^a 2×10^4 T cells plus 10^5 X-irradiated autologous PBMC per well were cultured for 3 days in the presence of viral antigen or individual gC complexes and glycoproteins. [³H]thymidine was added for the final 16 to 20 h, and cells were harvested and counted in a liquid scintillation counter. Results are expressed as mean counts per minute of triplicate wells and typically have a standard error of less than 10% of the specific response.

^b The gC-specific T-cell line, KM anti-gC, was obtained by repeated stimulation of PBMC with biochemically purified gC, autologous X-irradiated feeder cells ($5,000$ rad), and interleukin-2 (13). WRC-T3-3 and WRC-L26 were obtained as stated previously (13). WRC-T3-3 is a gB-specific T-cell clone, and WRC-L26 is an HCMV-specific T-cell clone which does not react with gB, on the basis of the observation that the former reacted with a rabbit reticulocyte lysate containing gB mRNA while the latter did not (data not shown).

^c Heat-inactivated HCMV virions and dense bodies purified from supernatants of infected fibroblasts by sucrose-gradient centrifugation at a protein concentration of $10 \mu\text{g/ml}$.

^d Purified by immunoaffinity from detergent extracts of HCMV virions; final protein concentration was $1 \mu\text{g/ml}$.

^e Obtained by electroelution from bands separated by SDS-PAGE; protein concentration could not be determined because of the presence of Coomassie blue.

^f BSA, Bovine serum albumin.

gp93 MAbs (F15-3G2) (11). Among the six donors, KM was seronegative for vaccinia virus and the rest were seropositive for vaccinia virus. Proliferation assays were set up using 10^5 PBMC per well, and either sucrose gradient-purified Towne strain HCMV or WR strain vaccinia virus was antigen at $2 \mu\text{g}$ of protein per well and $3 \mu\text{g}$ of protein per well, respectively. Immunoaffinity-purified vac-gB and vac-gBm165 were used at $1 \mu\text{g}$ per well. The cultures were incubated for 7 days, and $1 \mu\text{Ci}$ of [³H]thymidine was included for the final 16 h. The cells were collected on glass fiber filter paper, and radioactivity was measured in a 1205 Betaplate (LKB, Gaithersburg, Md.). Donor AK (HCMV seronegative and vaccinia virus seropositive) responded well to vaccinia virus and did not respond to glycoproteins purified from either vac-gB or vac-gBm165, indicating that these affinity-purified glycoproteins did not trigger proliferation responses which cross-reacted with vaccinia virus. PBMC from four of five HCMV-seropositive donors proliferated in response to glycoproteins expressed by both

recombinant viruses, but the magnitude of the response to glycoproteins expressed by vac-gB was higher than that to the truncated glycoprotein encoded by vac-gBm165 in all cases, suggesting that T_h precursors recognizing epitopes in regions of gB spanning both amino acids 1 to 513 and amino acids 514 to 907 contribute to the gB response. Seven gB-specific T_h clones derived from these four donors were also tested for their reactivity to the recombinant proteins. In these assays, lysates of 10^3 CV-1 cells infected with recombinant vaccinia virus containing vector pSC11 only, pSC11-gB, or pSC11-gBm165 were used as antigen. Our results indicate that the proliferation responses to lysate of cells infected with vaccinia virus containing pSC11 without a foreign gene insert were not significantly above background. All seven gB-specific T_h clones were reactive with the glycoprotein expressed by vac-gBm165, suggesting that epitope(s) in the N-terminal region of gB may be immunodominant in these donors. PBMC from donor SR were nonresponsive to glycoproteins expressed by vac-gB and

TABLE 2. Proliferative responses of PBMC and gB-specific T_h clones to HCMV and vaccinia virus recombinant proteins

T cell	Proliferative response ^a					
	No antigen	HCMV ^b	Vaccinia virus ^b	vac-pSC11	vac-gB (aa 1-907)	vac-gBm165 (aa 1-513)
Expt 1						
WRC-PBMC	165	141,359	15,592		67,929	29,236
KM-PBMC	835	281,169	872		171,223	133,634
DC-PBMC	797	94,355	1,794		101,936	26,598
SK-PBMC	338	141,359	6,625		67,929	29,236
SR-PBMC	515	88,614	2,305		553	587
AK-PBMC	289	420	43,166		333	333
Expt 2						
WRC-T3-3	913	35,647		ND ^c	40,862	57,321
WRC-T2-41	1,490	30,043		ND	10,922	25,793
KM-J	765	3,569		708	1,908	4,746
KM-M	81	10,110		179	1,331	5,719
DC-10	1,573	68,287		1,482	88,072	51,905
SK-9	781	37,707		794	62,196	26,075

^a 10^5 PBMC per well were cultured for 7 days (experiment 1) and 2×10^4 T_h clones plus 10^5 X-irradiated autologous PBMC per well were cultured for 3 days (experiment 2) in the presence of viral antigen or recombinant proteins. [³H]thymidine was added for the final 16 to 20 h, and cells were harvested and counted in a liquid scintillation counter. Results are expressed as mean counts per minute of triplicate wells and typically have a standard error of less than 10% of the specific response.

^b Heat-inactivated HCMV and vaccinia virus purified by sucrose-gradient centrifugation were used at protein concentrations of 10 and $15 \mu\text{g/ml}$, respectively.

^c ND, Not determined.

FIG. 2. gB-specific T_c responses. Cytotoxic assays were set up in V-bottom plates, using polyclonal T-cell lines raised against cell-associated virus. (a) WRC anti-HCMV, (b) WRC anti-gBm165, (c) SR anti-HCMV, and (d) SR anti-gBm165 as effector cells. The target cells added at 10^3 per well were uninfected autologous human skin fibroblasts (SF) (●—●), HCMV-infected autologous SF (■—■), vac-pSC11-infected autologous SF (+----+), or vac-gBm165-infected autologous SF (●----●). The supernatants were harvested 6 to 7 h after incubation. Results are expressed as percent lysis ± 1 standard deviation of quadruplicate wells.

vac-gBm165 but did respond to HCMV (Table 2, experiment 1).

Cytotoxicity was detected using a 6-h ^{51}Cr release assay (2) in V-bottom plates. Human skin fibroblast cells (i) uninfected, (ii) infected with HCMV (moi, 10; 24 h postinfection), (iii) infected with vac-pSC11 (moi, 10; 12 h postinfection), or (iv) infected with vac-gBm165 (moi, 10; 12 h postinfection) were labeled with ^{51}Cr and used as target cells. T_c lines established by initial stimulation with HCMV-infected autologous fibroblasts, followed by restimulation at day 10 with autologous fibroblasts infected with either HCMV (anti-HCMV T_c) or vac-gBm165 (anti-gBm165 T_c) at a T-cell-fibroblast ratio of 20:1, were used as effector cells. Percent specific cytolysis was computed according to the formula: $[(\text{CPM experimental release} - \text{CPM spontaneous release}) / (\text{CPM maximum release} - \text{CPM spontaneous release})] \times 100$, where CPM is counts per minute. The spontaneous release for all assays was less than 20% of maximum release.

Both the anti-HCMV and anti-gBm165 T_c lines from donor WRC, a gBm165 T_h responder, exhibited cytotoxic activity against both HCMV- and vac-gBm165-infected target cells (Fig. 2a and b). In contrast, the anti-HCMV T_c line from donor SR, a gBm165 T_h nonresponder, lysed HCMV-infected target cells but not target cells expressing gBm165 (Fig. 2c). Further more, the SR anti-gBm165 T_c line did not exhibit specific cytotoxic activity against any of the target cells (Fig. 2d).

Borysiewicz et al. demonstrated that HCMV-specific T-cell lines stimulated with autologous HCMV-infected fi-

broblasts recognize target cells infected with vac-gB recombinant virus (2). In the present report, we have extended these observations to show that the gB-specific cytotoxic response includes T_c recognizing epitopes on the N-terminal 513 amino acids of gB. Since vac-gBm165 has deleted the predicted transmembrane region of gB, cells infected with this recombinant virus presumably secrete the truncated gB glycoprotein but do not express this protein on the cell membrane. Therefore, it appears that surface expression of glycoprotein is not required for T_c recognition, consistent with previous observations that internal proteins can be recognized by T_c (22).

Although the N-terminal portion of gB appears to be immunogenic in most individuals, the importance of gB-specific humoral and cell-mediated immune responses in recovery from replicating HCMV infection and suppression of latent virus is unknown. It is likely that immune responses to other HCMV proteins are also protective, since healthy individuals lacking gB-specific antibodies or T_h and T_c responses do not appear to be at significant risk of serious HCMV disease.

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REFERENCES

1. Banks, T., M. Huo, K. Kousoulas, R. P. Spaete, and L. Pereira. 1989. A major neutralizing domain maps within the carboxyl-

- terminal half of the cleaved cytomegalovirus B glycoprotein. *J. Gen. Virol.* **70**:979–985.
2. Borysiewicz, L. K., J. K. Hickling, S. Graham, J. Sinclair, M. P. Cranage, G. L. Smith, and J. G. P. Sissons. 1988. Human cytomegalovirus-specific cytotoxic T cells: relative frequency of stage-specific CTL recognizing the 72-kD immediate early protein and glycoprotein B expressed by recombinant vaccinia viruses. *J. Exp. Med.* **168**:919–931.
 3. Britt, W. J. 1984. Neutralizing antibodies detect a disulfide-linked glycoprotein complex within the envelope of human cytomegalovirus. *Virology* **135**:369–378.
 4. Britt, W. J., and D. Auger. 1986. Synthesis and processing of the envelope gp55-116 complex of human cytomegalovirus. *J. Virol.* **58**:185–191.
 5. Britt, W. J., L. Vugler, E. J. Butfiloski, and E. B. Stephens. 1990. Cell surface expression of human cytomegalovirus (HCMV) gp55-116 (gB): use of HCMV-recombinant vaccinia virus-infected cells in analysis of the human neutralizing antibody response. *J. Virol.* **64**:1079–1085.
 6. Bruijn, F. J., and J. R. Lupski. 1984. The use of transposon Tn5 mutagenesis in the rapid generation of correlated physical and genetic maps of DNA segments cloned into multicopy plasmids—a review. *Gene* **27**:131–149.
 7. Cranage, M. P., T. Kouzarides, A. T. Bankier, S. Satchwell, K. Weston, P. Tomlinson, B. Barrell, H. Hart, S. E. Bell, A. C. Minson, and G. L. Smith. 1986. Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. *EMBO J.* **5**:3057–3063.
 8. Gehrz, R. C. 1991. Human cytomegalovirus: biology and clinical perspectives. *Adv. Pediatr.* **38**:190–219.
 9. Gretch, D. R., R. C. Gehrz, and M. F. Stinski. 1988. Characterization of a human cytomegalovirus glycoprotein complex (gcI). *J. Gen. Virol.* **69**:1205–1215.
 10. Gretch, D. R., B. Kari, L. Rasmussen, R. C. Gehrz, and M. F. Stinski. 1988. Identification and characterization of three distinct families of glycoprotein complexes present in the envelopes of human cytomegalovirus. *J. Virol.* **62**:875–881.
 11. Kari, B., Y.-N. C. Liu, R. Goertz, N. Lussenhop, M. F. Stinski, and R. Gehrz. 1990. Structure and composition of a family of human cytomegalovirus glycoprotein complexes designated gC-I (gB). *J. Gen. Virol.* **71**:2673–2680.
 12. Kari, B., N. Lussenhop, R. Goertz, M. Wabuke-Bunoti, R. Radeke, and R. Gehrz. 1986. Characterization of monoclonal antibodies reactive to several biochemically distinct human cytomegalovirus glycoprotein complexes. *J. Virol.* **60**:345–352.
 13. Liu, Y.-N. C., J. Eckhardt, B. Kari, and R. C. Gehrz. 1988. General characterization of human cytomegalovirus-specific proliferative CD4⁺ T cell clones. *Int. J. Cell Cloning* **6**:352–364.
 14. Mach, M., U. Utz, and B. Fleckenstein. 1986. Mapping of the major glycoprotein gene of human cytomegalovirus. *J. Gen. Virol.* **67**:1461–1467.
 15. Mackett, M., G. L. Smith, and B. Moss. 1987. The construction and characterization of vaccinia virus recombinant expressing foreign genes, p. 191–200. *In* D. M. Glover (ed.), *DNA cloning*, vol. 2. IRL Press, Washington, D.C.
 16. Pereira, L., M. Hoffman, M. Tatsuno, and D. Dondero. 1984. Polymorphism of human cytomegalovirus glycoproteins characterized by monoclonal antibodies. *Virology* **139**:73–86.
 17. Rasmussen, L., M. Nelson, M. Neff, and T. C. Merigan, Jr. 1988. Characterization of two different human cytomegalovirus glycoproteins which are targets for virus neutralizing antibody. *Virology* **163**:308–318.
 18. Smiley, B. L., J. R. Lupski, P. S. Svec, R. McMacken, and G. N. Godson. 1982. Sequences of the E coli DNA G pronase gene and regulation of its expression. *Proc. Natl. Acad. Sci. USA* **79**:4550–4554.
 19. Spaete, R. R., R. M. Thayer, W. S. Probert, F. R. Masiarz, S. H. Chamberlain, L. Rasmussen, T. C. Merigan, and C. Pachl. 1988. Human cytomegalovirus strain Towne glycoprotein B is processed by proteolytic cleavage. *Virology* **167**:207–225.
 20. Utz, U., W. Britt, L. Vugler, and M. Mach. 1989. Identification of a neutralizing epitope on glycoprotein gp58 of human cytomegalovirus. *J. Virol.* **63**:1995–2001.
 21. Weber, P. C., M. Levine, and J. C. Glorioso. 1987. Rapid identification of nonessential genes of herpes simplex virus type 1 by Tn5 mutagenesis. *Science* **236**:576–579.
 22. Yewdell, J. W., J. R. Bennink, G. L. Smith, and B. Moss. 1985. Influenza A virus nucleoprotein is a major target antigen for cross-reactive anti-influenza A virus cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. USA* **82**:1785–1789.