JOURNAL OF VIROLOGY, Nov. 1995, p. 7147–7151 Vol. 69, No. 11
0022-538X/95/$04.00+0

Emergence of Virus Escape Mutants after Immunization with Epitope Vaccine

GUNNAR WEIDT, WOLFGANG DEPPERT, OLAF UTERMÖHLEN, JOCHEN HEUKESHOVEN, AND FRITZ LEHMANN-GRUBE*

Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Hamburg, Germany

Received 5 April 1995/Accepted 16 August 1995

BALB/c and C57BL/6J mice were immunized with recombinant vaccines consisting of lymphocytic choriomeningitis virus $CD8^+$ T-lymphocyte epitopes and a carrier protein. During challenge infection with WE strain lymphocytic choriomeningitis virus, mutants with alterations in distinct amino acid residues of the epitopic nonapeptides appeared and multiplied. Splenocytes from WE-infected BALB/c mice lysed cells coated with the WE-type epitope; lysis was considerably less effective when the epitopic nonapeptide with which the syngeneic cells had been sensitized was the mutated form. Neither target was lysed by splenocytes from BALB/c mice infected with the variant virus. Mutants were not detected in F_1 hybrid mice immunized with two viral epitopes that were restricted by class I molecules of both parents.

A salient property of viruses, especially if they belong to the RNA subgroup, is variability. Its basis is genetic instability (20), but how a particular phenotype is selected is in general not well understood. One mechanism is immune escape, which we studied in mice experimentally infected with lymphocytic choriomeningitis (LCM) virus.

For protective immunity against viruses, $CD8^+$ T lymphocytes are considered to be pivotal. These cells virtually alone terminate acute LCM virus infection in a mouse (25, 48) and mediate protection resulting from injection of recombinant viral proteins (4, 44). $CD8^+$ T-cell-mediated immunity against LCM virus is also induced with fusion proteins consisting of major histocompatibility complex class I molecule-restricted viral T-cell epitopes and non-LCM virus components (43).

Subsequently, it was observed that in these mice the infection is not well controlled when the quantity of challenge virus is increased, because of, as will be shown here, virus escape from immunologic control.

(This work is part of the Ph.D. thesis of G. Weidt.)

MATERIALS AND METHODS

Virus and peptides. LCM virus of strain WE (36) was propagated and titrated as PFU in L (929) cells (23). The nonapeptides for sensitizing target cells were synthesized by continuous flow Fmoc strategy (3), employing NovaSyn TG resin (Novabiochem GmbH, Bad Soden, Germany). They were cleaved from the resin with trifluoroacetic acid in the presence of thioanisol and purified by reverse-phase high-performance liquid chromatography on a C_{18} column with a shallow water-methanol gradient containing 0.1% trifluoroacetic acid. Subsequent analysis by the dabsyl-Cl method (10) and mass spectrometry (MALDI-TOF) (19) gave the expected values.

Mice. Female BALB/cAnNCrlBr, C57BL/6NCrlBR (B6), and [BALB/cAnNCrlBR × C57BL/6JNCrlBR]F_1 ($CB6F_1$) mice that were purchased specified pathogen-free from Charles River Deutschland, Sulzfeld, Germany. They were kept behind barriers and used when 8 to 12 weeks old.

Epitope vaccine and immunization. Vaccines consisting of nonapeptides corresponding to LCM virus T-lymphocyte epitopes and amino acid residues (aa) 2 to 270 of the simian virus large tumor antigen ($TAg_{2–270}$) were synthesized as described previously (43). cDNA coding for $TAg_{2–270}$ was cloned into the vector pH6EX3 (5) and expressed in *Escherichia coli*. Insertion of aa 118 to 126 of the LCM virus nucleoprotein (NP) and aa 34 to 42 of the LCM virus glycoprotein (GP), which are, respectively, L^d- and D^b-restricted epitopes (17, 38) was achieved by oligonucleotide cloning. These chimeric proteins were injected together with sodium dodecyl sulfate intraperitoneally in 5-μg portions into mice, which were subsequently challenged by virus infection.

Sequencing of viral cDNA corresponding to epitopes and their flanking regions. L cells were infected at a multiplicity of infection of 0.01. After incubation for 36 h at 37°C they were lysed in guanidium thiocyanate buffer. RNA was extracted with phenol-chloroform, and 0.5 μg of RNA was reverse transcribed. Viral cDNA was amplified by PCR with 35 cycles each for 15 s at 94°C, for 15 s at 55°C, and for 60 s at 72°C, by using primer pairs (CTCAGGAGTCTTAAC CAGACTGTG and TACTACACCACTACTTGCACCCTG and GGTCA GATTGTGACAATGTTT and GAGAAACTTTGTCTTTTCCTG) (37). The products were purified by agarose gel, T/A ligated into a derivative of pUC18 (9), and cloned in *E. coli*. The clones were sequenced by Sanger's chain-termination method.

Chromium release assay. The cytotoxic T-lymphocyte (CTL) activity of splenocytes was determined according to the method of Brunner et al. (7), with modifications (18). Target cells were simian virus 40-transformed BALB/c fetal fibroblasts, either incubated for 30 min at 37°C at known concentrations of nonapeptides or virus infected for 48 h at a multiplicity of infection of 0.01. Effector and target cells were incubated for 4 h at 37°C, and specific release was calculated by correcting for release from noninfected control target cells in the presence of effector cells.

RESULTS

Emergence of viral escape mutants in immunized mice. BALB/c mice were immunized with a protein consisting of $TAg_{2–270}$ and the immunodominant L^d-restricted T-cell epitope (aa 118 to 126) of the LCM virus NP (38). After 7 days they were injected intravenously (i.v.) with 10^6 PFU of strain WE of LCM virus, which is equal to 10^7 mouse infectious units (24), and 5 days later the infectious titers in spleens were determined. These titers were lower than those in mice not previously immunized but not as low as had been anticipated. We suspected immune escape, but in 21 of 21 virus clones recovered from the organs of immunized mice, the nucleotides corresponding to the chimeric protein's epitope had not changed.

The virus retrieved from immunized mice was passaged again in immunized mice, this time by inoculation with 10^3 PFU (Table 1). On day 5 the virus titers in the spleens were almost as high as those in nonimmune mice infected with the same dose of either first-mouse-passage or L-cell-derived original WE virus and approximately 1,000-fold higher than the titers in immunized mice undergoing infection with virus that had multiplied in nonimmune mice or in L cells. Further experiments of this kind led to similar results, and essentially the same results were observed when the virus was passaged in B6

* Corresponding author. Mailing address: Heinrich-Pette-Institut, Martinistrasse 52, 20251 Hamburg, Germany. Phone: 040-480510. Fax: 040-464709.

TABLE 1. Emergence of escape mutants during passage of LCM virus in BALB/c mice immunized with an epitope vaccine consisting of aa 118 to 126 of viral NP and TAg_{2-270}

Passage (PFU) and source of virus[a]	No. of mice	Immunization[b]	Titer[c]
One (10^6)	5	Yes	$1.45 \times 10^5 \pm 0.54 \times 10^5$
	5	No	$2.17 \times 10^7 \pm 0.32 \times 10^7$
Two (10^3)			
Previous passage in immunized mice	5	Yes	$1.97 \times 10^6 \pm 1.17 \times 10^6$
	3	No	$6.78 \times 10^6 \pm 1.14 \times 10^6$
Previous passage in nonimmunized mice	3	Yes	$4.77 \times 10^3 \pm 2.75 \times 10^3$
	3	No	$6.88 \times 10^6 \pm 1.06 \times 10^6$
L cells[d]	1	Yes	$<2 \times 10^3$
	1	No	2.17×10^6

[a] Infection by i.v. injection of 10^6 PFU of L-cell-grown WE strain virus for passage one and 10^3 PFU of virus from spleens of infected immunized or nonimmunized mice for passage two.
[b] Immunization by intraperitoneal injection of 5 μg of chimeric protein together with sodium dodecyl sulfate 7 days before injection of virus.
[c] Mean (± standard error of the mean) PFU per gram of spleen.
[d] Original WE strain virus (not passaged in mice).

mice that had been immunized with a chimeric protein containing the D^b-restricted T-cell epitope (aa 34 to 42) of the LCM virus GP (17) (data not shown).

The conclusion that the multiplying virus had undergone some change that enabled its escape from immunologic control was substantiated by sequencing cDNA corresponding to aa 67 to 157 of NP and aa 2 to 260 of GP derived from cloned virus of second passages in immune BALB/c and B6 mice, respectively. In each case, the nonapeptides representing the epitopes were altered in one amino acid (Fig. 1). In the flanking regions, aa 80 of the NP was consistently leucine rather than proline as reported previously (37). Further aberrations, probably representing mutations as well as PCR errors, were random and affected approximately 2 of 1,000 nucleotides.

Kinetics of emergence of virus variants. The finding that during infection for 5 days in epitope-immune mice viral epitope variants were not detected, although they were present on day 5 of second passage in similarly immunized mice, suggested that in order for mutants to attain detectable levels, some minimal virus multiplication had to occur; it also suggested that once mutants had appeared, they rapidly outgrew the original strain WE virus. For obtaining some quantitative information on this point, use was made of the fact that a *Hin*dIII restriction site in the region of the strain WE virus RNA that coded for aa 118 to 126 of the viral NP (the L^d-restricted epitope) was lost in the mutated form.

Amino Acid Residues 118-126 of Nucleoprotein

Wild Type	AGG CCT CAA GCT TCT GGA GTC TAC ATG Arg Pro Gln Ala Ser Gly Val Tyr Met	5/5
Mutant 1	... ... .G. ... ... Arg	26/26
Mutant 2	... ... .GG ... ... Arg	15/15

Amino Acid Residues 34-42 of Glycoprotein

Wild Type	GCT GTG TAC AAT TTC GCC ACC TGT GGG Ala Val Tyr Asn Phe Ala Thr Cys Gly	6/6
Mutant	... ... C.. ... ... Leu	7/7

FIG. 1. Mutations of LCM virus epitopes in LCM virus-infected BALB/c (*H-2*^{d}) (top panel) and B6 (*H-2*^{b}) (bottom panel) mice that had been immunized with fusion proteins consisting of LCM virus immunodominant L^d- or D^b-restricted T-cell epitopes and TAg_{2-270}. On the right, the number of mutants of the number of individually tested clones are indicated.

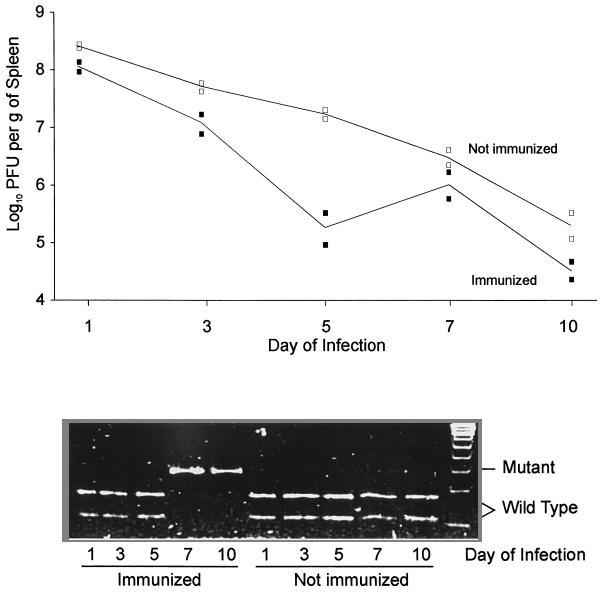

FIG. 2. Emergence of LCM virus variants in BALB/c mice immune to chimeric protein containing aa 118 to 126 of viral NP and infected with 10^6 PFU. Mice were vaccinated and 7 days later injected i.v. with virus. At intervals, infectious titers were determined in spleens of two individual mice and plotted (upper panel). Also, virus from the spleens of individual mice was used to infect L cells. After 44 h, RNA from the infected cells was reverse transcribed. The cDNA regions corresponding to the epitope were PCR amplified, and the fragments thus obtained treated with *Hin*dIII and separated by polyacrylamide gel electrophoresis. Each band (lower panel) represents the finding for one mouse, but similar results were obtained with spleens of the other animals sacrificed the same day.

BALB/c mice were immunized by injection of the epitope vaccine and infected by i.v. injection of 10^6 PFU of strain WE virus; infected control mice had not been vaccinated. At intervals, infectious titers were determined in the spleens of two mice from each group. In parallel, L cells were infected with virus from the spleen of each individual mouse; the cellular RNA was extracted, reverse transcribed, and amplified by PCR as has been done in the experiments for which the results are depicted in Fig. 1. The cDNA fragments thus obtained were treated with *Hin*dIII and subsequently separated by polyacrylamide gel electrophoresis. The results are depicted in Fig. 2.

As has been mentioned, multiplication of infectious virus was only slightly affected by epitope-specific immunity, although on day 5 the titers were approximately 100-fold lower than those in control mice. Thereafter, the infectivity rose and subsequently declined again. With regard to the viral PCR products, electrophoresis revealed that on day 5 of infection most were fragmented by *Hin*dIII, but on day 7 the wild-type form had become undetectable. Although these results do not allow exact quantitative evaluation, it can be estimated that 5 days after injection of 10^6 PFU in the epitope-immune mice greater than 95% of the epitope-encoding nucleotides were wild type (strain WE), whereas 2 days later greater than 95% were mutated.

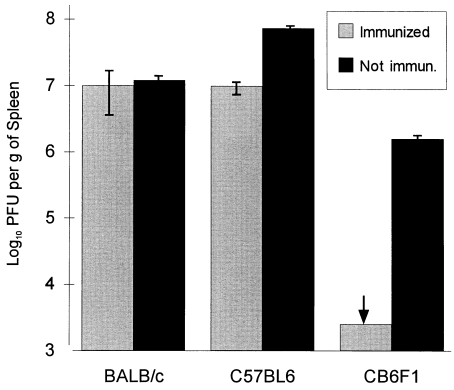

FIG. 3. Multiplication of LCM virus escape variants during second passage in BALB/c and B6 mice but not in $CB6F_1$ mice immunized with an epitope vaccine containing an H-$2L^d$-restricted epitope (aa 118 to 126) of the viral NP and an H-$2D^b$-restricted epitope (aa 34 to 42) of the viral GP. The mean titers of virus in five immunized and in five nonimmunized mice are shown, and error bars indicate standard errors.

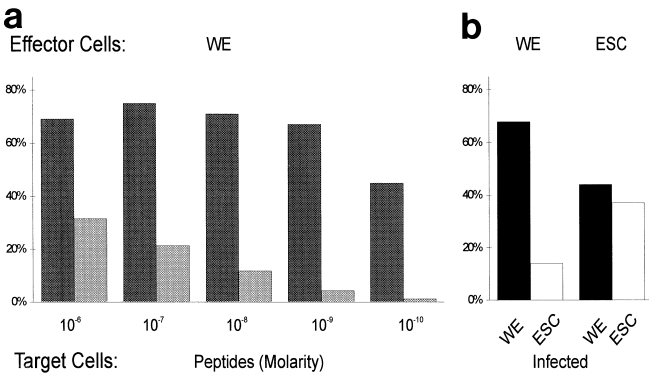

FIG. 4. CTL activity of splenocytes from BALB/c mice obtained 8 days after i.v. injection of WE strain or variant LCM virus. (a) BALB/c-SV40 cells were incubated with indicated concentrations of nonapeptide (aa 118 to 126) of NP (heavily stippled columns) and its mutated form (lightly stippled columns) and subsequently used as target cells for WE strain virus-induced spleen CTL. (b) Target cells were BALB/c-SV40 cells infected with WE strain (black column) or variant (ESC) (open column) virus. Lysis was determined with effector/target cell ratios of 100, 50, 25, and 12.5. Since the values thus obtained were proportional to the numbers of effector cells, only the results for ratios of 100 are presented.

Escape variants did not emerge in hybrid mice immunized with a vaccine containing haplotype-specific epitopes of both parents. Protective immunity against LCM virus also developed in BALB/c and B6 mice and their F_1 descendants injected with a recombinant vaccine containing the corresponding H-2^d- and H-2^b-restricted epitopes, aa 118 to 126 of NP and aa 34 to 42 of GP (43). In mice of either strain, immunized and infected as described for Table 1 and Fig. 1, variants with altered sequences emerged and multiplied to high titers despite epitope-specific immunity, but, in marked contrast, none could be detected in $CB6F_1$ mice (Fig. 3).

CTL activity directed against wild-type and mutated epitopes. The virus consisting predominantly of escape variants was eventually eliminated from the mouse (Fig. 2), indicating that antiviral effector cells were generated; this finding led to the question of which epitopes were recognized by these cells. The answer was obtained by performing cytotoxicity tests. BALB/c mice were injected with either the WE strain or variant virus, and 8 days later splenocytes were tested on syngeneic cells incubated with various concentrations of nonapeptides representing the wild-type epitope or its mutant. Throughout the dose range, the former sensitized the target cells much more efficiently than did the latter (Fig. 4a). However, in three trials there was not a trace of specific lysis when day-8 immune splenocytes from variant virus-infected mice were incubated with ^{51}Cr-labeled cells that had been coated with the wild-type or mutant epitope peptide, even when the concentration was increased to 10^{-4} M and preincubation was extended to 24 h. Obviously, the altered virus induced no $CD8^+$ T-cell immunity directed against either the wild-type epitope or its mutant; from this observation we deduce that clearance of the variant was mediated by effectors recognizing other viral epitopes. These epitopes have not yet been determined, but BALB/c mice are known to control the LCM virus by $CD8^+$ T lymphocytes specific for minor epitopes whenever the complex containing the immunodominant NP nonapeptide and L^d is poorly expressed or absent (18, 41). The conclusion that infection with the escape virus was terminated by $CD8^+$ T lymphocytes directed against minor LCM virus epitopes was strengthened by the results of parallel experiments in which both types of effector cells were tested on targets infected with each virus (Fig. 4b). The degree of lysis by WE-induced CTL was high in WE-infected cells but low in variant-infected cells. The degree of lysis by variant-induced CTL, on the other hand, was intermediate and about equally effective in both types of target cells, which is what one would expect if, in the functional absence of the major L^d-restricted epitope, other (minor) epitopes take over.

DISCUSSION

A priori, immunity is a strong force for suppressing virus variants, thereby allowing others to emerge, and in human infections antibody- or T-cell-driven selection has been reported to occur in individuals (2, 6, 8, 13, 32, 46) as well as in populations (14). In other similar cases, however, evidence for escape was not obtained (11, 28, 47). Obviously, pressure by immunity due to either vaccination or previous infection may be modified by other selecting influences, which makes its study under natural conditions a difficult task. A case in point is the appearance in patients of variants of human T-cell leukemia virus in which the transactivating function of the tax protein was impaired because of amino acid substitutions in tax CTL epitopes (30), and the practical consequences are well illustrated in a report on the spread of poliovirus in Finland during the years 1984 and 1985 (22). Immune escape of virus variants in vivo is not yet well understood, and we propose that the LCM virus-infected mouse is a suitable model for gaining an insight.

Inasmuch as the genomic information of LCM virus is encoded in RNA, it is not surprising that the emergence of variants both in vitro and in vivo has frequently been documented (21, 27, 34, 39). Also, LCM virus has been shown to evade in vivo control by antiviral $CD8^+$ T lymphocytes, although the experimental set-up was rather complex (33). In B6 mice transgenic for an antigen receptor with specificity for the class I molecule D^b plus aa 32 to 42 of the LCM virus GP, variants mutated in the region coding for the epitope appeared; aa 34, 35, and 36 were affected rather than aa 38 as was found by us. $CD8^+$ T-cell-driven selection of LCM virus variants has also been observed in vitro by cultivating infected cells together with cloned CTL. In every case those epitopes that represented the specific targets had changed (1, 29, 31). Similar findings have been reported for tumor cells in vitro expressing the simian virus 40 large tumor antigen (26).

Proline and methionine in positions 2 and 9, respectively,

have been identified as anchor residues for L^d-restricted 9-aa-long $CD8^+$ T-cell epitopes (12). These were unaltered in 41 of 41 escape clones of the LCM virus NP epitope that we have sequenced, and the wild-type epitope as well as its mutated form has glycine in position 6, rather than tryptophan, which has also been recognized as relevant for binding to $H\text{-}2L^d$ (40). Furthermore, the LCM virus GP epitope used here does not correspond to the motif of a D^b-restricted nonapeptide (16). Moskophidis and Zinkernagel (29), who studied a similar epitope (aa 32 to 42 of GP), reported that a CTL-induced change from valine to leucine at position 35 did not affect binding to major histocompatibility complex class I molecules but impaired recognition by D^b-restricted CTL. Since the peptide was 11 aa long (rather than 9 aa as used here) and the substitution was at position 35 of GP (rather than at position 38), no comparative conclusion is possible, and with current knowledge we remain ignorant as to the level at which the variants observed by us have escaped: failure of the altered nonapeptide to associate with its class I molecule or inability of effector $CD8^+$ T cells to recognize the complex. Alternatively, is processing affected? It is certain, though, that in the BALB/c mouse the mutated peptide, even when associated with infectious virus, does not trigger the generation of antivirally active T lymphocytes.

One further detail of our observations deserves being stressed. String-of-beads vaccines encoding T-cell epitopes (45) or containing them in the form of peptides (43) are being studied experimentally. Their use is regarded as problematic for exactly the reason we have observed, namely the emergence of immunologic escape variants (15). We did not detect escape variants in F_1 mice immunized against two different LCM virus epitopes restricted by parental class I molecules. This finding might have been expected, because of the low probability for the occurrence of double mutations leading to immune resistance, which has also been shown to be the case in vitro (35, 42). If they appear later, their multiplication is probably curtailed by the host's immune response directed against other epitopes. The diploid human major histocompatibility complex encodes six class I molecules. Provided that the number of epitopes in a vaccine is as large as is needed to protect the majority of or even all vaccinated individuals against a virus, each one is likely to develop immunity that is based on responses to more than one epitope. Thus, should it be possible to overcome the difficulty of identifying all the T-cell epitopes of a virus that associate in an immunogenic form with a minimal number of the known class I molecules and to combine these in a vaccine, the danger of the emergence of escape variants may not be as serious as has been feared.

ACKNOWLEDGMENTS

We sincerely thank Heike Dralle for excellent technical help and Holger Bartsch from Beiersdorf AG, Hamburg, for performing the mass spectrometry.

This work was financially aided by research grants from Bundesgesundheitsamt, AIDS-Zentrum, BMFT-Forschungsförderung, and Gemeinnützige Hertie-Stiftung, Frankfurt am Main. The Heinrich-Pette-Institut is supported by Freie und Hansestadt Hamburg and Bundesministerium für Gesundheit.

REFERENCES

1. **Aebischer, T., D. Moskophidis, U. Hoffmann Rohrer, R. M. Zinkernagel, and H. Hengartner.** 1991. *In vitro* selection of lymphocytic choriomeningitis virus escape mutants by cytotoxic T lymphocytes. Proc. Natl. Acad. Sci. USA **88:**11047–11051.
2. **Arendrup, M., A. Sönnerborg, B. Svennerholm, L. Akerblom, C. Nielsen, H. Clausen, S. Olofsson, J. O. Nielsen, and J.-E. S. Hansen.** 1993. Neutralizing antibody response during human immunodeficiency virus type 1 infection: type and group specificity and viral escape. J. Gen. Virol. **74:**855–863.
3. **Atherton, E., and R. C. Sheppard.** 1989. Solid phase peptide synthesis: a practical approach. IRL Press, Oxford.
4. **Bachmann, M. F., T. M. Kündig, G. Freer, Y. Li, C. Y. Kang, D. H. Bishop, H. Hengartner, and R. M. Zinkernagel.** 1994. Induction of protective cytotoxic T cells with viral proteins. Eur. J. Immunol. **24:**2228–2236.
5. **Berthold, H., M. Scanarini, C. C. Abney, B. Frorath, and W. Northemann.** 1992. Purification of recombinant antigenic epitopes of the human 68-kDa (U1) ribonucleoprotein antigen using the expression system pH6EX3 followed by metal chelating affinity chromatography. Protein Expr. Purif. **3:**50–56.
6. **Bertoletti, A., A. Costanzo, F. V. Chisari, M. Levrero, M. Artini, A. Sette, A. Penna, T. Giuberti, F. Fiaccadori, and C. Ferrari.** 1994. Cytotoxic T lymphocyte response to a wild type hepatitis B virus epitope in patients chronically infected by variant viruses carrying substitutions within the epitope. J. Exp. Med. **180:**933–943.
7. **Brunner, K. T., J. Mauel, J.-C. Cerottini, and B. Chapuis.** 1968. Quantitative assay of the lytic action of immune lymphoid cells on ^{51}Cr-labelled allogeneic target cells *in vitro*; inhibition by isoantibody and by drugs. Immunology **14:**181–196.
8. **Carman, W. F., A. R. Zanetti, P. Karayiannis, J. Waters, G. Manzillo, E. Tanzi, A. J. Zuckerman, and H. C. Thomas.** 1990. Vaccine-induced escape mutant of hepatitis B Virus. Lancet **336:**325–329.
9. **Cha, J., W. Bishai, and S. Chandrasegaran.** 1993. New vectors for direct cloning of PCR products. Gene **136:**369–370.
10. **Chang, J.-Y., R. Knecht, and D. G. Braun.** 1983. Amino acid analysis in the picomole range by precolumn derivatization and high performance liquid chromatography. Methods Enzymol. **91:**41–48.
11. **Chen, Z. W., L. Shen, M. D. Miller, S. H. Ghim, A. L. Hughes, and N. L. Letvin.** 1992. Cytotoxic T lymphocytes do not appear to select for mutations in an immunodominant epitope of simian immunodeficiency virus gag. J. Immunol. **149:**4060–4066.
12. **Corr, M., L. F. Boyd, S. R. Frankel, S. Kozlowski, E. A. Padlan, and D. H. Margulies.** 1992. Endogenous peptides of a soluble major histocompatibility complex class I molecule, $H\text{-}2L^d_s$: sequence motif, quantitative binding, and molecular modeling of the complex. J. Exp. Med. **176:**1681–1692.
13. **Couillin, I., B. Culmann-Penciolelli, E. Gomard, J. Choppin, J.-P. Levy, J.-G. Guillet, and S. Saragosti.** 1994. Impaired cytotoxic T lymphocyte recognition due to genetic variations in the main immunogenic region of the human immunodeficiency virus 1 NEF protein. J. Exp. Med. **180:**1129–1134.
14. **de Campos-Lima, P. O., V. Levitsky, J. Brooks, S. P. Lee, L. F. Hu, A. B. Rickinson, and M. G. Masucci.** 1994. T cell responses and virus evolution: loss of HLA A11-restricted CTL epitopes in Epstein-Barr virus isolates from highly A11-positive populations by selective mutation of anchor residues. J. Exp. Med. **179:**1297–1305.
15. **Domingo, E., and J. J. Holland.** 1994. Mutation rates and rapid evolution of RNA viruses, p. 161–184. *In* S. S. Morse (ed.), The evolutionary biology of viruses. Raven Press, Ltd., New York.
16. **Falk, K., O. Rötzschke, S. Stevanović, G. Jung, and H.-G. Rammensee.** 1991. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature (London) **351:**290–296.
17. **Gairin, J. E., and M. B. A. Oldstone.** 1992. Design of high-affinity major histocompatibility complex-specific antagonist peptides that inhibit cytotoxic T-lymphocyte activity: implications for control of viral disease. J. Virol. **66:**6755–6762.
18. **Gegin, C., and F. Lehmann-Grube.** 1992. Control of acute infection with lymphocytic choriomeningitis virus in mice that cannot present an immunodominant viral cytotoxic T lymphocyte epitope. J. Immunol. **149:**3331–3338.
19. **Hillenkamp, F., M. Karas, R. C. Beavis, and B. T. Chait.** 1991. Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers. Anal. Chem. **63:**1193A–1203A.
20. **Holland, J. J., J. C. de la Torre, and D. A. Steinhauer.** 1992. RNA virus populations as quasispecies. Curr. Top. Microbiol. Immunol. **176:**1–20.
21. **Hotchin, J., W. Kinch, and L. Benson.** 1971. Lytic and turbid plaque-type mutants of lymphocytic choriomeningitis virus as a cause of neurological disease or persistent infection. Infect. Immun. **4:**281–286.
22. **Hovi, T.** 1989. The outbreak of poliomyelitis in Finland in 1984-1985: significance of antigenic variation of type 3 polioviruses and site specificity of antibody responses in antipolio immunizations. Adv. Virus Res. **37:**243–275.
23. **Lehmann-Grube, F., and J. Ambrassat.** 1977. A new method to detect lymphocytic choriomeningitis virus-specific antibody in human sera. J. Gen. Virol. **37:**85–92.
24. **Lehmann-Grube, F., U. Assmann, C. Löliger, D. Moskophidis, and J. Löhler.** 1985. Mechanism of recovery from acute virus infection. I. Role of T lymphocytes in the clearance of lymphocytic choriomeningitis virus from spleens of mice. J. Immunol. **134:**608–615.
25. **Lehmann-Grube, F., D. Moskophidis, and J. Löhler.** 1988. Recovery from acute virus infection. Role of cytotoxic T lymphocytes in the elimination of lymphocytic choriomeningitis virus from spleens of mice. Ann. N. Y. Acad. Sci. **532:**238–256.

26. **Lill, N. L., M. J. Tevethia, W. G. Hendrickson, and S. S. Tevethia.** 1992. Cytotoxic T lymphocytes (CTL) against a transforming gene product select for transformed cells with point mutations within sequences encoding CTL recognition epitopes. J. Exp. Med. **176:**449–457.
27. **Matloubian, M., S. R. Kolhekar, T. Somasundaram, and R. Ahmed.** 1993. Molecular determinants of macrophage tropism and viral persistence: importance of single amino acid changes in the polymerase and glycoprotein of lymphocytic choriomeningitis virus. J. Virol. **67:**7340–7349.
28. **Meyerhans, A., G. Dadaglio, J.-P. Vartanian, P. Langlade-Demoyen, R. Frank, B. Asjö, F. Plata, and S. Wain-Hobson.** 1991. *In vivo* persistence of a HIV-1-encoded HLA-B27-restricted cytotoxic T lymphocyte epitope despite specific *in vitro* reactivity. Eur. J. Immunol. **21:**2637–2640.
29. **Moskophidis, D., and R. M. Zinkernagel.** 1995. Immunobiology of cytotoxic T-cell escape mutants of lymphocytic choriomeningitis virus. J. Virol. **69:** 2187–2193.
30. **Niewiesk, S., S. Daenke, C. E. Parker, G. Taylor, J. Weber, S. Nightingale, and C. R. M. Bangham.** 1995. Naturally occurring variants of human T-cell leukemia virus type I Tax protein impair its recognition by cytotoxic T lymphocytes and the transactivation function of Tax. J. Virol. **69:**2649–2653.
31. **Oldstone, M. B. A., H. Lewicki, A. Tishon, J. E. Gairin, P. Borrow, and C. Evans.** 1994. CTL escape viral variants, abstr. 154, p. 119. *In* Abstracts of the Ninth International Conference on Negative Strand Viruses, 1994.
32. **Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard, C. R. M. Bangham, C. R. Rizza, and A. J. McMichael.** 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. Nature (London) **354:**453–459.
33. **Pircher, H., D. Moskophidis, U. Rohrer, K. Bürki, H. Hengartner, and R. M. Zinkernagel.** 1990. Viral escape by selection of cytotoxic T cell-resistent virus variants *in vivo*. Nature (London) **346:**629–633.
34. **Popescu, M., and F. Lehmann-Grube.** 1976. Diversity of lymphocytic choriomeningitis virus: variation due to replication of the virus in the mouse. J. Gen. Virol. **30:**113–122.
35. **Portner, A., R. G. Webster, and W. J. Bean.** 1980. Similar frequencies of antigenic variants in Sendai, vesicular stomatitis, and influenza A viruses. Virology **104:**235–238.
36. **Rivers, T. M., and T. F. M. Scott.** 1935. Meningitis in man caused by a filterable virus. Science **81:**439–440.
37. **Romanowski, V., Y. Matsuura, and D. H. L. Bishop.** 1985. Complete sequence of the S RNA of lymphocytic choriomeningitis virus (WE strain) compared to that of Pichinde arenavirus. Virus Res. **3:**101–114.
38. **Schulz, M., P. Aichele, M. Vollenweider, F. W. Bobe, F. Cardinaux, H. Hengartner, and R. M. Zinkernagel.** 1989. Major histocompatibility complex-dependent T cell epitopes of lymphocytic choriomeningitis virus nucleoprotein and their protective capacity against viral disease. Eur. J. Immunol. **19:**1657–1667.
39. **Stocker, C., L. Martínes Peralta, T. Kratzberg, F. Lohmann, and M. Bruns.** 1994. Characterization of a virus variant produced by L cells persistently infected with lymphocytic choriomeningitis virus. J. Gen. Virol. **75:**3431–3439.
40. **Van den Eynde, B., H. Mazarguil, B. Lethé, F. Laval, and J. E. Gairin.** 1994. Localization of two cytotoxic T lymphocyte epitopes and three anchoring residues on a single nonameric peptide that binds to H-2L^d and is recognized by cytotoxic T lymphocytes against mouse tumor P815. Eur. J. Immunol. **24:**2740–2745.
41. **von Herrath, M. G., J. Dockter, M. Nerenberg, J. E. Gairin, and M. B. A. Oldstone.** 1994. Thymic selection and adaptability of cytotoxic T lymphocyte responses in transgenic mice expressing a viral protein in the thymus. J. Exp. Med. **180:**1901–1910.
42. **Webster, R. G., and W. G. Laver.** 1980. Determination of the number of nonoverlapping antigenic areas on Hong Kong (H3N2) influenza virus hemagglutinin with monoclonal antibodies and the selection of variants with potential epidemiological significance. Virology **104:**139–148.
43. **Weidt, G., W. Deppert, S. Buchhop, H. Dralle, and F. Lehmann-Grube.** 1995. Antiviral protective immunity induced by major histocompatibility complex class I molecule-restricted viral T lymphocyte epitopes inserted in various positions in immunologically self and nonself proteins. J. Virol. **69:**2654–2658.
44. **Weidt, G., O. Utermöhlen, J. Zerrahn, J. Reimann, W. Deppert, and F. Lehmann-Grube.** 1994. CD8$^+$ T lymphocyte-mediated antiviral immunity in mice as a result of injection of recombinant viral proteins. J. Immunol. **153:**2554–2561.
45. **Whitton, J. L., N. Sheng, M. B. A. Oldstone, and T. A. McKee.** 1993. A "string-of-beads" vaccine, comprising linked minigenes, confers protection from lethal-dose virus challenge. J. Virol. **67:**348–352.
46. **Yamamoto, K., M. Horikita, F. Tsuda, K. Itoh, Y. Akahane, S. Yotsumoto, H. Okamoto, Y. Miyakawa, and M. Mayumi.** 1994. Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. J. Virol. **68:**2671–2676.
47. **Yasumoti, Y., S. Koenig, R. M. Woods, J. Madsen, N. M. Wassef, C. R. Alving, H. J. Klein, T. E. Nolan, L. J. Boots, J. A. Kessler, E. A. Emini, A. J. Conley, and N. L. Letvin.** 1995. A vaccine-elicited, single viral epitope-specific cytotoxic T-lymphocyte response does not protect against intravenous, cell-free simian immunodeficiency virus challenge. J. Virol. **69:**2279–2284.
48. **Zinkernagel, R. M., and P. C. Doherty.** 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. Adv. Immunol. **27:**51–177.