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Immunology

Enhanced immunogenicity of a sequence derived from hepatitis B virus surface antigen in a composite peptide that includes the immunostimulatory region from human interleukin 1

(synthetic peptides/IgG class antibodies/enzyme-linked immunosorbent assay/inbuilt adjuvanticity)

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ABSTRACT **The effect on immunogenicity of coupling the immunostimulatory nonapeptide sequence (residues 163–171) from human interleukin 1β (IL-1β) to a small immunogen was examined. A 21-amino acid sequence spanning positions 12–32 on the large protein of hepatitis B surface antigen was chosen as a model. Three peptides were synthesized corresponding to the IL-1β-derived sequence [peptide IL-(163–171)], the hepatitis B surface antigen-derived sequence [peptide S1-(12–32)] and a composite peptide that included both these sequences separated by a spacer of two glycine residues [peptide S1-(12–32)–IL-(163–171)]. In an *in vitro* thymocyte proliferation assay, both peptides S1-(12–32)–IL-(163–171) and IL-(163–171) showed comparable activity, whereas peptide S1-(12–32) was inactive. Groups of five to seven mice each from C3H/CH, BALB/c, SJL/J, and C57BL/6 strains were immunized with equimolar amounts of either peptide S1-(12–32), peptide S1-(12–32)–IL-(163–171), or a mixture of peptides S1-(12–32) and IL-(163–171), and sera were screened for anti-S1-(12–32) antibodies. In all strains, peptide S1-(12–32)–IL-(163–171) elicited an increased primary and secondary anti-S1-(12–32) antibody response compared to the other two groups. Further, peptide S1-(12–32)–IL-(163–171) also induced an increased number of responders to primary immunization, though the number of responders was quantitative in all groups following secondary immunization. At least part of the enhanced immunogenicity of the S1-(12–32) sequence in peptide S1-(12–32)–IL-(163–171) appears to be due to augmented T-helper cell activity. These results suggest that coupling of the immunostimulatory IL-1β-derived sequence in tandem with an immunogen may confer inbuilt adjuvanticity.**

The cytokine interleukin 1 (IL-1) has been credited with a wide array of biological properties, which include amplification of both cellular and humoral immune responses to infectious or foreign agents (reviewed in refs. 1 and 2). Although precise details at the molecular level remain obscure, it is now widely believed that IL-1 plays an important role in T- and B-cell activation (1, 2). The immunoregulatory effects of IL-1 on T cells include enhanced proliferation in response to mitogen or antigen (3–5) and enhanced production of autocrine growth factors (3, 6). IL-1 has also been shown to act directly on B cells. It augments primary antibody responses both *in vivo* and *in vitro* (7–10) and can prime human B cells for subsequent activation (11). A B-cell growth and differentiation activity for IL-1 has also been described (12). Recently Uhl *et al.* (13) have identified IL-1 receptors on human monocytes, suggesting that initiation of the immune response by IL-1 can also be at the level of accessory cells.

Though it has been suggested that IL-1 could serve as an adjuvant in conjunction with weakly immunogenic vaccines (14), its potent inflammatory activity (1, 2) restricts this application. Antoni *et al.* (15) have synthesized several short peptide fragments from hydrophilic sequences on human and murine IL-1 to determine the minimal structure responsible for immunostimulatory activity. Of these, a fragment of nine amino acid residues corresponding to the sequence spanning positions 163–171 of human IL-1β was found to have high *in vitro* T-cell activation capacity but was devoid of any IL-1-associated inflammatory activity at the concentrations tested (15). Subsequently (16), it was shown that this nonapeptide could also potentiate *in vivo* antibody responses to both T-helper-dependent and T-helper-independent antigens, albeit at very high doses of the peptide (100 mg of peptide per kg of body weight). On the basis of these results and on the reported lack of IL-1-associated inflammatory activity, Nencioni *et al.* (16) proposed that the nonapeptide derived from residues 163–171 on human IL-1β represents a promising candidate adjuvant for use in poorly immunogenic vaccines.

The excessive amounts of the IL-1-derived peptide (residues 163–171) required to elicit a significant enhancement in antibody response against a given immunogen would appear to limit its applicability as an adjuvant. A dose ratio of 100 mg of peptide per kg of body weight clearly limits its use in humans. To overcome this, we decided to investigate whether direct coupling of the human IL-1β-derived sequence (residues 163–171) in tandem with a given immunogen would lead to enhanced immunogenicity. A known immunogenic sequence from the hepatitis B virus (HBV) surface antigen (HBsAg) was chosen as a model. This sequence corresponds to residues 12–32 on the pre-S1 region of the large protein and contains overlapping T-helper and B-cell determinants (17, 18). The results described in this report demonstrate that a composite synthetic peptide containing the HBsAg-derived pre-S1 sequence (residues 12–32) and the human IL-1β-derived sequence (residues 163–171) elicits enhanced primary and secondary antibody responses towards the HBsAg-derived sequence.

MATERIALS AND METHODS

Animals. C3H/CH, BALB/c, C57BL/6, and SJL/J mice were obtained from the breeding facility at the National Institute of Immunology (New Delhi). All mice used for immunization were between 6 and 8 weeks of age, weighed between 10 and 20 g, and were either female (C3H/CH and BALB/c) or male (SJL/J and C57BL/6).

Peptide Synthesis and Purification. Peptides were synthesized by the solid-phase method (19) on an Applied Biosys-

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Abbreviations: IL, interleukin; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus.

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tems model 430A peptide synthesizer by using 4-methylbenzhydrylamine resin. The following side-chain protecting groups were used: *o*-benzyl for glutamic acid, aspartic acid, serine, and threonine; and *o*-bromobenzyloxycarbonyl for lysine. At the end of the synthesis, peptides were simultaneously deprotected and cleaved from the resin with trifluoromethanesulfonic acid in the presence of thioanisole and 1,2-ethanedithiol as scavengers (20–23). Crude peptides were then purified by preparative HPLC on an Aquapore C-8 column (100 × 10 mm; Applied Biosystems). Purified peptides were characterized by amino acid sequencing and amino acid analysis, and results obtained were in good agreement with expected values. All peptides used in this study were at least 95% pure by HPLC.

Murine Thymocyte Proliferation Assay. Thymocytes from 4- to 6-week-old male C3H/CH mice were cultured at 2×10^5 cells per well in Falcon 96-well plates (Becton Dickinson) for 72 hr in culture medium [RPMI 1640 (Sigma) containing 2 mM L-glutamine, 25 mM Hepes buffer, and gentamycin sulfate at 50 μg/ml] supplemented with 5% heat-inactivated fetal bovine serum (Hybrimax, Sigma) and purified phytohemagglutinin (Sigma at 0.5 μg/ml). Synthetic peptides were added at the beginning of culture. Peptide-stimulated thymocyte proliferation was assessed as radioactivity incorporated following an 18-hr pulse with 1 μCi of [^{3}H]thymidine (New England Nuclear, specific activity 2 Ci/mmol; 1 Ci = 37 GBq) per well.

***In Vivo* Immunizations.** One micromole of either peptide S1-(12–32) or peptide S1-(12–32)–IL-(163–171) or a mixture of 1 μmol each of peptides S1-(12–32) and IL-(163–171) were adsorbed onto 1 ml of alum (hydrated aluminum hydroxide, a gift from Raj Raghupathy, National Institute of Immunology, New Delhi) at 4°C overnight. Prior to immunization, the antigen–alum complex was diluted in phosphate-buffered saline to a final peptide concentration of 100 μM, and 200 μl of this solution was injected i.p. into mice (day 0) followed by a booster with an identical dose on day 21. Mice from various groups were bled from the retroorbital plexus on days 14, 26, 35, 42, 49, and 56.

Measurement of *in Vivo* Anti-S1-(12–32) Production. Sera from individual mice were titrated by using direct ELISA. Plates (Titertek) were coated with 2 μg of peptide S1-(12–32) per well after first establishing that coating with either 1 or 2 μg of peptide per well gave optimal results in this assay. Coated plates were incubated with 50 μl of mouse serum of appropriate dilution per well at 37°C for 2 hr followed by three washes with 200 μl per well per wash. A 1:1000 dilution of peroxidase-conjugated goat anti-mouse IgG (Sigma) was added to each well in 100 μl, and the plate was incubated for an additional 2 hr at 37°C. After an additional three washes (200 μl per well per wash), bound secondary antibody was detected at 405 nm by using the diammonium salt of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) as chromogen. Data are expressed as the antibody titer representing the highest dilution to yield 2.5 times the OD units obtained with preimmunization sera at a dilution of 1:5.

RESULTS

Choice and Design of Synthetic Peptides. For the present study, we chose a synthetic peptide derived from the surface antigen sequence of HBV as our model immunogen. This peptide corresponds to the amino acid sequence spanning residues 12–32 on the pre-S1 region coded for by the HBV envelope gene [peptide S1-(12–32)] (17, 24) and contains overlapping T-helper and B-cell recognition sites within residues 12–21 and 16–27, respectively (18). Consequently peptide S1-(12–32) is immunogenic (25, 26); it is capable of priming murine T-helper cell activity for subsequent HBsAg challenge in a variety of *H-2* haplotypes (18, 27).

To test our hypothesis that coupling of the immunostimulatory human IL-1β sequence (residues 163–171) to an immunogen would lead to enhanced immunogenicity, three peptides were synthesized as depicted in Fig. 1. All of these were synthesized as the carboxyl-terminal amides. Peptide S1-(12–32) corresponds to the HBsAg-derived sequence of subtype adw (17), whereas peptide IL-(163–171) is the immunostimulatory sequence derived from human IL-1β (15). Peptide S1-(12–32)–IL-(163–171) represents a composite sequence containing both the HBsAg and the IL-1β-derived sequences separated by a spacer of two glycine residues (Fig. 1). A two-glycine spacer between the immunogenic and immunostimulatory functional regions was chosen for the following reasons: (*i*) since glycine is a strong disrupter of α-helix secondary structure formation and indifferent toward β-sheet secondary structure formation (28, 29), a two-glycine-residue spacer would effectively prevent any secondary structural overlap between the S1-(12–32) and IL-(163–171) sequences, thereby maintaining their discrete identities; (*ii*) given the zero hydrophilicity value of glycine (30), the spacer used should have minimal influence on the hydrophilicity profile of the immunogenic S1-(12–32) sequence; and (*iii*) since they lack a side chain, the glycines were not expected to add any novel antigenic features to the S1-(12–32) region.

Peptides IL-(163–171) and S1-(12–32)–IL-(163–171) Show T-Cell Activation Capacity. Antoni *et al.* (15) have demonstrated that peptide IL-(163–171) is capable of stimulating phytohemagglutinin-activated thymocyte cultures in a dose-dependent manner and that the stimulatory capacity was not due to IL-2-like activity. To test our preparation of IL-(163–171) and also peptide S1-(12–32)–IL-(163–171), phytohemagglutinin-activated C3H/CH thymocyte cultures were incubated with various concentrations of either peptide IL-(163–171), peptide S1-(12–32)–IL-(163–171), or peptide S1-(12–32), followed by a [^{3}H]thymidine pulse (see *Materials and Methods*). The results of this experiment are shown in Fig. 2. Both peptides IL-(163–171) and S1-(12–32)–IL-(163–171) displayed significant dose-dependent thymocyte stimulatory activity while, as might be expected, peptide S1-(12–32) was inactive (Fig. 2).

On a molar basis, the potency of peptides IL-(163–171) and S1-(12–32)–IL-(163–171) was comparable (Fig. 2), indicating that the human IL-1β-derived sequence retained its activity on incorporation into the composite peptide sequence.

Peptide S1-(12–32)–IL-(163–171) Elicits Enhanced Anti-S1-(12–32) Antibody Levels in Mice. We next decided to examine the immunogenicity of the S1-(12–32) sequence in peptide S1-(12–32)–IL-(163–171). For this, 0.02 μmol of either peptide S1-(12–32), peptide S1-(12–32)–IL-(163–171), or an equimolar mixture of 0.02 μmol each of peptide S1-(12–32) and peptide IL-(163–171) were adsorbed onto alum and injected i.p. into three separate groups of five to seven mice each. In addition, four different strains of mice were included in this study (namely, C3H/CH, BALB/c, SJL/J, and C57BL/6). Mice from all groups were immunized with the respective antigen–alum complex followed by a

PEPTIDE	SEQUENCE
S1[12-32]	MGTNLSVPNPLGFLPDHQLDP
IL[163-171]	VQGEESNDK
S1[12-32]-IL[163-171]	MGTNLSVPNPLGFLPDHQLDP<u>GG</u>VQGEESNDK

FIG. 1. Amino acid sequence of the synthetic peptides used in this study. Amino acids are identified by the one-letter code. All peptides were synthesized as the carboxyl-terminal amides. The two-glycine-residue spacer in peptide S1-(12–32)–IL-(163–171) is underlined.

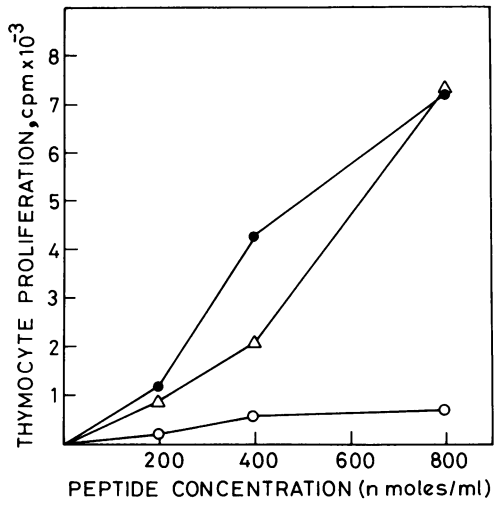

FIG. 2. Thymocyte proliferation in response to peptides IL-(163–171), S1-(12–32)–IL-(163–171), and S1-(12–32). Synthetic peptides were added at the concentrations indicated to phytohemagglutinin-activated C3H/CH thymocyte cultures. ○, Peptide IL-(163–171); ●, peptide S1-(12–32)–IL-(163–171); △, peptide S1-(12–32). Values represent the average ± SEM of triplicate cultures after correction for background obtained in the absence of peptide.

secondary immunization with an identical amount 3 weeks later (day 21, see *Materials and Methods*). Sera collected from individual mice at various times were assayed for IgG class antibodies specific for the S1-(12–32) sequence, and these results are presented in Fig. 3. In all strains tested, peptide S1-(12–32)–IL-(163–171) elicited a higher anti-S1-(12–32) antibody response as compared to either peptide

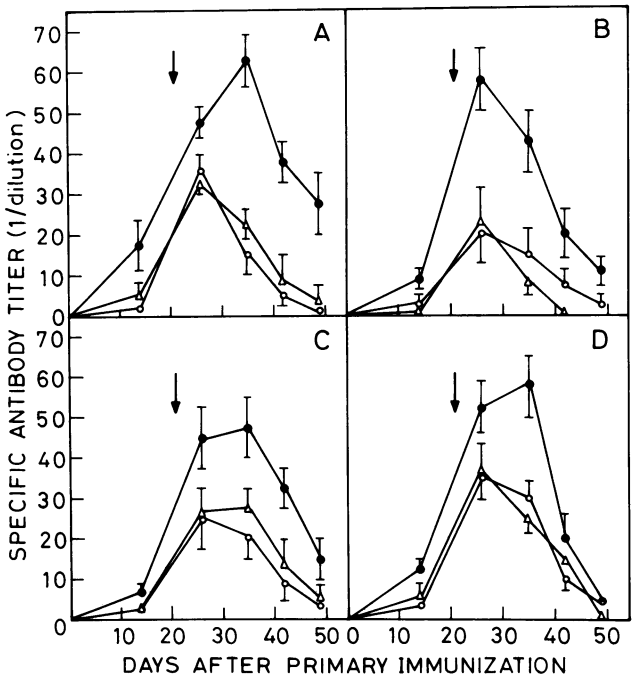

FIG. 3. Peptide S1-(12–32)–IL-(163–171) elicits enhanced anti-S1-(12–32) antibody levels in mice. Groups of five to seven mice each were immunized with either peptide S1-(12–32) (○), peptide S1-(12–32)–IL-(163–171) (●), or an equimolar mixture of peptides S1-(12–32) and IL-(163–171) (△). These animals were boosted on day 21 (indicated by arrow), and sera collected on the days indicated were assayed for anti-S1-(12–32) antibodies. Strains of mice used were C3H/CH (*A*), BALB/c (*B*), SJL/J (*C*), and C57BL/6 (*D*). Values presented are the mean ± SD of all mice in a group that gave positive antibody titer values.

S1-(12–32) alone or the mixture of peptides S1-(12–32) and IL-(163–171) (Fig. 3). Although the magnitude of this effect varied between different strains, increased antibody levels induced by peptide S1-(12–32)–IL-(163–171) were observed in response to both primary and secondary immunizations. Stimulation in primary response induced by peptide S1-(12–32)–IL-(163–171) versus peptide S1-(12–32) among the various strains varied from about 2-fold to as much as 8-fold in the case of C3H/CH mice (Fig. 3*A*), whereas that for the corresponding secondary response varied from about 1.5- to 3-fold. Antibody levels were down to background by day 56 in all cases except the C3H/CH group immunized with peptide S1-(12–32)–IL-(163–171), where a titer of 1:20 ± 4 was obtained (data not shown).

Antisera obtained on day 26 from S1-(12–32)–IL-(163–171)-immunized groups also showed lower levels of anti-IL-(163–171) antibodies, corresponding to titers of about 1:10 in C3H/CH and BALB/c strains and about 1:5 in SJL/J and C57BL/6 strains. The antibody response observed was peptide-specific since antisera obtained from all strains were found to be incapable of recognizing a synthetic peptide corresponding to residues 120–145 within the pre-S2 region of HBsAg (data not shown).

An additional group of six C3H/CH mice were also immunized with a composite peptide containing the S1-(12–32) sequence and a separate nonapeptide sequence from human IL-1β (residues 189–197) that has been shown to be devoid of immunostimulatory activity (15). This composite peptide [peptide S1-(12–32)–IL-(189–197)] failed to elicit detectable levels of anti-S1-(12–32)-specific antibodies on day 14. Anti-S1-(12–32) antibody titers on days 26 and 35 were 1:28 ± 8 and 1:16 ± 5, respectively.

It is not surprising that the mixture of peptides S1-(12–32) and IL-(163–171) did not significantly stimulate anti-S1-(12–32) antibody response over that obtained with peptide S1-(12–32) alone (Fig. 3). The amount of peptide IL-(163–171) employed in these studies was suboptimal; it was at least 50 times lower than that used by Nencioni *et al.* (16). Since peptide S1-(12–32)–IL-(163–171) was also used in identical amounts (0.02 μmol per mouse), the IL-1β-derived sequence (residues 163–171) appears to have gained severalfold in *in vivo* antibody potentiating activity when present in the composite peptide sequence.

Increased Number of Responders in Groups Immunized with Peptide S1-(12–32)–IL-(163–171). In the experiment described in Fig. 3, we observed that the number of primary responders in groups immunized with peptide S1-(12–32)–IL-(163–171) was significantly higher as compared to those immunized with either peptide S1-(12–32) or the S1-(12–32) and IL-(163–171) peptide mixture when a cut-off value of 1:4 for titer was used (Table 1). The only exception to this was C3H/CH mice where the mixture of S1-(12–32) and IL-(163–171) peptides also elicited an increased number of responders (Table 1). After secondary immunization, however, the number of responders was near 100% in all strains tested (Table 1).

DISCUSSION

The peptide S1-(12–32)–IL-(163–171) has several distinct properties. It is bifunctional: it displays an immunostimulatory capacity due to the IL-1β-derived sequence at its carboxyl end and immunogenicity due to the HBsAg-derived sequence toward its amino terminus. Its design included a spacer of two glycine residues by virtue of which the functional domains appear to have retained their discrete identities. The S1-(12–32) sequence was found to acquire enhanced immunogenicity in the composite peptide S1-(12–32)–IL-(163–171). Finally, the number of primary responders to S1-(12–32) was also higher in groups immunized with S1-(12–32)–IL-(163–171) as

Table 1. Peptide S1-(12–32)–IL-(163–171) induces an increased number of primary anti-S1-(12–32) responders

Strain	*H-2* haplotype	Immunogen	Number of responders 1°	2°
C3H/CH	*k*	S1-(12–32)	0/6	5/5
		S1-(12–32) + IL-(163–171)	4/6	4/4
		S1-(12–32)–IL-(163–171)	4/6	5/5
BALB/c	*d*	S1-(12–32)	2/7	5/6
		S1-(12–32) + IL-(163–171)	1/6	6/6
		S1-(12–32)–IL-(163–171)	5/7	6/6
SJL/J	*s*	S1-(12–32)	1/5	5/5
		S1-(12–32) + IL-(163–171)	0/5	5/5
		S1-(12–32)–IL-(163–171)	4/5	5/5
C57BL/6	*b*	S1-(12–32)	1/3	3/3
		S1-(12–32) + IL-(163–171)	2/5	5/5
		S1-(12–32)–IL-(163–171)	4/5	4/4

Data obtained from Fig. 3 were analyzed to determine the number of responders to primary (1°) and secondary (2°) immunization in each group. A cut-off value for titer of 1:4 was used because this was the lowest antiserum dilution employed in our assays. Numbers represent the number of responder mice out of the total number of mice in each group.

opposed to those immunized with either S1-(12–32) alone or the S1-(12–32) and IL-(163–171) mixture. Notably, our results also indicate that the IL-(163–171) sequence gains severalfold in *in vivo* antibody potentiating activity when present in the composite peptide. For instance, the level of IL-(163–171) sequence used here as peptide S1-(12–32)–IL-(163–171) is at least 50 times lower than that of the free peptide IL-(163–171) used by Nencioni *et al.* (16).

The major proportion of antibodies obtained in response to S1-(12–32)–IL-(163–171) was found to be directed against the S1-(12–32) sequence, though a minority population of anti-IL-(163–171) antibodies was also detected. This response to the IL-1β-derived sequence might well be expected for the following reasons: (*i*) there is a species-specific variation in sequence within this region between human and murine IL-1β (31), and (*ii*) the IL-1β-derived sequence was linked to a nonself sequence.

The choice of a weak adjuvant such as alum in these studies was deliberate in order to maximize sensitivity toward the influence of the IL-1β-derived sequence. Thus while anti-S1-(12–32) antibody titers obtained were relatively low, nevertheless, the increased immunogenicity of the S1-(12–32)–IL-(163–171) sequence in peptide S1-(12–32)–IL-(163–171) was clearly apparent. The S1-(12–32)-specific T-helper cell recruitment in C3H/CH mice immunized with either peptide S1-(12–32) or peptide S1-(12–32)–IL-(163–171) was also examined. However, as might be expected from the low titers obtained, priming of lymphocytes from either peripheral blood mononuclear cells or popliteal lymph nodes was very weak as observed upon subsequent challenge with peptide S1-(12–32) (data not shown). Nevertheless incorporation of [^{3}H]thymidine in S1-(12–32)–IL-(163–171)-primed cells was about 1.5- to 2-fold greater than that obtained for S1-(12–32)-primed cells (data not shown).

Although the mechanism by which the S1-(12–32) sequence acquires enhanced immunogenicity in the composite peptide S1-(12–32)–IL-(163–171) is not clear at the present time, the simplest explanation could lie in the immunostimulatory properties of the adjacent IL-(163–171) sequence (15, 16, 32). This is supported by our observations that, at least in C3H/CH mice, the composite peptide S1-(12–32)–IL-(189–197) did not display any enhanced S1-(12–32)-specific immunogenicity.

It remains to be seen if the results described here could also be extended to other immunogens. Of particular interest would be the effect of coupled IL-1β-derived immunostimulatory sequence on immunogenicity of larger immunogens. If it is indeed found to have broad applicability, this approach suggests a strategy for conferring inbuilt adjuvanticity to immunogens including vaccines.

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1. Dinarello, C. A. (1989) *Adv. Immunol.* **44,** 153–205.
2. Oppenheim, J. J., Kovacs, E. J., Matsushima, K. & Durum, S. K. (1986) *Immunol. Today* **7,** 45–56.
3. Mizel, S. B. (1987) *Immunol. Today* **8,** 330–332.
4. Gery, I., Gershon, R. K. & Waksman, B. H. (1972) *J. Exp. Med.* **136,** 128–142.
5. Mizel, S. B. & Ben-zui, A. (1980) *Cell. Immunol.* **54,** 382–389.
6. Tartakovsky, B., Finnegan, A., Muegge, K., Brody, D. T., Kovacs, E. J., Smith, M. R., Berzofsky, J. A., Young, H. A. & Durum, S. K. (1988) *J. Immunol.* **141,** 3863–3867.
7. Wood, D. D. & Gaul, S. L. (1974) *J. Immunol.* **113,** 925–933.
8. Leibson, H. J., Marrak, P. & Kappler, J. (1983) *J. Immunol.* **129,** 1398–1403.
9. Frasca, D., Boraschi, D., Baschieri, S., Bossa, P., Tagliabue, A., Adorini, L. & Doria, G. (1988) *J. Immunol.* **141,** 2651–2655.
10. Reed, S. G., Pihl, D. L. & Grabstein, K. (1989) *J. Immunol.* **142,** 2067–2071.
11. Freedman, A. S., Freeman, G., Whitman, J., Segil, J., Daley, J. & Nadler, L. M. (1988) *J. Immunol.* **141,** 3398–3404.
12. Pike, B. L. & Nossal, G. J. V. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8153–8157.
13. Uhl, J., Newton, R. C., Giri, J. G., Sandlin, G. & Horuk, R. (1989) *J. Immunol.* **142,** 1576–1581.
14. Reed, S. G., Pihl, D. L., Conlon, P. J. & Grabstein, K. H. (1989) *J. Immunol.* **142,** 3129–3133.
15. Antoni, G., Presentini, R., Perin, F., Tagliabue, A., Ghiara, P., Censini, S., Volpini, G., Villa, L. & Boraschi, D. (1986) *J. Immunol.* **137,** 3201–3204.
16. Nencioni, L., Villa, L., Tagliabue, A., Antoni, G., Presentini, R., Perin, F., Sivestri, S. & Boraschi, D. (1987) *J. Immunol.* **139,** 800–804.
17. Neurath, A. R. & Kent, S. B. H. (1988) *Adv. Virus Res.* **34,** 64–142.
18. Milich, D. R., McLachlan, A., Moriarty, A. & Thornton, G. B. (1987) *J. Immunol.* **138,** 4457–4465.
19. Kent, S. B. H. & Clarke-Lewis, I. (1985) in *Synthetic Peptides in Biology and Medicine*, eds. Alitalio, K., Partanen, P. & Vaheri, A. (Elsevier, Amsterdam), pp. 29–57.
20. Yajima, H., Fujii, N., Ogawa, H. & Kawatani, H. (1974) *J. Org. Chem.* 107–108.
21. Kiso, Y., Ukawa, K., Nakamura, S., Ito, K. & Akita, T. (1980) *Chem. Pharm. Bull.* **28,** 673–676.
22. Yajima, H. & Fujii, N. (1981) *J. Am. Chem. Soc.* **103,** 5867–5871.
23. Yajima, H., Minamitake, Y., Funakoshi, S., Katayama, I., Fujii, N., Segawa, T., Nakata, Y., Yasuhara, T. & Natajima, T. (1982) *Chem. Pharm. Bull.* **30,** 344–348.
24. Neurath, A. R., Kent, S. B. H., Strick, N., Taylor, P. & Stevens, C. E. (1985) *Nature (London)* **315,** 154–156.
25. Neurath, A. R., Kent, S. B. H., Parker, K., Prince, A. M., Strick, N., Brotman, B. & Sprout, P. (1986) *Vaccine* **4,** 35–37.
26. Neurath, A. R., Kent, S. B. H., Strick, N. & Parker, K. (1988) *Appl. Virol. Res.* **1,** 107–128.
27. Milich, D. R. (1988) *Immunol. Today* **9,** 380–386.
28. Chou, P. Y. & Fasman, G. D. (1978) *Adv. Enzymol.* **47,** 45–148.
29. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47,** 251–276.
30. Hopp, T. P. & Woods, K. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3824–3828.
31. March, C. J., Mosely, B., Larsen, A., Cerretti, D. P., Braedt, G., Price, V., Gillis, S., Henney, C. S., Kronheim, S. R., Grabstein, K., Conlon, P. J., Hopp, T. P. & Cosmon, D. (1985) *Nature (London)* **315,** 641–647.
32. Boraschi, D., Volpini, G., Villa, L., Nencioni, L., Scapigliati, G., Nucci, D., Antoni, G., Matteuci, G., Cioli, F. & Tagliabue, A. (1989) *J. Immunol.* **143,** 131–134.