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FINE SPECIFICITY OF THE MURINE ANTIBODY RESPONSE TO HIV-1 gp160 DETERMINED BY SYNTHETIC PEPTIDES WHICH DEFINE SELECTED EPITOPES*

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Abstract—In this report, we assess the humoral immune response in inbred strains of mice immunized with baculovirus-derived recombinant HIV-1 gp160 (rgp160). Six inbred strains of mice were each immunized with two different concns (5 and 50 μg) of rgp160, and the antibody response to rgp160 and synthetic peptides which define distinct gp160 epitopes was examined. Within a given inbred strain of mice, no significant difference in antibody titers to gp160 was observed in those groups receiving either 5 or 50 μg of rgp160 per injection. Following three immunizations with rgp160, differences in anti-gp160 titers were observed among the various inbred strains; however, these differences became less apparent after additional injections with rgp160. In addition, each mouse strain exhibited a unique reactivity pattern to seven gp160 epitopes defined by synthetic peptides. Multiple injections with rpg160 were required to induce responses to certain gp160 epitopes. The observed differences in the fine specificity of the humoral immune response to distinct gp160 epitopes among the six inbred strains suggest a genetic basis for regulating the antibody response to these epitopes. This apparent regulation can be overcome by multiple injections with rgp160.

INTRODUCTION

The envelope of human immunodeficiency virus type 1 (HIV-1), a human retrovirus that is the etiological agent of AIDS (Barre-Sinoussi *et al.*, 1983; Gallo *et al.*, 1984), contains a viral encoded molecule synthesized within infected cells (Allan *et al.*, 1985). The envelope gene product is a glycosylated polyprotein, gp160, which is cleaved into an outer membrane glycoprotein, gp120, and a transmembrane portion, gp41. The envelope heterodimer appears to play a critical role in the induction of HIV-1 specific immune responses. Numerous studies have identified epitopes on gp160 that induce virus neutralizing antibodies (Putney *et al.*, 1986; Chanh *et al.*, 1986; Ho *et al.*, 1987), mediate antibody-dependent cellular cytotoxicity (ADCC) (Blumberg *et al.*, 1987), or invoke a variety of T-cell responses (reviewed by Mills *et al.*, 1989).

Studies using inbred strains of mice have provided evidence that an immune response, which may determine protective immunity or susceptibility to a variety of infectious agents, including retroviruses, is genetically regulated (Vlug *et al.*, 1981; Zijlstra and Melief, 1986; Morrison *et al.*, 1987; Milich, 1987; Hamelin-Bourassa *et al.*, 1989). To date, studies utilizing inbred mice to examine the genetic restriction of the murine immune response to HIV-1 gp160 have focused on T-cell responses (Cease *et al.*, 1987; Takahasi *et al.*, 1988; Michel *et al.*, 1988). To our knowledge, no data are available on the genetic regulation of the humoral immune response to HIV-1 in murine systems. In view of the relevance that humoral immunity may have in controlling HIV infection and the use of HIV-1 gp160 and fragments thereof as putative vaccine candidates in phase I and II trials (reviewed by Kennedy and Koff, 1990), it is important to examine the potential genetic regulation of the antibody response to this viral antigen. Inbred strains of mice represent the animal model of choice to examine potential genetic regulation of the antibody response to HIV-1 gp160.

Synthetic peptides corresponding to amino acid sequences from HIV-1 gp160 have been previously used to define B and T-cell epitopes on gp160 (reviewed by Kennedy *et al.*, 1988; Cease, 1990) and to identify differences in the fine specificity of the humoral immune response between HIV-1 infected humans and chimpanzees (Warren *et al.*, 1990). These studies indicate that synthetic peptides which define HIV-1 gp160 epitopes represent useful tools in analyzing the fine specificity of a given immune response to the cognate viral antigen.

In this report, we analyze the humoral immune response of six inbred strains of mice immunized with

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Abbreviations—ADCC, antibody-dependent cellular cytotoxicity; HIV-1, human immunodeficiency virus type 1; MHC, major histocompatibility complex; NGS, normal goat serum; PBS/T, phosphate-buffered saline containing Tween-20; rgp160, recombinant gp160; SV40 T-ag, simian virus 40 large tumor antigen.

two different concns of baculovirus-derived recombinant HIV-1 gp160 (rgp160), and assessed the fine specificity of the anti-gp160 responses using seven synthetic peptides corresponding to selected epitopes of HIV-1 gp160. These studies indicate that differences in antibody titer and fine specificity among strains with different major histocompatibility complex (MHC) haplotypes may reflect a genetic regulation of the humoral immune response to HIV-1 gp160.

MATERIALS AND METHODS

Animals

Inbred mouse strains BALB/cByJ, A/J, CBA/CaJ, C57BL/10SnJ, DBA/1J and SJL/J were obtained from Jackson Laboratory, Bar Harbor, NE. Female mice between 6 and 8 weeks of age at the time of first injection were used in these studies. All mice were housed and fed under conventional conditions.

Antigens

Purified baculovirus-derived rgp160, precipitated in alum, was kindly provided by Dr Gale Smith (MicroGeneSys Inc., West Haven, CT). The sequence of rgp160 was derived from the LAV-1 isolate (Wain-Hobson *et al.*, 1985). A control baculovirus derived SV40 large T antigen (SV40 T-ag) was produced as previously described (Landford, 1988).

Immunizations

Groups of five mice each received a total of six i.p. injections consisting of either 5 or 50 μg of alum-precipitated rgp160 in 0.2 ml borate-buffer saline (BBS). The first three injections were performed bi-weekly, and subsequent injections (up to six) at monthly intervals.

Antisera

Two to four weeks after each injection, mouse blood was collected from the tail vein and allowed to clot at room temp. Serum was obtained from the whole clotted blood by centrifugation and stored at $-20°C$ until use. Serum obtained prior to rgp160 immunization was used as a control.

Peptides

Amino acid sequences for gp160 peptides 304–321, 425–448, 503–528, 616–632, 735–752 and 846–860 were derived from the HIV-IIIB (B10) isolate (Ratner *et al.*, 1985). Previous studies had indicated that each of these peptide sequences defined an epitope on HIV-1 gp160 (reviewed by Kennedy *et al.*, 1988, Cease, 1990). Peptides were assembled by solid-phase peptide synthesis on Merrifield polystyrene resin as previously described (Chanh *et al.*, 1986, 1988; Kennedy *et al.*, 1986) and, following HF cleavage, were purified by reverse-phase high-performance liquid chromatography. Peptide 600–611, also derived from the IIIB (B10) sequence, was purchased from Cambridge Research Biochemicals (Valley Stream, NY). The amino acid sequences of the synthetic gp160 peptides used in this study are described in Table 1. A control peptide, designated Hep122, corresponded to amino acids 122–137 of the S region of hepatitis B surface antigen (Dreesman *et al.*, 1985) was also used in this study.

Western blot

Western blot analysis was performed using the Bio-Rad Immunoblot System (Bio-Rad Laboratories, Richmond, CA) according to the manufacturer's directions with the following modifications. Briefly, bound human (1:100) or mouse (1:50) serum antibodies were detected with alkaline phosphatase conjugated goat anti-human or anti-mouse Ig (Sigma Chemical Co., St Louis, MO), respectively. The substrate used was provided by Bio-Rad Laboratories.

ELISA

Peptide 600–611, insoluble in aqueous buffers, was solubilized in 35% acetic acid and 250 ng in 50 μl per well was coated onto microtiter plates (Corning Glass Works, Corning, NY) by overnight incubation at 37°C. The remaining peptides (250 ng/well), rgp160 (20 ng/well) or control SV40 T-Ag (50 ng/well) were dissolved in bicarbonate buffer, pH 9.6, and were coated onto microtiter wells by overnight incubation at 4°C. This assay was performed by methods previously described in detail utilizing a goat anti-mouse

Table 1. Amino acid sequences of the synthetic peptides used in this study to define HIV-1 gp160 epitopes

Amino acid residues	Sequence[a]
304–321	(CGY)TRPNNNTRKSIRIQRGPG
425–448	CRIKQIINMWQEVGKAMYAPPISG
503–528	(CGY)VAPTKAKRRVVQREKRAVGIGALFLG
600–611	LGIWGCSGKLIC
616–632	(C)PWNASWSNKSLEQIWNN(G)
735–752	(Y)DRPEGIEEEGGERDRDRS(GC)
846–860	(CAY)AIRHIPRRIRQGLER(G)

[a]Amino acids in parentheses were added to facilitate coupling to carrier proteins for related immunization studies.

immunoglobulin conjugated with horseradish peroxidase (Cappel, Westchester, PA) (Warren *et al.*, 1990). The cut-off for a positive reaction was established by multiplying the mean optical density value obtained with a 1:50 dilution of the six pooled preimmune mouse sera by three. This cut-off value was greater than the optical density value obtained from the mean of the six individual preimmune control sera plus three standard deviations, and represents greater than a 99% confidence level. End-point titers were considered to be the reciprocal of the highest serum dilution that resulted in an optical density at least three times that obtained with a 1:50 dilution of the pooled preimmune sera from a given inbred strain. End-point titers are expressed as either the reciprocal dilution of the five individual sera pooled within a given group or the mean value ± standard error of the mean obtained with the five individual sera.

Neutralization assay

Mouse immune sera were tested for HIV-1 neutralizing activity by an assay that has been previously described (Robertson *et al.*, 1988). Briefly, 50 μl of heat-inactivated mouse immune serum at 2-fold dilutions ranging from 1:5 to 1:320 was mixed in individual microculture wells with 50 μl of HIV-1 IIIB culture supernatant containing approximately 100 $TCID_{50}$ and incubated for 1 hr at room temp. To each well, 5×10^4 SUP-T1 cells in 100 μl were added, and the cell cultures were maintained at 37°C and 5% CO_2 for 7 days. Cells were stained with MTT [3-(4,5-dimethylthiazol 2-yl)-2,5-diphenyltetrazolium bromide; thiasolyl blue; Sigma Chemical Co., St Louis, MO] and complete virus neutralization and the corresponding end-point titer was determined as the highest serum dilution that yielded an O.D. at 570 nm equivalent to uninfected cells.

Statistical analysis

To determine whether a statistically significant difference was observed in the antibody response between two groups of mice the arithmetic mean of five individual end-point titers was calculated. Statistical analysis to determine the level of significance was performed using the two-tailed Student's *t*-test (Downie and Heath, 1965).

RESULTS

To examine the epitope specificity and the potential for genetic regulation of the humoral immune response to HIV-1 gp160, we measured the specific binding of antisera obtained from six inbred strains of mice immunized with two different concns of rgp160 to seven synthetic peptides which define HIV-1 gp160 epitopes and to rgp160 by ELISA. First, we examined mean end-point titers of the five individual sera within the six different inbred strains to rgp160 and the seven synthetic peptides corresponding to gp160 epitopes. These titers were compared to end-point titers obtained by pooling the five individual mouse sera within a given inbred strain. No apparent difference was observed between the end-point titers obtained with individual sera vs end-point titers of pooled sera within a given inbred strain (data not shown). Based on this observation, sera obtained following each immunization with rgp160 were pooled to ensure that sufficient quantities were available for the subsequent serologic characterization.

The ability of the six inbred strains of mice to produce anti-gp160 responses was examined following the third and fifth immunizations with either 5 or 50 μg or rgp160 (Table 2). Based on end-point titers, the highest anti-gp160 responses were detected in SJL/J, A/J and CBA/CaJ strains of mice following three injections with rgp160. Lower levels of anti-gp160 were observed in BALB/c, C57BL/10SnJ and DBA/1J. The three higher responding strains had end-point titers to rgp160 equal to or greater than 1:3200 when immunized three times with either concn of rgp160. This represented a 2- to 16-fold increase in anti-gp160 titers compared to those from the three strains that exhibited the lower titers. The differences between the six inbred strains became less apparent after two additional immunizations. In five of the six inbred strains, the two additional injections with rgp160 increased the anti-gp160 end-point titers from 2- to 8-fold. Only in SJL/J mice did the anti-gp160 response not increase.

We also examined the effects of immunizing with different concns of rgp160 on the anti-gp160 titer (Table 2). These data indicated that increasing the dose of rgp160 from 5 to 50 μg for each injection did not significantly affect the levels of antibodies to rgp160 as assessed by end-point titers. Three immunizations with 50 μg generated greater anti-gp160 responses in one strain, an equal response in one strain, and lower responses in four strains, when compared to those strains of mice that received 5 μg of rgp160. These

Table 2. Anti-rgp160 reactivity of mouse sera following multiple injections with baculovirus-derived recombinant gp160

Mouse strain	H-2 haplotype	Dose (μg)	Number of injections 3	5
BALB/cByJ	d	5	1600[a]	6400
		50	800	6400
A/J	a	5	12,800	>12,800
		50	3200	>12,800
C57BL/10SnJ	b	5	800	6400
		50	1600	3200
CBA/CaJ	k	5	6400	12,800
		50	3200	12,800
DBA/1J	q	5	800	3200
		50	800	1600
SJL/J	s	5	12,800	6400
		50	6400	6400

[a]End-point titer calculated as the reciprocal of the highest dilution that resulted in an optical density (O.D.) exceeding the cut-off. The cut-off for positive reactivity was three times the mean O.D. of the pooled preimmune sera obtained at a dilution of 1:50. Control antigens included a baculovirus expressed recombinant SV40 large T-antigen that resulted in end-point titers of less than 50 for each of the six inbred strains.

differences in anti-gp160 levels between groups of mice immunized with 5 or 50 μg of rgp160 became less apparent in sera obtained after two additional injections. Four of the six inbred strains demonstrated similar anti-gp160 titers when given five injections of either 5 or 50 μg of rgp160. In the other two inbred strains (C57BL/10 and DBA/1J), sera obtained following the fifth injection with 5 μg of rgp160 had 2-fold higher titers when compared to sera from mice receiving the 50 μg dose. Based on these observations and the lack of major differences in anti-peptide reactivity between groups of mice immunized with the two different doses of rgp160 (data not shown), we arbitrarily selected sera from mice immunized with 50 μg of rgp160 for further serologic characterization.

To further assess the HIV-1 gp160 reactivity of the antisera from rgp160 immunized mice, Western blot analysis was performed. As shown in Fig. 1, antisera obtained following the fifth immunization from each of the six inbred strains of mice recognized HIV-1 gp160, gp120 and gp41 (lanes 3–8, mol. wt species 160, 120 and 41 kDa). No reactivity was observed with a representative mouse serum obtained prior to immunization with rgp160 (lane 2). A mol. wt species corresponding to approximately 70 kDa was also recognized by the antisera obtained from mice immunized with rgp160. Since a similar mol. wt species band was also detected by serum from an AIDS patient (Lane 1), this reactivity is either an unglycosylated form of gp120 (Allan *et al.*, 1985), a cleavage product of gp120 (Berman *et al.*, 1990), gp41 dimers (Pinter *et al.*, 1989), or possibly non-HIV in nature. Together these data indicate that immunization

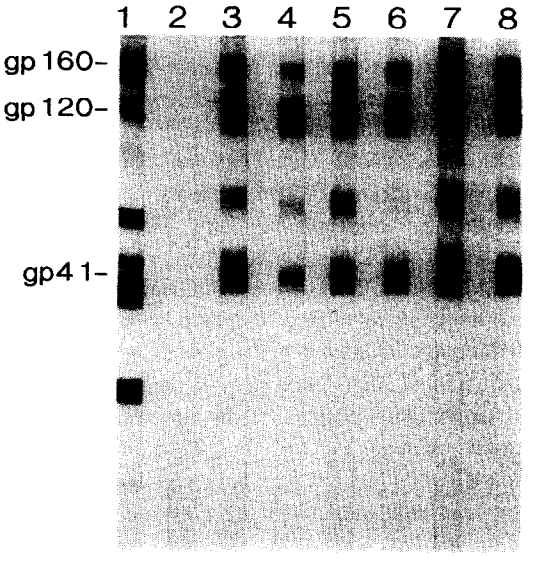

Fig. 1. Western blot analysis of pooled sera from six inbred mouse strains following five immunizations with 50 μg of recombinant gp160. Control serum from an HIV-1 infected human (lane 1). A representative preimmune serum from strain A/J (lane 2). Immune sera of strains BALB/c ByJ (lane 3), DBA/1J (lane 4), C57BL/10SnJ (lane 5), CBA/CaJ (lane 6), A/J (lane 7) and SJL/J (lane 8).

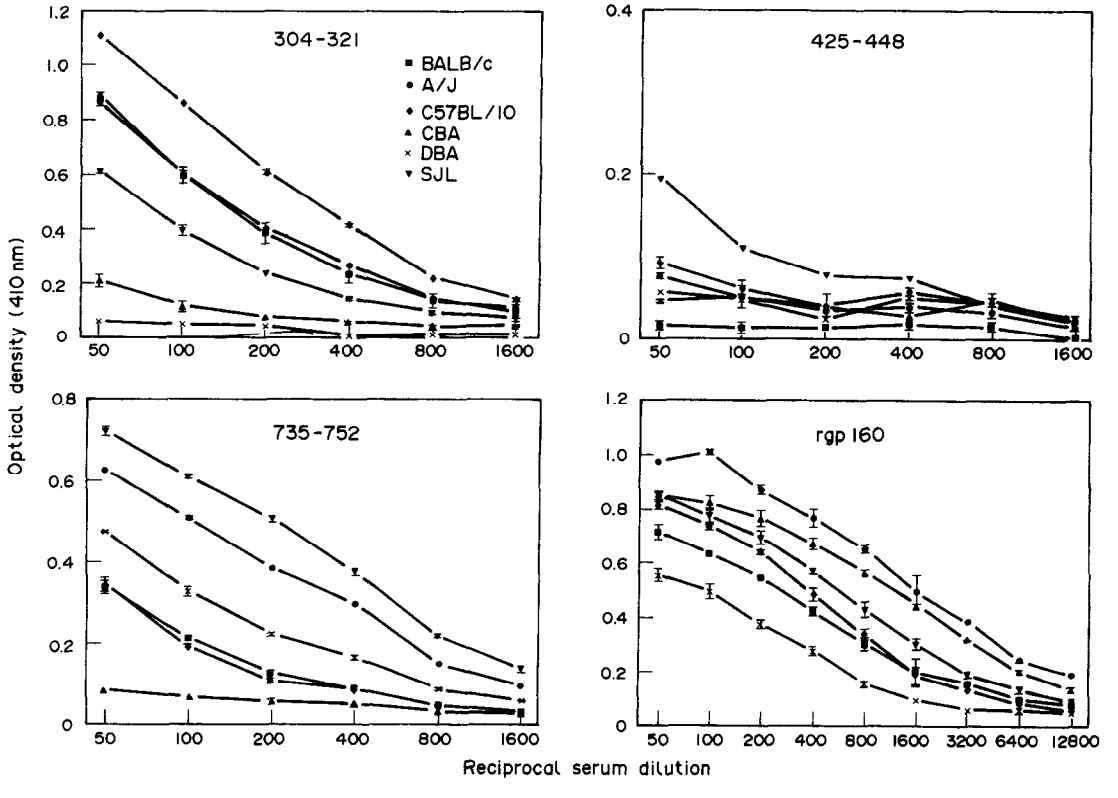

Fig. 2. Binding curves of sera obtained from six inbred mouse strains after five immunizations with 50 μg of rgp160. Sera were reacted to rgp160 and to selected gp160 epitopes defined by synthetic peptides 304–321, 425–448 and 735–752. Each point represents the mean of duplicate values. Standard deviations for the duplicate measurements ranged from O.D. values 0.000–0.061 (not shown).

of mice with rgp160 induces antibodies that recognize epitopes on gp160, gp120 and gp41.

In the next set of experiments, we analyzed the ability of the anti-gp160 containing sera to bind the seven synthetic peptides representing gp160 epitopes. Representative binding curves of pooled anti-gp160 containing sera obtained after five immunizations are depicted in Fig. 2. We selected three of the seven synthetic peptides which correspond to two previously defined gp120 epitopes (peptides 304–321 and 425–448) along with a gp41 epitope (peptide 735–752). Antisera from CBA and DBA/1J mice exhibited low or undetectable end-point titers (1:50 and <1:50, respectively) to the epitope defined by peptide 304–321. The four other strains of mice had variable end-point titers ranging from 1:400 (SJL) to 1:1600 (C57BL/10). Similar variability was observed in the antibody response to the gp41 epitope defined by peptide 735–752, with end-point titers ranging from 1:100 to 1:1600. Considerably lower reactivity was oberved to the gp120 epitope defined by peptide 425–448, with only one mouse strain, SJL, recognizing the epitope defined by this peptide. Antibody titers to rgp160 were higher (end-point titers ranging from 1:1600 to >12,800) and the variation in antibody levels among the six inbred strains was markedly reduced compared to antibody responses to the selected epitopes. The binding curves depicted in Fig. 2 illustrate the specificity of the various antibody–antigen interactions and the extent of interstrain variation in antibody levels to rgp160 and the three representative rgp160 epitopes as defined by peptides.

The end-point titers of antisera obtained following the third and fifth injection with rgp160 against each of the seven gp160 epitopes are shown in Table 3. In four of the six strains, the antibody titer to the peptides, along with the number of peptides that were recognized by the antisera, was related to the anti-rgp160 titer. For example DBA/1J mice, which are weak responders to rgp160 following three immunizations, recognized only two epitopes (peptides 600–611 and 735–752), whereas A/J mice, which produced a higher anti-gp160 response, recognized six of the seven gp160 peptides. The gp160 epitopes represented by peptides 425–448 and 616–632 seemed to be the least immunogenic in mice, as only one strain each (SJL and A/J mice, respectively) developed detectable responses against the gp160 epitope defined by each of these peptides. Antibody titers to peptides 304–321 and 735–752 were relatively high when compared to titers to the other five peptides. Antisera from C57BL/10 and SJL mice exhibited end-point titers up to 1:1600 for peptides 304–321 and 735–752, respectively. Detectable responses to gp160 epitopes defined by peptides 304–321 and 735–752 developed in five of the six rgp160 immunized inbred strains. Utilizing peptide based immunoaffinity chromatography, the anti-peptide component for selected peptide reactive epitopes was estimated to represent from 2 to 10% of the total anti-gp160 response (data not shown).

In all six strains examined, the level of antibodies following three immunizations to at least one of the gp160 peptides increased after two additional immunizations by 2- to 8-fold. For example, antisera from A/J mice showed an increase in titer after two additional injections with rgp160 to five of the gp160 epitopes defined by peptides. Following two additional immunizations, lower titers were observed in two of the inbred strains for specific gp160 epitopes. Antisera from C57BL/10 and CBA mice exhibited a 2-to 4-fold decrease in antibody titers to two (503–528 and 735–752) and one (600–611) peptide, respectively. All of the six mouse strains formed antibodies to at least two of the seven gp160 epitopes that were examined, whereas no

Table 3. Recognition of gp160 epitopes defined by synthetic peptides following three and five injections of recombinant gp160 in six inbred strains of mice[a]

Mouse strain	No. of injections	gp160 epitopes defined by synthetic peptides						
		304	425	503	600	616	735	846
BALB/cByJ	3	800[b]	—[c]	400	—	—	100	50
	5	800	—	200	—	—	100	100
A/J	3	200	—	200	100	100	400	50
	5	800	—	400	100	400	800	400
C57BL/10SnJ	3	1600	—	800	50	—	400	100
	5	1600	—	400	100	—	100	400
CBA/CaJ	3	—	—	100	200	—	—	—
	5	50	—	100	100	—	—	100
DBA/1J	3	—	—	—	200	—	200	—
	5	—	—	—	400	—	400	—
SJL/J	3	400	100	50	—	—	400	50
	5	400	100	50	—	—	1600	100

[a]Antisera from each of the six inbred strains of mice gave end-point titers of less than 50 when examined with the control Hep122 peptide preparation.

[b]End-point titer calculated as the reciprocal of the highest dilution that resulted in an optical density at least three times the mean of preimmune sera at a dilution of 1:50.

[c]Denotes undetectable antibody levels at a dilution of 1:50.

Table 4. Kinetics of the humoral immune response of individual C57BL/10SnJ mice immunized with recombinant gp160[a]

Antigen	No. of injections 1	2	3	4	5	6
304–321	< 50	60 ± 10[b]	810 ± 600	1430 ± 1244	1430 ± 1244	1470 ± 1235
425–448	< 50	< 50	< 50	< 50	< 50	< 50
600–611	< 50	< 50	90 ± 29	115 ± 33	120 ± 30	225 ± 54
846–860	< 50	< 50	60 ± 15	70 ± 11	180 ± 18	300 ± 50
gp160	< 50	70 ± 11	1760 ± 351	2560 ± 351	2560 ± 351	3600 ± 872
Hep122[c]	< 50	< 50	< 50	< 50	< 50	< 50

[a]Five mice were immunized with 50 μg of recombinant gp160 and serum was obtained between 14 and 28 days after injection.

[b]Mean end-point titer ± standard error, calculated as the reciprocal of the highest dilution that resulted in an optical density at least three times the mean of preimmune sera, tested at a dilution of 1:50.

[c]Peptide Hep122 represents residues 122–137 of the hepatitis B surface antigen.

strain exhibited reactivity to all gp160 epitopes following a total of five injections with rgp160. This pattern of reactivity illustrates the extensive variability in antibody responses to selected rgp160 epitopes among different inbred strains of mice.

In the next set of experiments, we examined the intrastrain variability of the antibody response to selected gp160 epitopes during the course of the murine immune response to rgp160. For this purpose, the antibody response to four representative epitopes (two each from gp120 and gp41) in five individual C57BL/10 mice was assessed (Table 4). Antisera were obtained following each of six injections with rgp160 and reactivity was determined to: (i) gp120 epitopes defined by peptides 304–321 and 425–448; (ii) gp41 epitopes defined by peptides 600–611 and 846–860; and (iii) rgp160. The selection of which peptides to include in this analysis was based on the reactivity of the pooled C57BL/10 antisera following five immunizations: epitopes defined by peptides demonstrated high (304–321), intermediate (846–860), low (600–611) and no (425–448) reactivity afgter immunization with rgp160 (Table 3). Following six injections with rgp160, no antibodies were detected to peptide 425–448 and to control peptide Hep122 (Table 4). An antibody response to gp160 epitopes defined by peptides 600–611 and 846–860 was first observed after three injections with rgp160 and increased by approximately 2- and 5-fold with additional immunizations. Antibodies specific for peptide 304–321 were first detected after two injections. The mean antibody titers increased following two additional injections and remained at that level upon subsequent immunizations with rgp160. The antibody response to rgp160 was detectable after two injections and increased following additional injections. These data, which include the mean endpoint titers of the five individual C57BL/10 mice to gp160 and to the four gp160 peptides, did not differ from end-point titers obtained using pooled C57BL/10 antiserum (see Table 3). A/J mice also exhibited significant increases in antibody titer to several peptides. However, unlike C57BL/10 and the other inbred strains, A/J mice reacted to peptide 304–321 with increasing titers following each additional immunization (data not shown).

A summary of the kinetics of the antibody response to each of the seven rgp160 epitopes in all six inbred mouse strains immunized with 50 μg of rgp160 is given in Table 5. The number of injections with rgp160 required to induce an antibody response to synthetic peptides defining gp160 epitopes varied greatly among both the

Table 5. Number of immunizations required to induce an antibody response to gp160 epitopes defined by synthetic peptides[a]

Mouse strain	gp160 epitopes 304	425	503	600	616	735	846	rgp160
BALB/cByJ	3[b]	—[c]	3	—	—	4	4	2
A/J	3	6	3	3	3	2	3	2
C57BL/10SnJ	2	6	4	5	—	3	3	2
CBA/CaJ	5	—	3	3	—	—	3	2
DBA/1J	—	—	6	3	—	2	6	2
SJL/J	3	3	2	6	—	2	3	2

[a]Mice were immunized with 50 μg of rgp160 and serum was obtained between 14 and 28 days after injection. No reactivity was observed with control peptide Hep122 at a serum dilution of 1:50.

[b]Number of injections required to stimulate production of anti-peptide antibodies.

[c]No antibody response to that epitope was detected following six injections with recombinant gp160.

seven peptides and the six inbred strains of mice. Reactivity to the gp160 epitope defined by peptide 735–752 required the least number of immunizations (a mean of 2.6 injections for the five responding strains). The rgp160 epitopes defined by peptides 425–448 and 616–632 appear to be the least immunogenic: epitope 425–448 required between three and six immunizations (a mean of five) to elicit detectable antibody levels in the three strains of mice that responded (A/J, C57BL/10 and SJL) while only one of the six inbred strains examined (A/J) developed detectable responses to epitope 616–632. The most immunogenic gp160 epitopes in the six inbred strains of mice examined were defined by peptides 503–528 and 846–860. Following six immunizations, all six inbred strains of mice produced detectable antibody responses to these two peptide epitopes. Alternatively, only A/J mice responded with antibodies to each of the gp160 epitopes defined by the seven peptides. Together, these results indicate that each of the six inbred strains exhibited distinct kinetics and patterns of antibody reactivity to this set of gp160 epitopes.

DISCUSSION

Our laboratory has previously examined the fine specificity of the anti-gp160 response of HIV-1 infected humans and chimpanzees (Warren *et al.*, 1990, 1991). Considerable variation in antibody reactivity to the seven gp160 epitopes was observed between the HIV-1 infected humans and chimpanzees that were examined. Thus, it was of interest to determine whether a similar variability might occur in the anti-gp160 response following immunization with a recombinant gp160 preparation. The antibody response following three injections with rgp160 was characterized and, based on endpoints titers, A/J (haplotype H-2^a), CBA/CaJ (H-2^k) and SJL (H-2^s) mice responded more strongly to rgp160 than did BALB/c ByJ (H-2^d), C57BL/10 SnJ (H-2^b) and DBA/1J (H-2^q) mice. Differences in the levels of antibodies to a given antigen in inbred strains of mice with distinct H-2 haplotypes has been used to define MHC linked restriction of an immune response (reviewed by Klein, 1975). The observed interstrain variability in the anti-gp160 response, as assessed by end-point titers, suggests a possible genetic basis for the differences in the immune response to rgp160. Following two additional injections with rgp160 (a total of five injections), the anti-gp160 titers increased in five out of six inbred strains. In addition, the interstrain variation in antibody levels to gp160 became less apparent after five immunizations. A similar observation that additional immunizations with staphylococcal nuclease decreased the interstrain differences in the antibody response has also been reported (Sachs *et al.*, 1978). In this latter system it was suggested that both H-2 linked and non-H-2 gene(s) could contribute to the regulation of the antibody responses after multiple immunizations with staphylococcal nuclease. A similar situation may exist for an antibody response to rgp160 in inbred strains of mice receiving multiple injections.

Table 6. Summary of HIV-1 gp160 reactivity to selected peptide epitopes by anti-gp160 antisera

Species	*n*	Percent reactivity to HIV-1 gp160 epitopes 304	425	503	600	616	735	846
Mouse[a]	6	83	17	83	67	17	83	83
Chimpanzee	23	30	13	22	39	4	22	48
Human[b]	160	16	6	41	93	2	6	12

[a]Mice from six inbred strains received five injections of 50 μg of rgp160 prior to assessing for reactivity to HIV-1 gp160 epitopes.

[b]All classified stages of HIV-1 infection are included (Warren *et al.*, 1991).

To confirm the HIV-1 gp160 specificity of the humoral immune response in mice immunized with rgp160, Western blot analysis was performed. Antisera from all six mouse strains obtained after five immunizations recognized gp160, gp120 and gp41 by Western blot analysis. The detection of an additional band by the mouse anti-gp160 response most likely represents a modified form of the HIV-1 envelope protein, since a similar reactivity was observed with serum from an AIDS patient. This reactivity by the human serum supplied by the manufacturer as a positive control for the Western blot analysis and the fact that little if any reactivity of the mouse antisera was observed by ELISA using a control baculovirus-derived SV40 T-ag (data not shown) suggests that the additional band detected in the Western blot was not the result of baculovirus contaminants that induced cross-reactive antibody responses.

Variability in antibody responses among the six inbred mouse strains following rgp160 immunization was observed when binding to rgp160 epitopes defined by synthetic peptides was examined. In five of the six strains, immunization with rgp160 generated antibodies to the HIV-1 V3 epitope defined by peptide 304–321. In C57BL/10SnJ mice, these anti-V3 antibodies could be detected after two injections with rgp160, while in the other four reactive strains three or more immunizations were required. Conversely, only six out of 19 animals (32%), experimentally infected with either the HTLV-IIIB or LAV isolated of HIV, developed antibodies to this epitope. This peptide defines a type-specific neutralizing epitope (Putney *et al.*, 1986); however, no *in vitro* neutralizing activity was observed with any of the mouse antisera that were examined (data not shown).

Following five immunizations with rgp160, only one of the six mouse strains, SJL/J, developed low, albeit detectable antibody levels to peptide 425–448, an epitope that corresponds to the putative gp120 binding site to the CD4 receptor (Laskey *et al.*, 1987). Two additional mouse strains (A/J and C57BL/10SnJ) developed an antibody response to this epitope following the sixth immunization with rgp160. Similarly, only one of six inbred mice (A/J) developed a detectable antibody response to the epitope defined by peptide 616–632. These data suggest that the epitopes defined by peptides 425–448 and 616–632 are weakly immunogenic in rgp160

immunized mice. Among 160 HIV-1 seropositive individuals and 23 HIV-1 infected chimpanzees, only 6 and 13%, respectively, exhibited weak reactivity to 425–448 by ELISA (Warren *et al.*, 1990, 1991). Similarly, the immunorecessive nature of the epitopes defined by peptide 616–632 was also observed in HIV-1 infected humans and chimpanzees. These data suggest that both the putative CD4 binding site and the neutralizing gp41 epitope defined by peptide 616–632 are immunorecessive in humans and chimpanzees infected with HIV-1 as well as in mice immunized with rgp160.

Five of the six strains tested exhibited reactivity with variable antibody titers to the gp120 carboxy-terminal epitope defined by peptide 503–528. This peptide represents a conserved region on gp120 which is recognized by sera from 40 to 50% of HIV-1 infected humans (Palker *et al.*, 1987). Peptide 600–611 represents the most immunogenic gp160 site for HIV seropositive humans, with >95% reactivity (Wang *et al.*, 1986; Gnann *et al.*, 1987), whereas reactivity to this epitope was observed in 39% of HIV-1 infected chimpanzees that were examined (Warren *et al.*, 1990). Of the six mouse strains tested, four developed antibodies to this site on gp160 after five immunization with rgp160. Five of six mouse strains reacted with epitopes defined by peptides 735–752. This high degree of reactivity among different inbred mouse strains immunized with rgp160 is in contrast with the low incidence of antibody reported in humans and chimpanzees to this gp41 epitope (Warren *et al.*, 1990, 1991). A similar difference between human and murine antibody response was observed with peptide 846–860. Only 8% of seropositive HIV-1 infected individuals had specific antibodies to this conserved, *C*-terminal epitope of gp41 (Warren *et al.*, 1991), while each of the six mouse strains developed antibodies following multiple immunizations with rgp160. Forty-eight percent of HIV-1 infected chimpanzees developed antibodies to this epitope (Warren *et al.*, 1990). The differences between human, chimpanzee and murine antibody responses to gp160 epitopes defined by synthetic peptides 735–752 and 846–860 may be attributed to a different pathway of antigen presentation during a natural infection process versus immunization with a recombinant subunit vaccine (Bolognesi, 1990).

An analysis of the fine specificity and possible genetic regulation of the humoral immune response to gp160 is warranted in light of the fact that this rgp160 preparation in alum ("VaxSyn") has been approved by the FDA to be tested as a vaccine candidate in a multi-center phase I and II safety and efficacy trails (Dolin *et al.*, 1991). In addition, this recombinant gp160 preparation has also been utilized in phase I trials as an active immunotherapeutic strategy in HIV-1 seropositive individuals (Redfield *et al.*, 1991).

The results presented herein indicate that within a given inbred strain the concn of rgp160 used to immunize mice had no significant effect on the anti-gp160 levels that were induced. Differences in anti-gp160 titers were observed among the various inbred strains following three injections with rgp160; however, these differences were not statistically significant. Additional injections with rgp160 decreased the observed differences in anti-gp160 levels among the six inbred strains. Each inbred strain exhibited a unique pattern of reactivity to seven synthetic peptides which define gp160 epitopes. This variability in the fine anti-gp160 specificity among different inbred strains of mice suggests a possible genetic basis for regulating the antibody response to selected gp160 epitopes.

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