Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5537–5541, July 1989
Immunology

Development of biologically active peptides based on antibody structure

(reovirus type 3/receptor/idiotype)

WILLIAM V. WILLIAMS*, DAVID A. MOSS†, THOMAS KIEBER-EMMONS‡, JEFFREY A. COHEN§, JEFFREY N. MYERS†, DAVID B. WEINER†, AND MARK I. GREENE†

Departments of *Medicine, †Pathology and Laboratory Medicine, and §Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6082; and ‡IDEC Pharmaceuticals Corporation, La Jolla, CA 92037

Communicated by Peter C. Nowell, April 7, 1989

ABSTRACT **Antibody molecules are composed of several functional domains, including a variable domain that contacts antigen and a constant domain. The hypervariable regions of antibody molecules play an integral role in determining their specificity. However, the delineation of specific residues most critical in binding is difficult. We have been studying a monoclonal antibody (87.92.6) that binds to the reovirus type 3 receptor on a number of cell types, down-modulates the receptor, and inhibits DNA synthesis in the cells. We have shown that a peptide derived from the second complementarity-determining region of the monoclonal antibody 87.92.6 light-chain variable region can reproduce both of these effects. We were also able to demonstrate specific amino acid residues and structural features involved in producing these effects. The study of antibody structure, coupled with molecular synthetic techniques, can lead to the development of biologically active substances with potential clinical use.**

The monoclonal antibody (mAb) 87.92.6 was developed to mimic the cell attachment site of reovirus type 3 (1–3). This site is specifically recognized by another mAb termed 9BG5 (4–6). mAb 9BG5 binds to the reovirus type 3 hemagglutinin, prevents reovirus type 3 from binding to cells (6), and neutralizes reovirus infectivity (4, 5). The reovirus type 3 hemagglutinin is also responsible for attaching to specific cellular receptors (7, 8) and for determining the tissue tropism of the various reovirus serotypes (9, 10). mAb 87.92.6 mimics the reovirus type 3 hemagglutinin by binding to both mAb 9BG5 and the reovirus type 3 receptor and by competing with reovirus type 3 for binding to specific cellular receptors (1–3).

Reovirus type 3 inhibits host-cell DNA synthesis upon receptor perturbation (11). This effect is not due to infection of cells, as replication-defective reovirus type 3 particles retain this property (12). We recently have demonstrated that mAb 87.92.6 similarly inhibits DNA synthesis in fibroblasts, neuronal cells, and lymphocytes (13). Data from one such experiment are shown in Fig. 1. Murine fibroblasts [which possess specific receptors for reovirus type 3 (15)] (L cells) were incubated with reovirus type 3 or left untreated (Fig. 1, experiment 1), and the level of DNA synthesis was measured. Reovirus type 3 markedly inhibited DNA synthesis by these cells. mAb 87.92.6 had a similar effect, as shown in Fig. 1, experiment 2. mAb 87.92.6 markedly inhibited DNA synthesis, whereas a control antibody (mAb HO22.1) had no effect.

The amino acid sequences of the mAb 87.92.6 heavy-chain variable regions and light-chain variable regions (V_L) have recently been deduced (16). These sequences have been compared with the sequence of the reovirus type 3 hemagglutinin (17–19). Homology was found between amino acids 317–332 of the hemagglutinin and a combined determinant comprised of the heavy and light chain second complementarity-determining regions (CDR IIs). We have constructed synthetic peptides corresponding to these areas and found that one of these peptides (from the mAb 87.92.6 light-chain CDR II) can inhibit specifically the interactions of both mAb 87.92.6 and reovirus type 3 with the reovirus type 3 receptor (14, 20), indicating that this peptide (termed V_L peptide) specifically interacts with the receptor. We have used these data to construct a structural model for this site in both the reovirus type 3 hemagglutinin and in the mAb 87.92.6 V_L CDR II (14, 20).

We reasoned that V_L peptide might exhibit biologic effects similar to those exhibited by reovirus type 3 and mAb 87.92.6. mAb 87.92.6 is effective only as a native antibody, whereas monomeric Fab fragments have no effect (13). Therefore, we synthesized V_L peptide with an additional amino-terminal cysteine residue to form a dimeric peptide (V_LSH; see Scheme 1). When L cells were treated with V_LSH, marked inhibition of DNA synthesis was observed. Fig. 2 shows a representative experiment. V_L peptide (which lacks the added cysteine residue) had no effect on L-cell proliferation. Several control peptides used also had no effect in these assays (Fig. 2 and data not shown). This result indicates that aggregation of the reovirus type 3 receptors on L cells is essential for the inhibition of DNA synthesis by these peptides.

Aggregation of the reovirus type 3 receptors on some cells by mAb 87.92.6 leads to disappearance of that receptor from the cell surface (21). We reasoned that V_LSH peptide might similarly down-modulate this receptor. Murine thymoma (R1.1) cells were treated with peptides, washed, and the level of expression of both the reovirus type 3 receptors (recognized by mAb 87.92.6) and Thy 1.2 molecules (recognized by mAb HO 13.4) was assessed. A representative experiment is shown in Fig. 3. V_LSH peptide specifically down-modulates the reovirus type 3 receptor in a dose-dependent manner but does not effect the expression of Thy 1.2 molecules on these cells. Monomeric V_L peptide did not produce this effect (Fig. 4), indicating that competition for binding to the reovirus type 3 receptor is not responsible for the decreased staining with mAb 87.92.6.

We have recently been studying the role of specific residues involved in the interaction of V_L peptide with the reovirus type 3 receptor. These studies use variant peptides with substitutions at several positions in the putative binding domain of V_L peptide (14). These studies indicate that deletion of hydroxyl groups from positions 11 (tyrosine), 12

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CDR II, second complementarity-determining region; V_L, light-chain variable region; mAb, monoclonal antibody; V_L peptide, synthetic peptide constructed from the mAb 87.92.6 light-chain CDR II; V_LSH, dimer form of V_L peptide with additional amino-terminal cysteine.

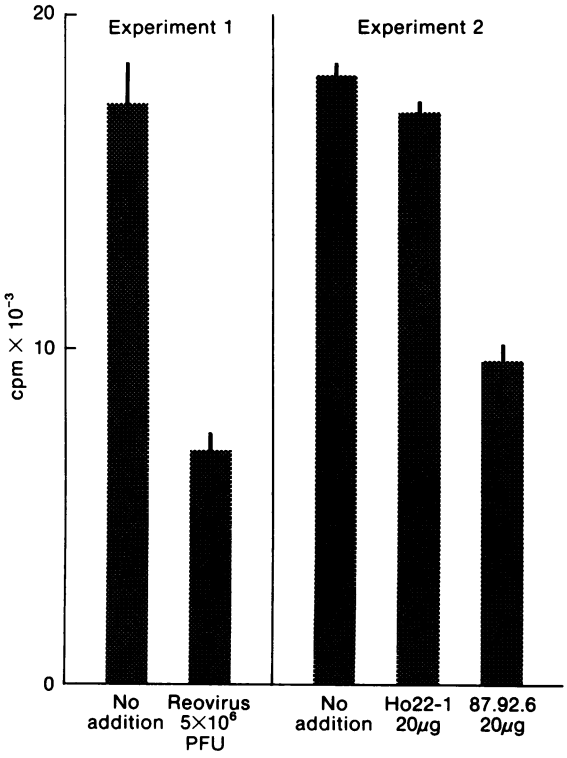

FIG. 1. Reovirus type 3 and mAb 87.92.6 inhibition of L-cell proliferation. L cells (13) were cultured at 5×10^4 cells per well of 96-well microtiter plates in 100 μl of medium for 24 hr. In experiment 1 infectious reovirus type 3 particles (final concentration, ≈400 pM of the reovirus type 3 hemagglutinin), was added and incubated for an additional 24 hr before adding ^{3}H-labeled thymidine. Prior studies had indicated that UV-inactivated reovirus type 3 is effective in inhibiting DNA synthesis by L cells at similar concentrations (12). In experiment 2 purified mAb 87.92.6 or control mAb HO22.1 (14) (final concentration, ≈400 nM) was added for 1 hr at 37°C; then the culture medium was removed and replaced with 100 μl of fresh medium for 24 hr before adding ^{3}H-labeled thymidine. Cells were incubated for an additional 4–6 hr and incorporated cpm of triplicate wells were determined. PFU, plaque-forming units.

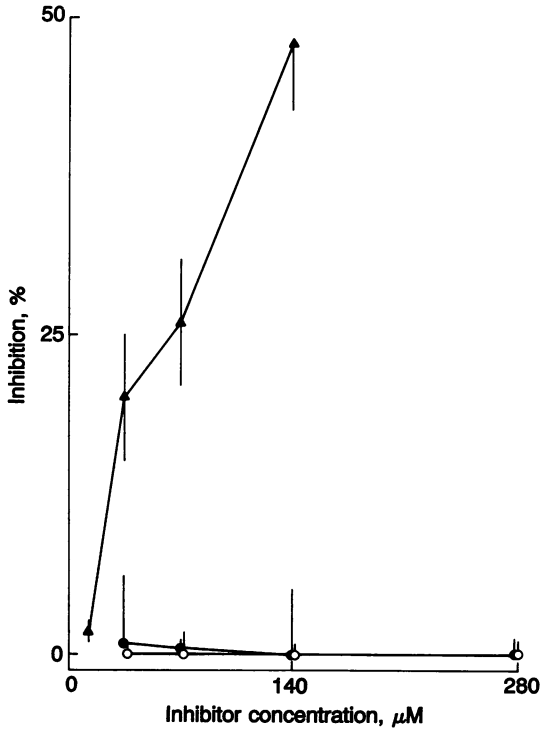

FIG. 2. Inhibition of L-cell proliferation by peptides. L cells were suspended at 10^6 cells per ml in Dulbecco's modified Eagle's medium/10% fetal bovine serum, and 50 μl was added to each well of 96-well microtiter plates. After 24-hr culture, control (●), V_L (○), or V_LSH (▲) peptides were added to the concentrations noted, and the cells were cultured for 24 hr. ^{3}H-labeled thymidine was added for an additional 4–6 hr, and incorporated cpm were determined. Percent inhibition was calculated by the formula: {1 − [(cpm without additive) − (cpm with additive)/(cpm without additive)]} × 100. The peptides used are shown in Scheme 1.

(serine), 14 (serine), or 15 (threonine) reduces the apparent affinity of these peptides for the reovirus type 3 receptor on some cells. Molecular modeling of the region of sequence similarity, based on a comparative modeling approach (14), suggests that the basic structure of this domain is a 3:5 β-hairpin loop (Fig. 5). In addition, in work described elsewhere we and others have shown that the reovirus type 3 receptor is sialylated (22–24), and removal of sialic acid residues limits virus binding. This fact may be relevant to recent studies that have molecularly characterized interactions of other hemagglutinins with sialic acid (25).

To study the effect of these variant peptides on cellular physiology, we have used lectin-induced mitogenesis to provide a system where we can induce both receptor perturbation (by the peptides) and aggregation (by the lectin). Reovirus type 3 and anti-reovirus type 3 receptor antibodies have both been demonstrated to inhibit Con A-induced lymphocyte proliferation (6, 11). We have investigated the effects of these peptides on lymphocyte proliferation both with and without Con A. Without Con A, V_LSH peptide (which is dimeric and can cross-link the receptor) markedly inhibited spontaneous lymphocyte proliferation, whereas V_L peptide (which is monomeric and cannot cross-link the receptors) had no significant effect. However, in the presence of Con A (which should aggregate the receptors), both V_LSH peptide and V_L peptide had similar effects in inhibiting lymphocyte proliferation (26).

When variant peptides were used that lacked hydroxyl groups from positions 12 and 15 (V_LA12 and V_LA15, respectively; see Scheme 1), the inhibition of Con A-induced lymphocyte proliferation was diminished (Fig. 6*A*). This result indicates that these amino acid residues are involved in interactions critical to receptor perturbation, which inhibits proliferation. In contrast, peptides that lacked the hydroxyl groups of positions 11 (tyrosine) and 14 (serine) had similar biologic activity to the unaltered V_L peptide (Fig. 6*B* and data not shown); these hydroxyl groups do not appear to be directly involved in mediating inhibition of DNA synthesis. The predicted positions of all the hydroxyl groups in the turn region are shown in Fig. 5. We also have used a peptide with

```
VL:        Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Thr-Leu-Gln
VLSH:   Cys-Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Thr-Leu-Gln
VLF11:     Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Phe-Ser-Gly-Ser-Thr-Leu-Gln
VLA12:     Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ala-Gly-Ser-Thr-Leu-Gln
VLA13:     Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Ala-Ser-Thr-Leu-Gln
VLA15:     Lys-Pro-Gly-Lys-Thr-Asn-Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Ala-Leu-Gln

Control: Cys-Thr-Tyr-Arg-Tyr-Pro-Leu-Glu-Leu-Asp-Thr-Ala-Asn-Asn-Arg
```

SCHEME 1. Peptide sequences.

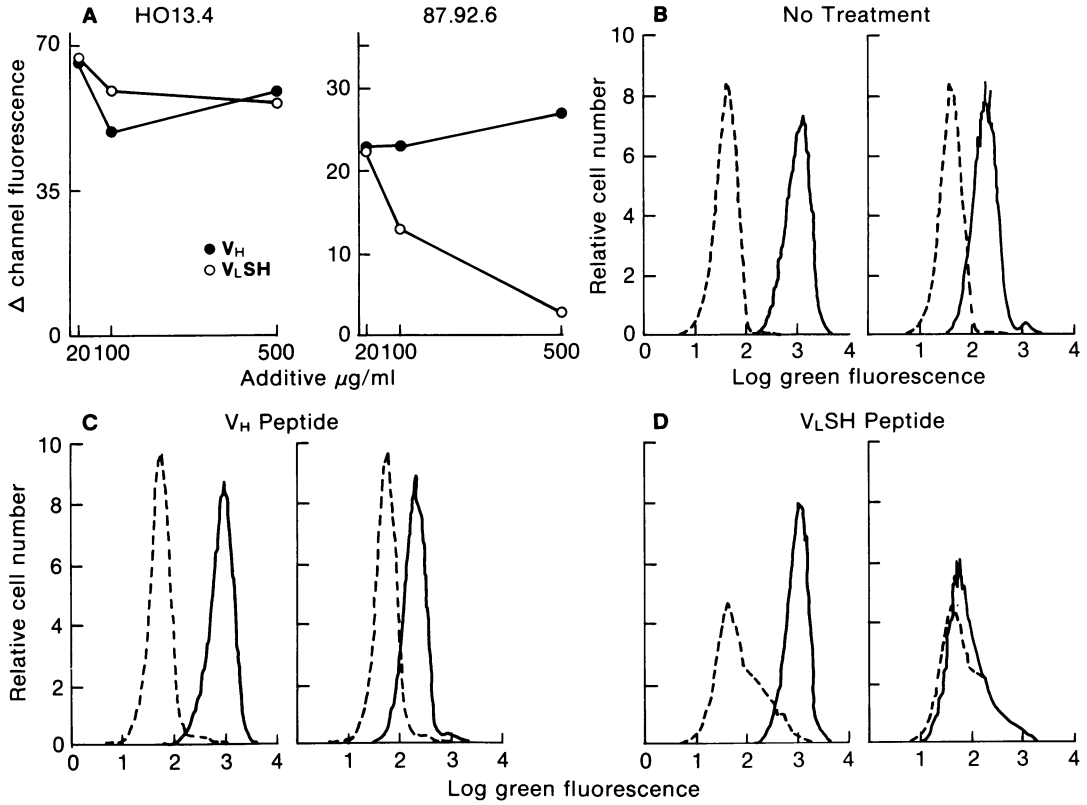

FIG. 3. Modulation of the reovirus type 3 receptor by peptides. R1.1 cells (21) were cultured with peptides at the concentration noted (*A*), left untreated (*B*), or treated with peptide at 500 μg/ml (*C* and *D*) for 1 hr at 37°C. Cells were centrifuged and washed three times in 1% bovine serum albumin in phosphate-buffered saline with 0.1% sodium azide (FACS medium). mAb 87.92.6 (100 μl of hybridoma-bearing ascites fluid) and mAb HO 13.4 (1 μg of affinity-purified antibody) were added for 30 min on ice. Cells were washed, and 100 μl of a 1:100 dilution of fluoresceinated goat anti-mouse immunoglobulin (Southern Biotechnology Associates, Birmingham, AL) was added for 30 min. Cells were washed, and fluorescence intensity was analyzed by flow cytometry. Mean channel fluorescence was compared for cells incubated with or without primary antibody to give the difference (Δ) in mean channel fluorescence (*A*). Cells were stained with mAb HO 13.4 (*A, B, C,* and *D Left*), or with mAb 87.92.6 (*A, B, C,* and *D Right*). Cells were treated with V_H peptide (*A Left*, and *C*) or V_LSH peptide (*A Right*, and *D*); V_H peptide is derived from the sequence of the mAb 87.92.6 heavy-chain CDR II and does not specifically interact with the reovirus type 3 receptor (14). The V_H peptide sequence was Cys-Gln-Gly-Leu-Glu-Trp-Ile-Gly-Arg-Ile-Asp-Pro-Ala-Asn-Gly. Other peptides are described in Scheme 1. (*B–D*) Broken lines, fluorescence without mAb; solid lines, fluorescence with mAb.

a Gly-Ala substitution at position 13 in the putative binding domain of V_L peptide (V_LA13). In contrast to the other

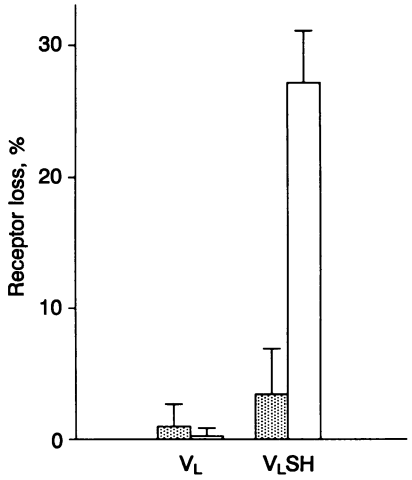

FIG. 4. V_LSH modulates the reovirus type 3 receptor. R1.1 cells were cultured with peptides V_L and V_LSH at 100 μg/ml for 1 hr at 37°C and stained with mAb HO 13.4 (▨) or mAb 87.92.6 (□) before flow cytometry analysis as described in the legend to Fig. 3. Decrease in mean channel fluorescence (MCF) was calculated according to the formula {1 − [Δ MCF without additive) − (Δ MCF with additive)/(Δ MCF without additive)]} × 100. The mean ± SD for three experiments is shown.

FIG. 5. Solid-sphere rendering of β-turn of V_L peptide. Color code: Blue, amino acids that interact directly with reovirus type 3 receptor: green, remaining β-turn and base residues; red, hydroxyl groups [Tyr-11, turn position 1 (lower left); Ser-12, turn position 2 (critical for DNA synthesis inhibition); Ser-14, turn position 4; Thr-15, turn position 5 (lower right; critical for DNA synthesis inhibition)].

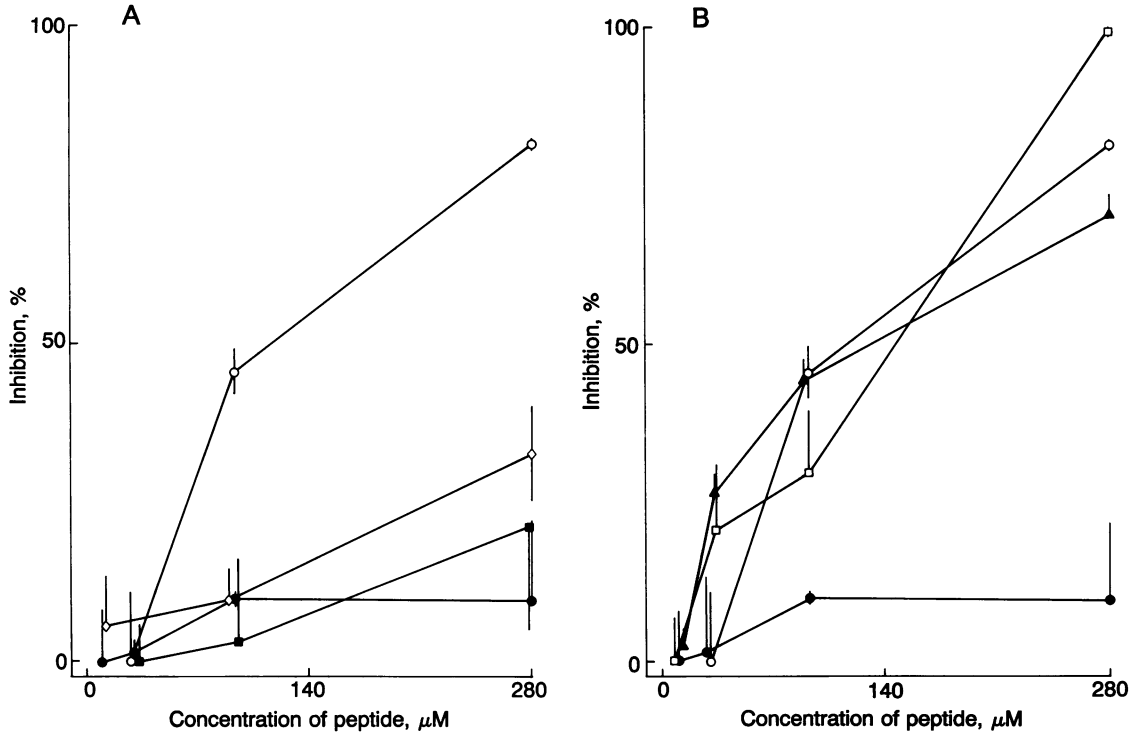

FIG. 6. Peptide inhibition of Con A-induced lymphocyte proliferation. C3H female mouse splenocytes were prepared as a single-cell suspension and cultured at 2.5×10^5 cells per well with peptides at the concentrations noted and with Con A at 2.5 μg/ml. Seventy-two hours later, ^{3}H-labeled thymidine was added; the cells were then harvested in 18 hr, and the incorporated cpm were determined. Percent inhibition was calculated as for Fig. 2. (*A*) Peptides compared were control peptide (●), V_L peptide (○), V_LA12 (■), and V_LA15 (◇). (*B*) Peptides compared were control peptide (●), V_L peptide (○), V_LF11 (▲), and V_LA13 (□). Sequences for these peptides are shown in Scheme 1.

substitutions described, V_LA13 had a slightly increased effect on the inhibition of Con A-induced lymphocyte proliferation at the highest concentration used, although this was not apparent at lower concentrations (Fig. 6*B*). These studies indicate that modification of the V_L peptide can identify specific residues required for receptor perturbation. Specifically, hydroxyl groups found on amino acids 12 (serine) and 15 (threonine), which are in a β-turn region (14, 20), may be most critical to interactions with the cell-bound receptor.

mAbs have been extremely useful in studying many aspects of a large number of biological systems. Although many biologically active mAbs have been described, clinical application has been limited (27). This is, in part, due to concerns about the development of immune responses to these mAbs, including both xenogeneic and anti-idiotypic responses. Our studies indicate that short, nonimmunogenic peptides, modeled after the hypervariable regions of antibodies, can exhibit biological activity. Similar observations have now been made in another system, wherein the biological effect of an anti-fibrinogen receptor mAb with antiplatelet activity was mimicked by a complementarity-determining region-derived peptide (28). Sequential modification of peptides developed by this strategy may lead to the development of substances with increased biologic activity that have potential clinical utility.

We acknowledge Subhashini Srinivasin for her help with calculations, Dr. Alan Pickard for flow cytometry analysis, Dr. Frank Robey for some of the peptides utilized, Dr. Glen Gaulton for providing reovirus type 3, Michael Merva for excellent technical assistance, Peggy Hanrahan for preparing this manuscript, and L. Marie for her comments. This work was supported by grants from the National Institutes of Health, National Cancer Institute, National Eye Institute, the Lucille Markey Charitable Trust (M.I.G.), and American Foundation for AIDS Research (D.B.W.). J.A.C. is the recipient of a clinician–investigator award from National Institute of Neurological and Communicative Disorders and Stroke; W.V.W. is the recipient of a National Institutes of Health Postdoctoral Fellowship and grants from the Measey and McCabe Foundations.

1. Noseworthy, J. H., Fields, B. N., Dichter, M. A., Sobotka, C., Pizer, E., Perry, L. L., Nepom, J. T. & Greene, M. I. (1983) *J. Immunol.* **131**, 2533–2538.
2. Kauffman, R. S., Noseworthy, J. H., Nepom, J. T., Finberg, R., Fields, B. N. & Greene, M. I. (1983) *J. Immunol.* **131**, 2539–2541.
3. Co, M. S., Gaulton, G. N., Fields, B. N. & Greene, M. I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1494–1498.
4. Burstin, S. J., Spriggs, D. R. & Fields, B. N. (1982) *Virology* **117**, 146–155.
5. Spriggs, D. R., Kaye, K. & Fields, B. N. (1983) *Virology* **127**, 220–224.
6. Nepom, J. T., Tarideau, M., Epstein, R. L., Weiner, H. L., Gentsch, J., Fields, B. N. & Greene, M. I. (1982) *Immunol. Res.* **1**, 255–261.
7. Lee, P. W. K., Hayes, E. C. & Joklik, W. K. (1981) *Virology* **108**, 156–163.
8. Masri, S. A., Nagata, L., Mah, D. C. W. & Lee, P. W. K. (1986) *Virology* **149**, 83–90.
9. Weiner, H. L., Drayna, D., Averill, D. R., Jr., & Fields, B. N. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5744–5748.
10. Fields, B. N. & Greene, M. I. (1982) *Nature (London)* **200**, 1–5.
11. Sharpe, A. H. & Fields, B. N. (1983) *J. Virol.* **38**, 389–392.
12. Hand, R. & Tamm, I. (1973) *J. Virol.* **11**, 223–231.
13. Gaulton, G. N. & Greene, M. I. (1989) *J. Exp. Med.* **169**, 197–212.
14. Williams, W. V., Guy, H. R., Rubin, D. H., Robey, F., Myers, J. N., Kieber-Emmons, T., Weiner, D. B. & Greene, M. I. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6488–6492.
15. Epstein, R. L., Powers, M. L., Rogart, R. B. & Weiner, H. L. (1984) *Virology* **133**, 46–55.
16. Bruck, C., Co, M. S., Gaulton, G. N., Smith, T., Fields, B. N. & Greene, M. I. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6578–6582.
17. Bassel-Duby, R., Jayasuriya, A., Chatterjee, D, Sonenberg, N., Maizel, J. V., Jr., & Fields, B. N. (1985) *Nature (London)* **315**, 421–423.

18. Cashdollar, L. W., Chmelo, R. A., Weiner, J. R. & Joklik, W. K. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 24–28.
19. Nagata, L., Masri, S. A., Mah, D. C. W. & Lee, P. W. K. (1984) *Nucleic Acids Res.* **12,** 8699–8710.
20. Williams, W. V., Guy, H. R., Weiner, D. B., Rubin, D. H. & Greene, M. I., in *Vaccines 88,* eds. Ginsberg, H., Brown, F., Lerner, R. A. & Chanock, R. M. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), in press.
21. Gaulton, G., Co, M. S. & Greene, M. I. (1985) *J. Cell. Biochem.* **28,** 69–78.
22. Co, M. S., Gaulton, G., Fields, B. N. & Greene, M. I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1494–1498.
23. Gentsch, J. R. & Pacitti, A. F. (1985) *J. Virol.* **56,** 356–364.
24. Pacitti, A. F. & Gentsch, J. R. (1987) *J. Virol.* **61,** 1407–1415.
25. Weis, W., Brown, J. H., Cusack, S., Paulson, J. C., Skehel, J. J. & Wiley, D. C. (1988) *Nature (London)* **333,** 426–431.
26. Williams, W. V., Guy, H. R., Weiner, D. B. & Greene, M. I. (1989) *Virol. Immunol.*, in press.
27. Martin, P. J., Hansen, J. A., Anasetti, C., Zutts, M., Durnam, D., Storb, R. & Thomas, E. D. (1988) *Am. J. Kidney Dis.* **11,** 149–152.
28. Taub, R., Gould, R. J., Garsky, V. M., Ciccarone, T. M., Hoxie, J., Friedman, P. A. & Shattil, S. J., (1989) *J. Biol. Chem.* **246,** 259–265.