

Genetic Control and Fine Specificity of the Immune Response to a Synthetic Peptide of Influenza Virus Hemagglutinin

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The immune response to a synthetic peptide, H3 HA₁(305-328), representing the C'-terminal 24 amino acid residues of the HA₁ chain of the hemagglutinin of the H3 subtype of influenza virus is controlled by genes in the I region of the major histocompatibility complex. Mice of the *H-2^d* haplotype are high responders and produce antibody for several months after a single injection of peptide without carrier. Mice of the *H-2^b*, *H-2^k*, and *H-2^q* haplotypes are low antibody responders. Investigation of recombinant and congenic mouse strains revealed that high responsiveness requires the genes that encode the I-E^d molecule. Immunoassays, involving direct binding to analogs of this peptide and inhibition by both these analogs and synthetic epitopes, were used to analyze the specificity of the polyclonal response. In BALB/c mice, the primary antibody response is directed principally against the antigenic site 314-LKLAT-318, whereas the secondary response after a boost is predominantly directed to a distinct site, 320-MRNVPEKQT-328. The T-cell response to the peptide H3 HA₁(305-328), as measured by antigen-induced proliferation of primed T cells in vitro, is also I-E^d restricted in high-responder *H-2^d* mice and is directed against an antigenic site that does not require the four C-terminal residues unique to the H3 influenza subtype. A different epitope appears to be recognized by T cells from CBA (*H-2^k*) mice, which proliferate to a moderate extent on exposure to the peptide but, nevertheless, do not provide help for an antibody response.

A synthetic peptide, H3 HA₁(305-328), representing the C-terminal 24 amino acid residues of the heavy chain (HA₁) of influenza virus hemagglutinin of the H3 subtype, elicits an antibody response in BALB/c mice and in rabbits without the need for coupling to a carrier (4, 13) and thus must contain epitopes for T cells as well as for B cells. We are using this peptide as a model antigen with which to delineate the sites recognized by T and B cells and to study the presentation of T-cell epitopes by antigen-presenting cells.

Although antisera raised to the intact virus do not bind to the peptide, antibodies elicited by peptide H3 HA₁(305-328) bind to viruses of the H3 subtype. No binding has been observed between anti-peptide antibodies and viruses belonging to the H2 subtype (4, 13). Comparison of the amino acid sequences of the region of the HA₁ molecule from representative strains of the H3 subtype, A/Memphis/1/71 (Mem71), and the H2 subtype, A/Japan/305/57 (Jap57) (Fig. 1), has revealed that the differences are localized within two clusters of amino acids. Two analogs of peptide H3 HA₁(305-328) were synthesized, each containing one of the H3 subtype-specific clusters of amino acids on the H2 backbone (Fig. 1). Monoclonal antibodies raised against H3 HA₁(305-328) were shown to bind to one or another, but not both, of these analogs, indicating the presence of at least two distinct epitopes for B cells on the peptide (4). More recently, we have examined the binding of monoclonal antibodies and immune serum from BALB/c mice to comprehensive sets of overlapping peptides of different lengths covering the sequence H3 HA₁(305-328) and have shown (13a) that it contains three separate antigenic sites for antibody, the boundaries of which are indicated in Fig. 1.

In the present study, we examined the genetic control of the antibody and T-cell responses to this peptide in mice. A

high antibody response was obtained only in mice of the *H-2^d* haplotype and was shown to be linked to the presence of the I-E^d allele. Mice of the *H-2^b*, *H-2^k*, and *H-2^q* haplotypes were low responders. T-cell response to the peptide, measured by a proliferation assay, was high (and I-E^d restricted) in *H-2^d* mice and low in *H-2^b* mice, but *H-2^k* mice showed a significant T-cell response despite being low antibody producers. The epitopes recognized by H3 HA₁(305-328)-specific B and T cells from high-responder mice were also studied, and the fine specificity of the antibody produced during the course of the primary and secondary responses was examined.

MATERIALS AND METHODS

Synthetic peptides. The synthetic peptides H3 HA₁(305-328) and H2 HA₁(305-328), which represent the C-terminal 24 amino acid residues of the HA₁ polypeptides from Mem71 and Jap57 viruses, respectively, together with analog A and analog B, were synthesized by using Applied Biosystems synthesizer model 430A as previously described (4). Briefly, synthesis was carried out in the solid phase by using a 1% cross-linked phenylacetamidomethylpolystyrene resin and *t*-butyloxycarbonyl derivatives of the amino acids. Peptides were cleaved from the resin by using hydrogen fluoride containing 10% anisole. After cleavage, peptides were extracted into 50% acetic acid and purified by gel filtration, followed by preparative high-pressure liquid chromatography. The purity of the peptides was monitored by amino acid analysis and analytical high-pressure liquid chromatography. Shorter peptides were synthesized manually by using a CRB flow synthesizer with polydimethylacrylamide as the support resin and fluorenylmethoxycarbonyl derivatives of the amino acids.

Virus. The virus designated Mem71 is a reassortant bearing the hemagglutinin of Mem71 (H3) and the neuraminidase

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C P K Y V K Q N T L K L A T G M R N V P E K Q T	H3 HA₁(305-328)
C P K Y V K S E K L V L A T G L R N V P Q I E S	H2 HA₁(305-328)
C P K Y V K S E K L V L A T G L R N V P E K Q T	Analog A
C P K Y V K Q N T L K L A T G L R N V P Q I E S	Analog B

FIG. 1. Amino acid sequences, in single-letter code, of the peptide H3 HA₁(305-328), representing the C-terminal 24 residues (305 to 328) of the HA₁ of Mem71 virus (subtype H3), and of the peptide H2 HA₁(305-328), representing the equivalent region of the HA₁ of Jap57 virus (subtype H2). Amino acid residues that differ between the two peptides are shown in boldface. The three antigenic sites on H3 HA₁(305-328) that are recognized by polyclonal BALB/c antiserum (13a) are boxed. Two synthetic analogs based on the H2 HA₁(305-328) sequence are also shown; analog A has the H3-specific sequence EKQT at the C terminus, and analog B has the H3-specific sequence QNTLK in the central region.

of A/Bellamy/42 (N1). The virus was grown in the allantoic cavities of 10-day embryonated chicken eggs, harvested from the allantoic fluid by precipitation with polyethylene glycol (2), and purified by centrifugation through 20 to 60% continuous sucrose gradients.

Mice and immunizations. All of the mice used were females between 6 and 8 weeks of age. BALB/c, CBA/H (CBA), C57BL/10 (B10), B10.D2, B10.BR, B10.A, and D2.GD mice were obtained from the animal facility of this department. BALB.K, BALB.B, and DBA/2 mice were obtained from the Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia, and B10.A(5R) and B10.Q mice were from Animal Resources Centre, Willetton, Western Australia. For antibody studies, mice were immunized intraperitoneally with peptide H3 HA₁(305-328) in complete Freund adjuvant (CFA).

MAbs. The preparation and properties of monoclonal antibodies (MAbs) 1/1 and 2/1 have been described elsewhere (4, 13a). The epitopes recognized by these MAbs are indicated in Fig. 1.

ELISA. Enzyme-linked immunosorbent assay (ELISA) was performed as previously described (6), by using polyvinyl chloride microtiter plates treated with coating solutions of either purified virus (1,000 hemagglutinating units/ml) or synthetic peptide (5 µg/ml). In some assays, the virus-coated wells were exposed to 0.1 M sodium acetate buffer (pH 4.9) for 5 min at room temperature and then returned to pH 7.4 by washing with phosphate-buffered saline (pH 7.4), containing 0.05% Tween 20. Antibody titers are expressed as the reciprocal of the antibody dilution giving an absorbance of 0.1, which represents at least five times the background. In inhibition experiments, dilutions of synthetic peptides were mixed with a dilution of antiserum that gave less-than-maximum binding to peptide H3 HA₁(305-328) in the absence of inhibitor. Mixtures (50 µl) of inhibitor and antiserum were then added to wells coated with peptide H3 HA₁(305-328), and the assay was completed in the usual way.

Isolation of T cells. Mice were inoculated in the hind footpad with 25 µg of peptide H3 HA₁(305-328) in CFA. After 7 days, the popliteal and inguinal lymph nodes were removed, cell suspensions were prepared, and T cells were isolated by passage through nylon wool columns (5).

T-cell proliferation assay. T cells (3×10^5 cells per well) were cultured in 96-well flat-bottom tissue culture plates (Nunc, Roskilde, Denmark) with an equal number of irradiated (2,200 R; ⁶⁰Co source) normal syngeneic spleen cells as a source of antigen-presenting cells, together with peptide or

virus, in a total volume of 250 µl. The culture medium was RPMI 1640 supplemented with 5% heat-inactivated (56°C, 30 min) fetal bovine serum–2 mM L-glutamine–2 mM sodium pyruvate–0.1 mM 2-mercaptoethanol–100 IU of penicillin per ml–100 µg of streptomycin per ml. Before use, virus was dialyzed against RPMI 1640 and inactivated by exposure to UV radiation (2 min at 14 cm from a 15 W source). The cultures were incubated for 96 h at 37°C in an atmosphere of 5% CO₂, with [³H]thymidine (0.5 µCi per well) present during the last 18 h. Cells were harvested onto glass fiber filters (Flow Laboratories, Inc.), and the incorporated radioactivity was measured by liquid scintillation spectrometry.

RESULTS

Antibody response to peptide H3 HA₁(305-328) in inbred strains of mice. Sera obtained from BALB/c (*H-2^d*), CBA (*H-2^k*), and B10 (*H-2^b*) mice at various times after inoculation with different doses of peptide H3 HA₁(305-328) were examined for their ability to bind to the peptide in ELISA. The results (Fig. 2) show that a single injection of 25 or 50 µg of peptide without carrier elicited high levels of specific antibody in BALB/c mice but not in CBA or B10 mice. The level of circulating antibody in BALB/c mice reached a peak by 35 days and remained high for at least a further 100 days. After a single boost on day 136, significant amounts of antibody were obtained in CBA and B10 mice, but the titers reached only 0.1 to 1% of those achieved in BALB/c mice.

These results indicate that different strains of mice respond in a quantitatively different way to inoculation with this peptide antigen; BALB/c mice are high responders, and CBA and B10 mice are low responders.

Identification of the gene required for high responsiveness. To further define the genetic control of the antibody response, a more extensive range of congenic and recombinant mice of various haplotypes was inoculated intraperitoneally with 25 µg of peptide H3 HA₁(305-328). Sera collected 35 days after injection were titrated individually by ELISA (Fig. 3). Only mice of the *H-2^d* haplotype (BALB/c and B10.D2) produced high titers of specific antibody, whereas mice of the *H-2^b*, *H-2^k*, and *H-2^a* haplotypes produced low levels of antibody. The mean antibody titer of DBA/2 mice was somewhat lower than that of the other *H-2^d* strains, possibly because of the influence of non-*H-2*-linked genes. The response in *H-2* recombinant mouse strains indicated that the presence of the *d* allele at the *K* and *A* loci (in D2.GD mice) or at the *D* locus [in B10.A and B10.A(5R) mice] does not confer high responsiveness. Presence of the genes encoding the I-E^d molecule is required for production of high levels of antibody to this peptide.

Specificity of the antibody response. The specificity of antibody produced by high-responder mice was examined in a direct binding assay. Individual sera obtained from animals 35 days after inoculation were titrated in ELISA against homologous antigen, analog A, analog B, and Mem71 virus (Table 1). The pattern of the response to the different antigens by individual animals was found to be similar for all of these high-responder strains. In each case, the antibody bound well to the parent peptide and to analog B, but titers against analog A were significantly lower. All sera bound to Mem71 virus, and the titer was augmented if the virus was previously exposed to pH 5.

To examine the kinetics of the appearance of antibodies with different specificities, sera taken at various times from mice inoculated with 50 µg of peptide H3 HA₁(305-328) were titrated against the peptide and its analogs. The results show

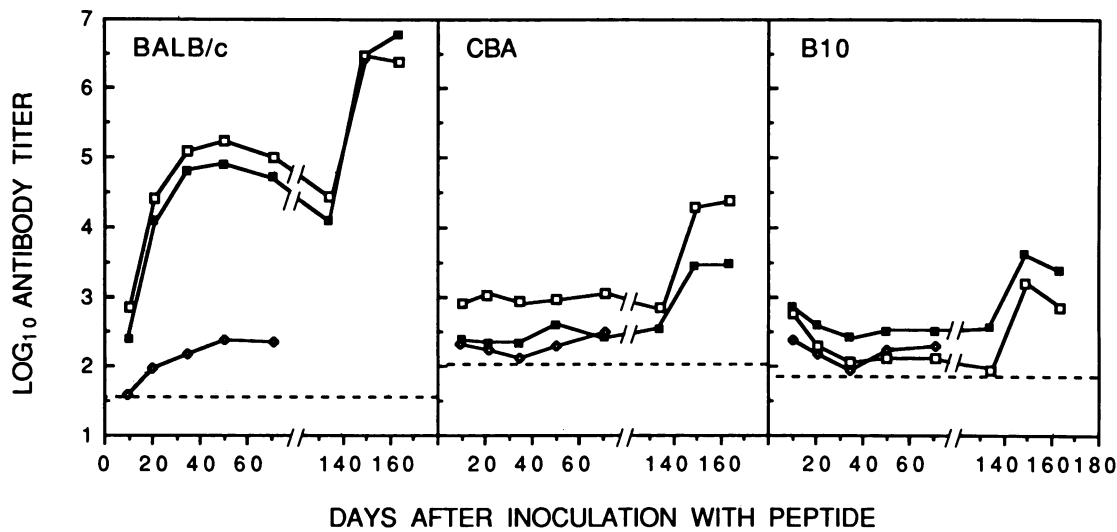


FIG. 2. Production of antibody to the peptide H3 HA₁(305-328) by mice of different strains. BALB/c, CBA, and B10 mice (five per group) were inoculated with 10 (◇), 25 (■), or 50 (□) µg of H3 HA₁(305-328) emulsified in CFA. On day 136 postinoculation, mice that initially received 25 or 50 µg were reimmunized with the same dose of peptide, also in CFA. The mice were bled at various time intervals, and equal volumes of serum from each group member were pooled. The serum pools were tested by ELISA for specific antibody to H3 HA₁(305-328). The dotted line represents the mean background level of binding of sera obtained from four uninoculated mice.

that, after primary immunization of BALB/c mice (Fig. 4A), the antibody response was directed principally to epitopes present in analog B. Antibody capable of binding to analog A appeared later in the primary response and predominated after the secondary immunization. In CBA mice, however, antibodies directed to epitopes present in analog B predominated during both the primary and secondary responses (Fig. 4B).

The specificity of antibodies produced after inoculation with the peptide H3 HA₁(305-328) was further defined in an inhibition assay in which the abilities of peptides, peptide analogs, and peptide fragments to interfere with the binding of antisera to the parent peptide were examined. The results from an experiment with serum taken from a BALB/c mouse after primary inoculation (Fig. 5A) showed that a mixture of analogs A and B can totally inhibit the interaction between antibody and peptide H3 HA₁(305-328). Therefore, these peptide analogs together contain all of the antigenic specificities of the parent peptide. Most of the antibody was directed to epitopes also present on analog B, as demonstrated by the finding that this analog alone very efficiently inhibited binding by up to 80%. The fact that the pentapeptide LKLAT, though 60-fold less efficient than analog B,

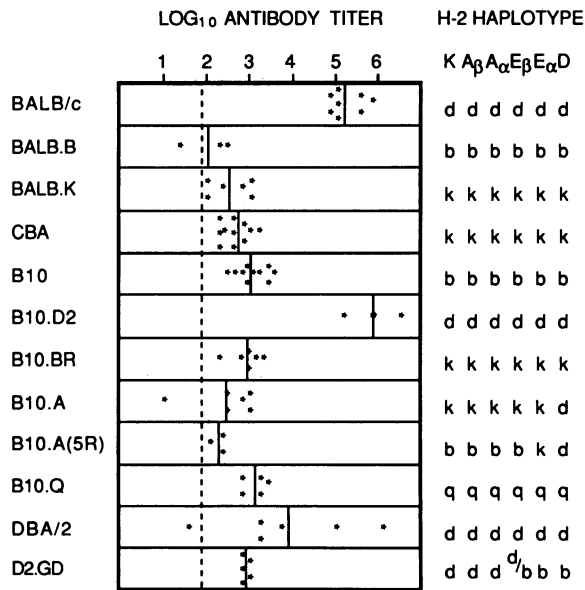


FIG. 3. Influence of genes in the *H*-2 complex on production of antibody to H3 HA₁(305-328). Mice of different strains, with the haplotypes shown (7), were inoculated with 25 µg of H3 HA₁(305-328) in CFA and bled 35 days later. The titer of antibody to H3 HA₁(305-328) in each individual serum (*) was determined by ELISA. The geometric mean titer of the sera from all individuals of each strain is shown as a solid line. The mean of prebleed titers is shown as a dotted line.

TABLE 1. Reactivity of sera from high-responder mice with peptide analogs and virus ^a					
Mouse strain and no.	Log ₁₀ titer of antibody against:				
	H3 HA ₁ (305-328)	Analog A	Analog B	Mem71 virus ^b pH 7	pH 5
BALB/C					
1	4.2	3.0	4.5	3.5	4.5
2	4.9	3.5	4.4	4.2	4.9
3	4.2	2.5	4.2	3.8	4.5
B10.D2					
1	4.6	1.6	3.4	4.1	4.8
2	4.4	2.7	4.4	3.8	4.7
3	3.8	2.3	3.7	3.5	4.1
DBA/2					
1	4.3	3.6	4.0	3.6	4.4
2	3.4	1.5	2.9	2.5	3.5
3	3.0	1.2	2.9	2.7	3.4

^a Antisera were collected from mice 35 days after inoculation with 25 µg of H3 HA₁(305-328) and examined for binding to different antigens by ELISA.
^b Virus was either untreated (pH 7) or pretreated at pH 5 before assay.

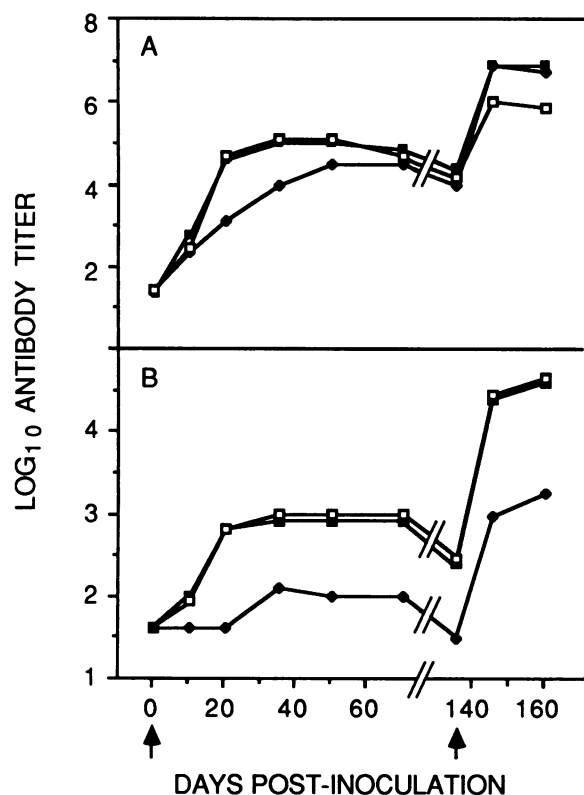


FIG. 4. Reactivity of anti-H3 HA₁(305-328) antisera with synthetic analogs of the peptide. Sera from a BALB/c (A) and a CBA (B) mouse, taken at different times after primary and secondary inoculations (arrows) with 50 µg of H3 HA₁(305-328) were tested by ELISA for binding to H3 HA₁(305-328) (■), analog A (◆), and analog B (□).

inhibited to a similar level indicates that virtually all of this antibody is directed to 314-LKLAT-318 and that there is very little antibody produced in the primary response against the antigenic site 305-CPKYVKQNTL-314, which has also been shown to be present in analog B (13a).

The inhibition curve for analog A was biphasic. Low concentrations of analog A gave a maximum inhibition of about 18%; at very high concentrations, however, both analog A and peptide H2 HA₁(305-328) were able to inhibit the binding of antibody to the parent peptide by at least 75%, but the concentrations of peptide required were approximately 400-fold and 2,000-fold higher, respectively, than that required for analog B.

A different pattern of inhibition was obtained by using the secondary-response serum from this mouse (Fig. 5B). Although, as before, a mixture of both analogs could totally inhibit interaction with the parent peptide, binding was efficiently inhibited by analog A but not B. The octapeptide RNVPEKQT also inhibited the interaction between antibody and parent peptide by about 60%. No inhibition was observed with analog B, LKLAT, or H2 HA₁(305-328).

These data indicate that the primary antibody response in BALB/c mice is principally directed against the antigenic site 314-LKLAT-318, whereas most of the antibodies produced during the secondary response are directed toward the site 320-MRNVPEKQT-328. To confirm this, a comparison was made between the inhibition patterns obtained for the polyclonal antisera and those obtained for MAbs known to

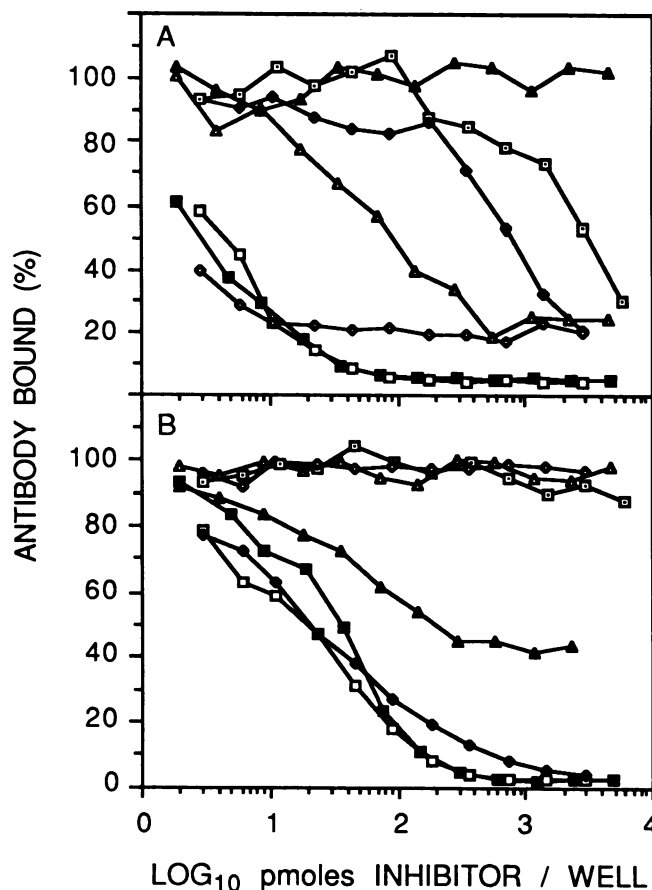


FIG. 5. Ability of different peptides to inhibit binding of BALB/c mouse antisera to H3 HA₁(305-328). Serum was taken from a BALB/c mouse at day 35 after a primary inoculation of 50 µg of H3 HA₁(305-328) (A) and at day 145, 9 days after secondary immunization with an equivalent dose (B). The sera were tested in ELISA for their ability to bind to H3 HA₁(305-328) in the presence of dilutions of H3 HA₁(305-328) (■), H2 HA₁(305-328) (□), analog A (◆), analog B (◇), analogs A plus B (□), or the short synthetic peptides RNVPEKQT (▲) and LKLAT (△). Antibody bound is expressed as a percentage of the mean amount bound to H3 HA₁(305-328) in 12 wells that received no inhibitor.

recognize these sites (Fig. 6). The epitope of MAb 2/1 is 314-LKLAT-318, in which every amino acid residue is essential, whereas MAb 1/1 recognizes 322-NVPEKQT-328, of which only N-322, E-325, and Q-327 are irreplaceable (13a). The binding of MAb 2/1 to H3 HA₁(305-328) was inhibited as efficiently by analog B as by the parent peptide, whereas LKLAT alone also inhibited completely at a molarity about 20-fold higher (Fig. 6A). Analog A and H2 HA₁(305-328) inhibited at concentrations 200 to 500-fold higher than that of the parent peptide. The overall pattern of inhibition observed with MAb 2/1 by using this panel of peptides closely resembled that seen with the polyclonal BALB/c primary-response serum (Fig. 5A), except that, as expected, inhibition with analog B and LKLAT was complete. Similarly, the pattern of inhibition of binding of MAb 1/1 paralleled that observed for the BALB/c secondary-response serum, except that complete inhibition was achieved with the octapeptide RNVPEKQT, as well as with analog A (Fig. 6B).

Genetic control of T-cell response to peptide H3 HA₁(305-328). Genetic restriction of the antibody response to animals

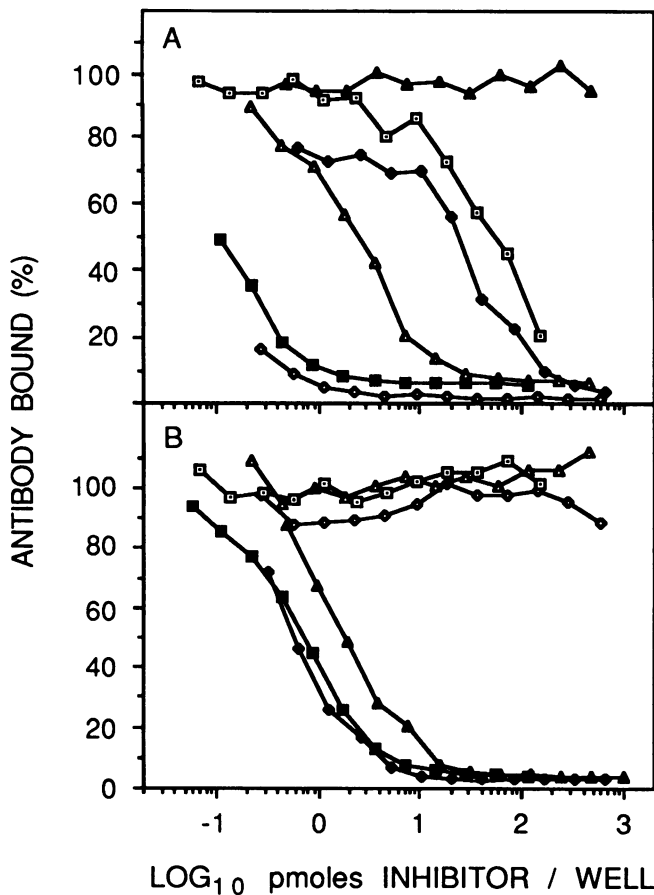


FIG. 6. Ability of different peptides to inhibit binding of MAbs to H3 HA₁(305-328). MAbs 2/1 (A) and 1/1 (B) were tested in ELISA for their ability to bind to H3 HA₁(305-328) in the presence of dilutions of H3 HA₁(305-328) (■), H2 HA₁(305-328) (□), analog A (◆), analog B (◇), RNVPEKQT (▲), and LKLAT (△). Antibody bound is expressed as a percentage of the mean amount bound to H3 HA₁(305-328) in 12 wells that received no inhibitor.

bearing a particular I-region gene is thought to be imposed by the requirement for helper T cells to recognize antigen in association with I-region gene products, i.e., class II major histocompatibility antigens (reviewed in references 3 and 14). Class II restricted T cells proliferating in response to antigen would, therefore, be expected to be under the same genetic control as antibody production. This was examined for peptide H3 HA₁(305-328) by inoculating different strains of mice in the footpads and examining the ability of T cells obtained from the draining lymph nodes of these animals to respond to antigen in vitro. The results (Fig. 7A) show that T cells obtained from peptide-primed BALB/c mice gave a strong in vitro response, whereas the proliferation of T cells from B10 mice was no greater than that of the control animals. T cells from CBA mice, which produced only low levels of antibody in response to peptide H3 HA₁(305-328), nonetheless proliferated strongly in vitro in response to this peptide.

A similar pattern of genetic restriction of the T-cell response was observed in congenic strains of mice bearing different *H-2* haplotypes on an identical (B10) background (Fig. 7B). T cells obtained from B10.D2 (*H-2^d*) mice responded strongly, whereas T cells from B10.BR (*H-2^k*) mice gave an intermediate response.

The particular *H-2* gene required for high responsiveness was identified by showing that D2.GD mice, which express I-A^d but not I-E^d, fail to mount a T-cell proliferative response to the peptide, whereas DBA/2 mice, which have the same genetic background and express both I-A^d and I-E^d, do respond (Fig. 7C). Thus, the high T-cell responsiveness, like high antibody responsiveness, of *H-2^d* mice is dependent on expression of I-E^d.

Specificity of T-cell response. T cells from different strains of mice which had been primed with H3 HA₁(305-328) were tested for their ability to proliferate in response to various antigens (Table 2). T cells from high-responder strains (BALB/c and B10.D2) proliferated well in response to the parent peptide and analog B. There was no significant response to analog A or H2 HA₁(305-328). Intact virus was capable of, at best, only very weak stimulation. B10.BR mice which, like CBA (*H-2^k*) mice, gave an intermediate response to the parent peptide (Fig. 7), responded poorly to both analogs A and B, suggesting that they do not recognize the same region of the molecule as do the high-responder strains.

DISCUSSION

The capacity of peptide H3 HA₁(305-328) to act as an immunogen is strictly governed by the genetic makeup of the recipient. Mice of the *H-2^d* haplotype produce high levels of specific antibody and show a strong T-cell proliferative response to the peptide. In both cases, high responsiveness was shown to be linked to the genes that encode the I-E^d molecule. It is therefore probable that H3 HA₁(305-328) or a processed form of this peptide binds to the I-E^d molecule for presentation to helper T cells (14). D2.GD mice, which express I-A^d but not I-E^d, and mice of the *H-2^b* haplotype both give very weak antibody and proliferative T-cell responses.

In mice of the *H-2^k* haplotype, however, the low antibody response observed was not associated with inability to mount a proliferative T-cell response. T cells from these mice recognized the peptide, although the resulting proliferation was not as high as that seen in *H-2^d* mice. Since B10.D2 mice, which have the same background genes as B10.BR mice, are high responders for antibody production, it is not likely that absence of the appropriate *V_H* genes is responsible for the specific antibody deficiency of B10.BR mice. Rather, the T cells that proliferate in response to the peptide in *H-2^k* mice may not have helper function (8), or the peptide may contain an epitope recognized by *H-2^k* suppressor T cells which is analogous to that described for hen egg white lysozyme and *H-2^b* T cells (1, 16).

We have shown elsewhere (13a) that BALB/c mice produce antibody that recognizes three distinct sites on H3 HA₁(305-328) (Fig. 1). The site 320-MRNVPEKQT-328 is represented by 320-LRNVPEKQT-328 in analog A; residue 320, which constitutes the N-terminal boundary of this site, is not an essential residue but augments binding of polyclonal sera (13a). Analog B contains site 314-LKLAT-318 and the minor antigenic region 305-CPKYVKQNTL-314 (Fig. 1). These analogs have the advantage over shorter synthetic peptides for use in direct binding assays, since they bind well to plastic and probably retain some of the structural features of the parent peptide. In the present study, it was confirmed that peptide-primed BALB/c mice produced antibody reactive with both analogs in the direct binding assay. When the specificities of sera taken at different times after primary and secondary immunizations of these animals were examined, it

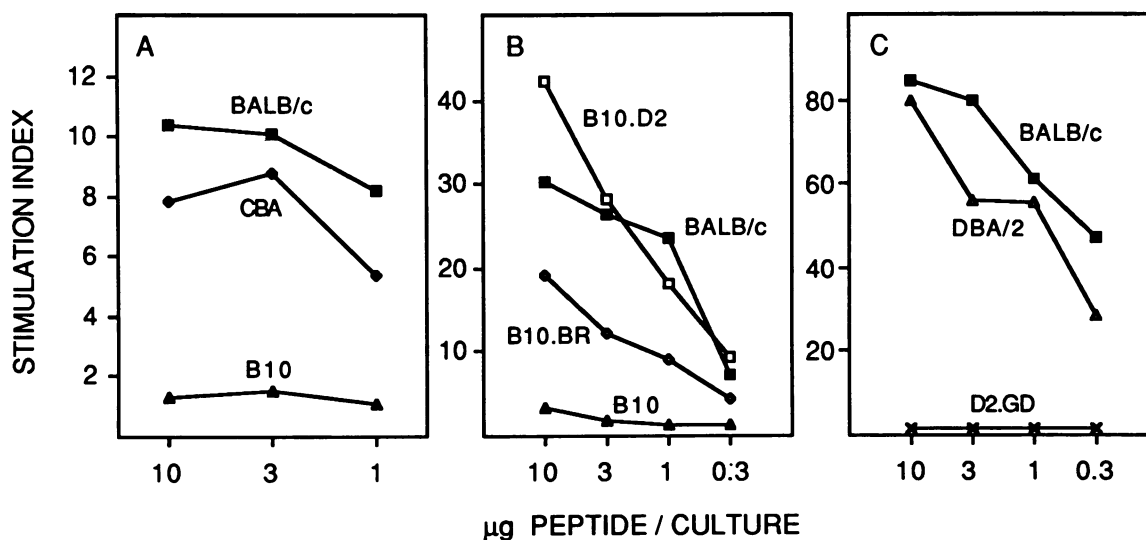


FIG. 7. Proliferative T-cell response to H3 HA₁(305-328) in mice of different haplotypes. BALB/c (■), CBA (◆), B10 (▲), B10.D2 (□), B10.BR (◇), DBA/2 (△), and D2.GD (×) mice were inoculated in the hind footpad with 25 μg of H3 HA₁(305-328) in CFA. After 7 days, the lymph node T cells from these mice were examined for their capacity to proliferate in response to different doses of H3 HA₁(305-328). The results are expressed as a stimulation index, which is the mean counts per minute incorporated in triplicate cultures containing antigen divided by the mean counts per minute in cultures without antigen.

was interesting that, in all four mice tested, the initial dominance of antibody reactive with analog B was followed after the boost by a predominance of antibody reactive with analog A.

This change in specificity of the antibody might reflect idiotype selection mediated by idiotype-specific helper T cells. Such a mechanism has been suggested to explain the shift in epitope specificity and emergence of a predominant idiotype in the secondary response to hen egg white lysozyme (11, 12, 15). Alternatively, our findings may be explained in terms of a model recently proposed by Manser et al. (9, 10) for the molecular evolution of the immune response. In this model, after initial clonal expansion and differentiation of primary B cells specific for antigen, the antibody response evolves as a consequence of random somatic mutation in V-region genes and selection of B-cell clones with the highest affinity for antigen. Not all B-cell clones show the same adaptability, i.e., ability to sustain random mutation without losing antigen specificity. Clones with a combination of high affinity and high adaptability

emerge to dominate the response. When this model is applied to the present study, it may be that in the preimmune repertoire, B-cell clones specific for 314-LKLAT-318 exist which have moderate to high affinity but low adaptability; such clones would participate early in the antibody response. B-cell clones specific for 320-MRNVPEKQT-328 may be initially of low affinity, but they possess a higher adaptability and thus a greater chance of increasing their affinity by somatic mutation and, by selection, would ultimately come to dominate the response. The low level of antibody produced by CBA mice was directed primarily to epitopes shared with analog B, and no change in specificity was observed on secondary immunization. This is also consistent with the model, since the low level of antibody produced reflects limited expansion of B-cell clones and, hence, limited opportunity for somatic mutation.

Use of the inhibition assay to study the specificity of BALB/c antibodies against H3 HA₁(305-328) allowed examination of their binding characteristics under conditions of antigen excess rather than antibody excess. These studies revealed that, at sufficiently high molar excess, both analog A and H2 HA₁(305-328) could inhibit the binding of primary BALB/c antiserum with the H3 HA₁(305-328) peptide by at least 80%. This was unexpected, because direct binding studies had shown no cross-reactivity with H2 HA₁(305-328) and also because this antiserum was found to be predominantly (80%) specific for epitopes on analog B. One interpretation of these findings is that antibodies specific for 314-LKLAT-318 can also bind with very low efficiency to 314-LVLAT-318, which is present on both analog A and H2 HA₁(305-328). This hypothesis was supported by data obtained with MA b 2/1, whose epitope corresponds precisely to 314-LKLAT-318 (13a), and whose binding to H3 HA₁(305-328) was also found to be inhibited by analog A and H2 HA₁(305-328) at concentrations several hundredfold higher than those required for analog B or the native peptide.

The inhibition studies also showed that the shorter peptides LKLAT and RNVPEKQT were capable of blocking antibodies to H3 HA₁(305-328). The peptide LKLAT

TABLE 2. Reactivity of T cells with peptides, peptide analogs, and virus^a

Antigen	Proliferative response of T cells ^b			
	BALB/c (H-2 ^d)	B10.D2 (H-2 ^d)	B10.BR (H-2 ^k)	B10 (H-2 ^b)
H3 HA ₁ (305-328)	28.1	39.2	17.2	0.4
Analog A	0.8	0.9	3.2	0
Analog B	17.3	29.4	2.3	0
H2 HA ₁ (305-328)	0.4	0.5	1.3	0
Mem71 virus	1.3	3.5	0.8	0

^a Lymph node T cells from mice primed with 25 μg of H3 HA₁(305-328) in CFA were tested for their ability to proliferate in response to 10 μg of peptide or 1,000 hemagglutinating units of virus.

^b Stimulation indices were calculated as the mean counts per minute of triplicate cultures that contained antigen divided by the mean counts per minute of cultures without antigen. The data are expressed as stimulation indices of cultures of peptide-primed T cells minus stimulation indices of cultures of CFA-primed T cells.

blocked the binding of polyclonal antisera to the same extent as that observed with analog B, indicating that it contained all of the antigenic information of the analog, even though the shorter peptide was considerably less efficient on a molar basis. The latter may indicate that the pentapeptide-antibody interaction is of lower affinity because of the absence of additional structural information which is provided by other residues within the longer analogs. Similarly, RNVPEKQT was less efficient at blocking than was analog A.

Antibody to H3 HA₁(305-328) from high-responder strains of mice was capable of binding to intact virus. As previously shown (4), this binding was enhanced by pretreatment of the virus at pH 5, under which conditions the C terminus of HA₁ is likely to become more exposed. In contrast, peptide-primed T cells react very poorly to intact virus (and to pH 5-treated virus [data not shown]). This may be due to differences in the processing or presentation of this region of HA; when given in the form of the peptide (305-328), a particular site is recognized, but when given in the form of intact HA or virus, processing may lead to production of different fragments such that this site is not intact or does not associate with class II molecules in the same way.

Less can be deduced from these data regarding the specificity of T cells that recognize H3 HA₁(305-328). T cells from high-responder strains of mice respond predominantly to analog B and not to analog A or H2 HA₁(305-328). It appears, therefore, that at least one of the amino acid residues in the H3-specific sequence 311-QNTLK-315 is required in the epitope recognized by these T cells or in the agretope (14), whereas residues 325-EKQT-328 are not required. T cells from *H-2^k* mice show a different specificity; neither the analogs nor H2 HA₁(305-328) stimulated these T cells very well. This observation suggests a requirement for M at position 320. Studies in progress with T-cell clones and a more extensive range of peptides of various lengths (R. Ffrench et al., manuscript in preparation) should enable us to locate and characterize the epitopes recognized by T cells in mice of different haplotypes.

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