CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Sept. 1994, p. 500–505 Vol. 1, No. 5
1071-412X/94/$04.00+0
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Epitope-Specific Antibody Response against Glycoprotein E of Pseudorabies Virus

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Received 28 January 1994/Returned for modification 24 March 1994/Accepted 25 April 1994

In this study we investigated the epitope-specific antibody response against glycoprotein E (gE) of pseudorabies virus. Epitope-specific antibody responses were investigated by enzyme-linked immunoperoxidase monolayer assays. In a vaccinated crossbred pig population, most pigs responded to antigenic domain E and to a lesser degree to antigenic domains C and D. Only few pigs responded to antigenic domains F, A, and B. Using vaccinated pigs, we investigated the influence of two different pseudorabies virus strains and the genetic background of the host on the epitope-specific antibody response. More pigs infected with the virulent NIA-3 strain had a detectable antibody response against antigenic domains C, F, and A than did pigs infected with the mildly virulent Sterksel strain ($P \leq 0.05$; Fisher's exact test). No differences in the epitope-specific antibody responses of two genetically different pig breeding lines were observed ($P \geq 0.1$; Fisher's exact test). In both breeding lines the incidence of the epitope-specific antibody response was comparable to that in the crossbred pig population. In addition, we studied the epitope-specific antibody response in genetically well-defined inbred mouse strains. The epitope-specific antibody responses were strikingly different and indicated that genetic background influenced the epitope-specific antibody response. Of the serum samples of mice with C57BL and a BALB background, 40 and 17%, respectively, were positive in one of the epitope-specific immunoassays. In contrast to pigs, mice responded predominantly to antigenic domain D and to a lesser degree to antigenic domains E and B. Only few mice had a detectable antibody response against antigenic domains C and A, and none had a detectable antibody response against antigenic domain F.

Pseudorabies (Aujeszky's disease) is one of the main costly diseases threatening the pig industry. The causative agent is pseudorabies virus (PRV), also known as suid herpesvirus-1 and Aujeszky's disease virus, a member of the family of the *Herpesviridae* (subfamily *Alphaherpesvirinae*). To control and eradicate PRV in most countries, glycoprotein E (gE)-negative marker vaccines are used (17, 18, 20). The use of gE-negative vaccines, in combination with a serological test, makes it possible to distinguish vaccinated from infected pigs (21). In the future, knowledge obtained by using these marker vaccines may be used to control other alphaherpesvirus infections such as bovine herpesvirus and equine herpesvirus infections in animals and herpes simplex virus and varicella-zoster virus infections in humans.

Detection of gE antibodies in infected pigs is crucial in monitoring the eradication or control of PRV. Although tests for the detection of gE antibodies are based on various principles (4, 10, 21), most rely on murine monoclonal antibodies (MAbs) to detect antibodies against one or two epitopes on gE. Consequently, it is crucial to know which epitopes on gE are recognized by the sera of infected pigs and if factors such as the infecting PRV strain or the genetic background of the host can influence the epitope-specific antibody response.

In this report we describe the epitope-specific antibody response in crossbred pigs from breeding farms in an area of the Netherlands that is endemically infected with PRV (14). We investigated the influence of the infecting PRV strain on the epitope-specific antibody response in vaccinated specific-pathogen-free (SPF) pigs infected with a virulent or mildly virulent PRV strain. The influence of the genetic background on the epitope-specific antibody response was investigated by comparing two pig breeding lines and by comparing six genetically well-defined mouse strains.

As decided at the 18th International Herpesvirus Workshop, Pittsburgh, Pa., in 1993, the nomenclature of the herpes simplex virus glycoproteins is used. PRV gE was previously called gI.

MATERIALS AND METHODS

Cells and viruses. A pig kidney cell line (SK6) was cultivated in Dulbecco's modified essential medium supplemented with 5% fetal calf serum, L-glutamine (0.3 mg/ml), penicillin (90 U/ml), streptomycin (100 U/ml), and nystatin (45 U/ml). The virulent NIA-3 strain (9) and the mildly virulent Sterksel strain (16) were used as challenge viruses in pigs.

Mice were infected with an NIA-3 mutant (M207) lacking the thymidine kinase protein (TK^-). This mutant virus is not lethal for mice and contains a 19-bp deletion in the thymidine kinase gene identical to the deletion in vaccine strain 783 (12). The mutant was constructed by using overlap recombination (22).

MAbs. MAbs were produced against PRV NIA-3 and Phylaxia, purified from ascites fluid by ammonium sulfate precipitation, and diluted in phosphate-buffered saline (PBS) to a final concentration of approximately 7 mg/ml. By using these MAbs, six antigenic domains were identified on gE (6). The MAbs and the antigenic domains which they represent are listed in Table 1.

Immunoperoxidase monolayer assay (IPMA). SK6 cells were seeded in culture dishes and grown to near confluence. When appropriate, the cells were infected with virus and incubated until plaques appeared. The monolayers were washed with PBS, and the plates were dried and frozen for at

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TABLE 1. Location and characteristics of antigenic domains on gE of PRV

Antigenic domain[a]	Location (amino acids)	Characteristics	MAbs[b]
E	78–239	Discontinuous[c]	2
C	78–239	Discontinuous	6, 9
D	68–82	Continuous[d]	7
F	—[e]	Discontinuous	10
A	64–73 and 75–84	Discontinuous	1, 3, 5
B	52–67	Continuous	4, 8, 11

[a] Antigenic domains were determined previously (6).
[b] Underlined MAbs are used in the ESB-IPMA and the ESC-ELISA.
[c] MAbs bind to a discontinuous sequence of amino acids (1).
[d] MAbs bind to a continuous sequence of amino acids (1).
[e] Location on gE is not known.

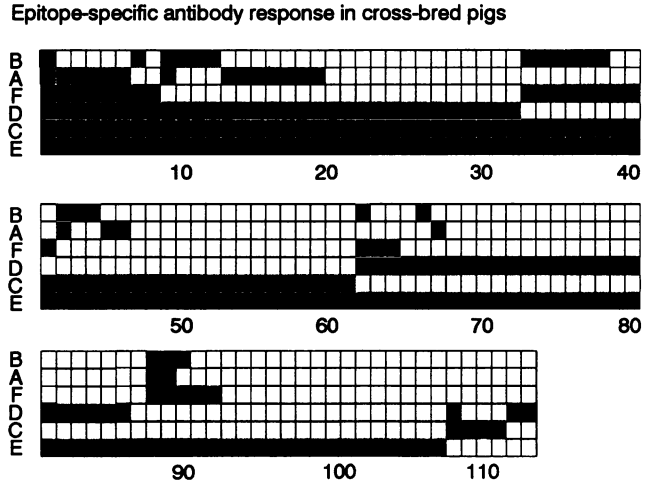

FIG. 1. Antibody responses against single antigenic domains of gE in individual pigs as measured by ESB-IPMA. Each horizontal bar represents one pig. A black box indicates that the pigs had detectable antibodies directed against the antigenic domain indicated at the left of the figure. Numbers indicate the number of pigs investigated.

least 60 min at −20°C. The monolayers were fixed with cold 4% (wt/vol) paraformaldehyde in PBS for 5 min at room temperature. After fixation, the plates were washed three times with PBS and incubated for 1 h at 37°C with the relevant MAb diluted in dilution buffer (PBS containing 0.1% bovine serum albumin and 0.01% Tween 80). The detecting antibody was rabbit anti-mouse immunoglobulins (mainly immunoglobulin G) conjugated to horseradish peroxidase (HRPO; DAKO, Copenhagen, Denmark) diluted 1:1,000 in dilution buffer, and the mixture was incubated for 1 h at 37°C. After each incubation period the plates were washed three times with PBS containing 0.05% Tween 80. Peroxidase activity was visualized by the addition of 2 mg of 3-amino-9-ethylcarbazole (Sigma) per ml in 0.05 M sodium acetate (pH 5.0) containing 0.005% H_2O_2 (13).

ESB-IPMAs. One day before infection, SK6 cells (10^6 cells per ml) were seeded in a microtiter plate (Greiner). Cells were infected with 10^4 PFU of PRV NIA-3 per ml and incubated for 15 h at 37°C. The plates were washed twice with PBS, dried, and stored at −20°C until use. Before use, the cells were fixed with an ice-cold solution of 4% paraformaldehyde in PBS for 15 min at room temperature. After fixation, the plates were washed three times with washing buffer (PBS supplemented with 0.66 M NaCl and 0.5% Tween 80). Pig sera were diluted 1:10 in serum buffer (PBS, 0.66 M NaCl, 1 mM EDTA, 0.1% Na_3N, 1% Tween 80, 5% fetal calf serum), and 100 μl of the diluted serum per well was incubated for 1 h at 37°C. After the incubation period, the plates were washed three times with washing buffer and then incubated with 100 μl of MAb diluted in serum buffer.

One MAb per antigenic domain was selected for use in the epitope-specific blocking (ESB)-IPMA. The selected MAbs were MAb 5 for antigenic domain A, MAb 8 for antigenic domain B, MAb 9 for antigenic domain C, MAb 7 for antigenic domain D, MAb 2 for antigenic domain E, and MAb 10 for antigenic domain F (6) (Table 1). The highest dilution of the MAb yielding a maximal A_{450} of 1.0 to 1.5 was selected. After incubation of the MAb, the plates were washed three times with washing buffer. The detecting antibody was rabbit anti-mouse immunoglobulins (mainly immunoglobulin G) conjugated to HRPO diluted 1:1,000 in conjugate buffer (PBS supplemented with 0.66 M NaCl, 1 mM EDTA, 1% Tween 80, 5% fetal calf serum). Then 100 μl of the conjugate was added per well, and the mixture was incubated for 1 h at 37°C. After the incubation period, the plates were washed three times with washing buffer. Then 100 μl of substrate (0.1% 5-amino-hydroxybenzoic acid dissolved in sodium phosphate buffer [pH 6.8] and supplemented with 0.005% H_2O_2) was added per well, and the mixture was incubated overnight at room temperature in the dark. The plates were shaken for 5 min and read at 450 nm in a Titertek multiscan (type 310-B; Flow Laboratories). Eight negative control sera were included in each test. When field sera were tested, the control sera were obtained from vaccinated pigs from a farm free of PRV. When sera from SPF pigs were tested, the control sera were obtained from SPF pigs in our institute. The control sera of SPF pigs did not contain antibodies directed against PRV, according to results of a commercial Aujeszky kit. The panel of control sera was used to calculate the mean A_{450} value and the standard deviation (SD) of the mean. A serum sample was considered to block the binding of the MAb if the A_{450} value was below the mean A_{450} value plus twice the SD of the eight control serum samples. The specificity of the ESB-IPMAs was tested by comparing the results obtained for 155 serum samples in the ESB-IPMAs with the results obtained in a previously described gE enzyme-linked immunosorbent assay (ELISA) (21), which detects antibodies against antigenic domain E, C, or both. Of 96 serum samples which were positive in the gE ELISA, 86 were positive in at least one of the ESB-IPMAs and 10 were negative. Of 28 serum samples which had a doubtful result in the gE ELISA, 23 were positive in one of the six ESB-IPMAs and 5 were negative. Two serum samples with detectable antibodies only against antigenic domain D had a doubtful result in the gE ELISA (Fig. 1). All 31 serum samples that were negative in the gE ELISA were negative in all of the ESB-IPMAs. We concluded that the six ESB-IPMAs (combined) had a lower detection level than but were at least as specific as the gE ELISA.

Epitope-specific competition (ESC) ELISAs. For mouse sera, commercial ELISA plates (Aujeszky-kit; Bommeli AG, Liebfeld-Bern, Germany) coated with PRV antigen gave the best results. Serial dilutions of MAb-HRPO conjugates in 0.05% Tween 80 in PBS were made to determine the highest dilution of conjugate yielding the maximal A_{450} value to use in the test. The same MAbs as those used in the ESB-IPMAs were used in this test. Mouse sera were diluted 1:10 in 0.05% Tween 80 in PBS, and 100 μl per well was transferred to the ELISA plate. After incubation for 30 min at 37°C, 100 μl of the

MAb-HRPO dilution was added and the plates were incubated again for 1 h at 37°C. Then the plates were washed with 0.05% Tween 80 in deionized water, and 100 μl of substrate (3,3′,5,5-tetramethylbenzidine, 1 mg/ml) was added. The reactions were stopped by addition of 100 μl of 0.5 M H_2SO_4, and the plates were finally read at 450 nm in a Titertek multiscan plate reader. In each test the mean A_{450} value and the SD of the mean were determined with eight control serum samples from SPF mice containing no antibodies directed against PRV. A serum sample was considered to block the binding of the MAb-HRPO conjugate if the A_{450} value was below the mean value plus twice the SD of the eight control serum samples.

Pig sera. (i) Pig sera from a crossbred pig population. We obtained 160 pig serum samples from pig farms in an endemically infected area which was intensively examined for the presence of PRV. All pigs were vaccinated intramuscularly with a gE-negative vaccine strain (vaccine strain 783) (14).

(ii) Pig sera from different breeding lines. We obtained 100 pig serum samples from two different breeding lines of Euribrid, Boxmeer, The Netherlands. Of these, 50 were from breeding line D and 50 were from breeding line C. Breeding line D descends from the Dutch landrace pig and Swedish landrace pig and has been a closed population since 1973. Breeding line C descends from the Large White pig and an American pig and has been a closed population since 1981. Pigs were vaccinated with a live gE-negative vaccine strain.

(iii) Pig sera from experimentally infected pigs. Dutch landrace pigs were obtained from the SPF herd of the Central Veterinary Institute. The pigs were born from unvaccinated sows and were free of antibodies against PRV before the start of the experiment. The pigs were vaccinated intramuscularly with 10^6 PFU of vaccine strain 783 per ml at the age of 10 and 14 weeks. They were challenged at 22 weeks of age with 10^5 PFU of the mildly virulent Sterksel strain (16) or 10^5 PFU of the virulent NIA-3 strain.

Collection of oropharyngeal fluid. Swab specimens of oropharyngeal fluid were collected from SPF pigs daily from 1 day before to 10 days after challenge infection with strain NIA-3 or Sterksel. Swabs were extracted with 4 ml of Dulbecco's modified essential medium supplemented with 5% fetal calf serum and antibiotics. To determine the virus content per gram of oropharyngeal fluid, we measured the weight of the collected fluid after centrifugation of the swabs in a special container (made at the Central Veterinary Institute).

Mouse experiments. Six inbred mouse strains from different genetic backgrounds were obtained from Harlan-CPB, Austerlitz, The Netherlands. The mouse strains were C57BL10.ScSn (*H-2*^{b}), B10.BR (*H-2*^{k}), B10.D2 (*H-2*^{d}), BALB/c (*H-2*^{d}), BALB.B (*H-2*^{b}), and BALB.K (*H-2*^{k}). Fifteen mice per strain were used for the experiment. To analyze immune responses, we immunized 10-week-old mice intraperitoneally with 10^8 PFU of PRV mutant M207 (TK^-) at days 1, 14, and 28 after the start of the experiment. At 2 weeks after the last immunization, we sedated the mice with ether and collected blood by heart puncture. The blood of three or four mice was pooled. Virus neutralization tests were performed in microtiter plates. Sera were heat inactivated, diluted in duplicate in twofold steps, and mixed with equal volumes (50 μl) of a virus suspension containing 100 to 200 PFU of NIA-3. The serum-virus mixtures were incubated for 24 h at 37°C (2). Then SK6 cells were added to the microtiter plates. After a 3-day incubation period at 37°C, cell monolayers were stained with amido black and the antibody titers were read. Neutralizing-antibody titers were expressed as $\log_{10}$ of the final serum dilution inhibiting the cytopathic effect in 50% of the cell culture.

Statistical calculations. StatXact, version 2.02, software (Cytel Software Corp., Cambridge) was used to perform all statistical calculations.

RESULTS

Epitope-specific antibody response in crossbred pigs. To study the antigenicity of single antigenic domains on gE, we investigated sera from a crossbred pig population in six ESB-IPMAs. This epitope-specific antibody response was determined for 160 serum samples obtained from pigs on farms located in an endemically PRV-infected area. Pigs responded predominantly to antigenic domain E (96%) and to a lesser extent to antigenic domains C (56%) and D (55%). Fewer pigs responded to antigenic domains F (22%), A (18%), and B (17%). In addition, the epitope-specific antibody responses varied considerably between the pigs (Fig. 1). In 1 pig, antibodies directed against all six antigenic domains were detected; in 7 pigs, antibodies against five antigenic domains were detected; in 21 pigs, antibodies against four antigenic domains were detected; in 24 pigs, antibodies against three antigenic domains were detected; in 39 pigs, antibodies against two antigenic domains were detected; and in 21 pigs, antibodies against one antigenic domain were detected.

Epitope-specific antibody response in SPF pigs infected with a virulent or mildly virulent PRV strain. PRV field isolates have been shown to differ in virulence (16). To investigate whether the virulence of the infecting PRV strain affects the epitope-specific antibody response against gE, we vaccinated 52 SPF pigs twice with vaccine strain 783 and challenged them with the virulent NIA-3 strain ($n = 28$) or with the mildly virulent Sterksel strain ($n = 24$). An IPMA confirmed that all antigenic domains were present on gE of both strains (results not shown). After challenge infection, virus was isolated from the oropharyngeal fluid. No significant differences in virus titers between pigs challenged with NIA-3 and pigs challenged with Sterksel were observed (2a).

The incidence of the epitope-specific antibody response was the same as in crossbred pigs irrespective of the strain used for challenge; again, pigs responded predominantly to antigenic domain E and to a lesser extent to antigenic domains C and D. Fewer pigs responded to antigenic domains F, A, and B (Fig. 2). With the exception of one pig challenged with the Sterksel strain, all pigs developed detectable antibodies to antigenic domain E. The epitope-specific antibody responses of pigs challenged with strain NIA-3 or strain Sterksel to antigenic domains E, D, and B did not differ significantly ($P \geq 0.05$; Fisher's exact test). In contrast, significantly more pigs challenged with strain NIA-3 responded to antigenic domains F, A, and C (F, $P \leq 0.05$; A, $P \leq 0.005$; C, $P \leq 0.01$; Fisher's exact test) than did pigs challenged with strain Sterksel.

We concluded that a virulent strain may induce more antibodies against some antigenic domains than a less virulent strain does. However, in both strains examined, the same antigenic domains appear immunodominant.

Epitope-specific antibody response in pigs of different genetic backgrounds. It is well established that genetic background (immune response genes) can influence the specificity of a B-cell response (8). To investigate if the genetic background of pigs affects the epitope-specific antibody response to gE, we investigated 100 pig serum samples from two different breeding lines. No differences ($P \geq 0.05$; Fisher's exact test) in the incidence of the epitope-specific antibody response were found between these two breeding lines or between the pigs from these two breeding lines and the crossbred pigs. Pigs responded predominantly to antigenic domain E (89%) and to

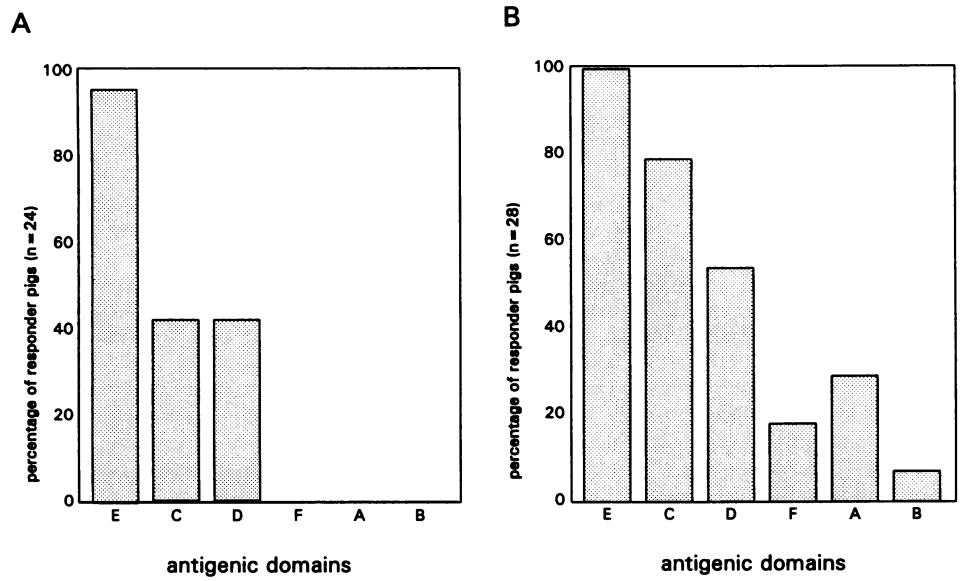

FIG. 2. Antibody responses against single antigenic domains of gE in vaccinated SPF pigs challenged with the mildly virulent Sterksel strain (n = 24) (A) or the virulent NIA-3 strain (n = 28) (B) as measured by ESB-IPMA. The number of pigs investigated was set at 100%. Each bar represents the percentage of pigs with detectable serum antibodies directed against the antigenic domain indicated at the bottom of the figure.

a lesser extent to antigenic domains C (64%) and D (75%). Fewer pigs responded to antigenic domains F (6%), A (18%), and B (12%).

Epitope-specific antibody response in different inbred mouse strains. Because it is difficult to obtain genetically well-characterized but genetically different pigs, we also tested mice for the influence of genetic background on the epitope-specific antibody response. Mice were vaccinated three times with the mutant strain M207 (TK^-), and sera were tested in six ESC-ELISAs.

Although all mice developed high PRV-specific neutralizing-antibody titers (≥3.1), we found that the epitope-specific antibody response differed remarkably between some of the mouse strains (Table 2). Of the serum samples of mice with a C57BL background, 40% were positive in at least one of the ESC-ELISAs, in contrast to only 17% of the serum samples of mice with a BALB background. In contrast to pigs, mice responded predominantly to antigenic domain D (97%) and to a lesser extent to antigenic domains E (74%) and B (70%). Fewer mice responded to antigenic domains C (48%) and A (37%), and none responded to antigenic domain F.

Comparing mouse strains BALB/c ($H\text{-}2^d$), BALB.B ($H\text{-}2^b$), and BALB.K ($H\text{-}2^k$) of different major histocompatibility complex (MHC) backgrounds, we found that significantly fewer BALB/c mice responded to antigenic domain E but significantly more BALB/c mice responded to antigenic domain B ($P \leq 0.05$; Fisher's exact test). Comparing mouse strains C57BL.ScSn ($H\text{-}2^b$), B10.D2 ($H\text{-}2^d$), and B10.Br ($H\text{-}2^k$), we found that significantly fewer B10.Br mice ($P \leq 0.05$; Fisher's exact test) responded to antigenic domain C. Thus, genetic background does affect the epitope-specific antibody response in mice, but no clear correlation between the epitope-specific antibody response and the MHC haplotype was found.

DISCUSSION

In the process of eradicating or controlling PRV, the ability to detect gE antibodies in infected pigs is crucial. Most tests to detect gE antibodies rely on detecting antibodies against one or two epitopes on gE (4, 5, 10, 15, 21). The antigenicity of these epitopes has, however, not been examined previously. In this study we first described the antibody response against

TABLE 2. Antibody response against antigenic domains of gE in inbred mouse strains immunized twice with strain M207 (TK^-)

Mouse strain[a]	Antibody response against antigenic domain[b]:						% Positive[c]	Mean neutralization titer ± SD
	E	C	D	F	A	B		
B10.D2 ($H\text{-}2^d$)	++++	++++	++++	----	++--	++++	75	3.6 ± 0.3
B10.Br ($H\text{-}2^k$)	++++	+---[d]	++++	----	+-+-	+--+	58	3.5 ± 0.2
C57BL.ScSn ($H\text{-}2^b$)	+++++	+++++	+++++	-----	++-+-	+++++	76	3.5 ± 0.5
BALB/c ($H\text{-}2^d$)	-----[e]	-----	+++++	-----	-----	+++++[f]	33	3.1 ± 0.0
BALB.K ($H\text{-}2^k$)	+++-	-+--	++-+	----	---+	---+	37	3.1 ± 0.0
BALB.B ($H\text{-}2^b$)	-+++++	-+--+	+++++	-----	-+--+	----+	46	3.3 ± 0.5

[a] MHC haplotype is given in parentheses.
[b] Serum samples from three or four mice were pooled. One + or − sign represents one pooled serum sample.
[c] (Number of positive serum pools/total number of serum pools per mouse strain) × 100%.
[d] Significantly less than other C57BL strains ($P \leq 0.05$; Fisher's exact test).
[e] Significantly less than other BALB strains ($P \leq 0.05$; Fisher's exact test).
[f] Significantly more than other BALB strains ($P \leq 0.005$; Fisher's exact test).

single antigenic domains on gE in crossbred pigs and then examined the influence of the two different PRV strains and the genetic background of the host on this response.

Antibody responses in pigs against a restricted number of six antigenic domains were determined (9). In fact, more (unidentified) antigenic domains may be present on gE, because murine MAbs may not fully detect the antigenic domains detected by pig antibodies either qualitatively or quantitatively.

Observed differences in epitope-specific antibody responses can be due to differences in sensitivity between the six epitope-specific assays. Differences in the affinity of the MAbs for their epitopes can influence the sensitivity of the assays. High-affinity MAbs compete more strongly for binding to their epitopes than low-affinity MAbs do. Consequently, antibodies in the pig sera can compete more easily for binding to their epitopes with low-affinity MAbs than with high-affinity MAbs. However, in previous studies the MAbs were used in a competition assay to map antigenic domains on gE (6) and no indications for differences in affinity were observed.

The results of our assays were not completely consistent with the results of the gE ELISA (21), which detects antibodies against antigenic domain C, E, or both. The gE ELISA appeared more sensitive than our tests. This could be due to the different dilutions of the sera used; in the ESB-IPMA and ESC-ELISA, serum was diluted 1:10, whereas in the gE ELISA, serum was diluted 1:1. This dilution was necessary to decrease nonspecific reactions of the pig sera in the assays.

Van Oirschot (16, 19) showed that pigs with high titers of maternal antibodies at the time of infection and pigs infected with a mildly virulent PRV strain responded poorly and inconsistently to gE. We demonstrated that fewer vaccinated SPF pigs had a detectable antibody response to antigenic domains C, F, and A after infection with a mildly virulent PRV strain than after infection with a virulent PRV strain. These two strains, however, did not appear to differ in their replication in the oropharyngeal region in this experiment. We found no qualitative differences in the expression of antigenic domains on gE between the virulent and mildly virulent PRV strains. Cells infected with the virulent or the mildly virulent PRV strain reacted in an IPMA with all our MAbs, indicating that all antigenic domains on gE are present. However, we did not compare the quantitative expression of gE in the two strains. Possibly the gE load in those two strains differ. Hence, the infecting PRV strain can influence the epitope-specific antibody response.

It is known that genetic background (MHC-linked immune response genes) can influence the B-cell response, but correlation of the antibody response to immune response genes is difficult to verify by using complex antigens such as gE (3, 8). The influence of these immune response genes on the antibody response to individual epitopes is obscured by reaction to the whole gE and can be seen only when the antigen dose administered is so low that just one immunodominant determinant is recognized by the immune system.

We detected significant differences in the epitope-specific antibody response between mice with a C57BL background and mice with a BALB background. Genetic background clearly influences the epitope-specific immune response. However, we were unable to correlate these differences with a specific MHC haplotype. BALB/c mice responded least well to antigenic domains E, C, F, and A. However, they are capable of producing antibodies against these antigenic domains, because all MAbs used in this study were produced in this mouse strain. The level of antibodies was probably too low to detect these epitopes in our assays.

We detected no differences in the epitope-specific antibody response between two genetically different pig breeding lines. However it is not known to what extent the two breeding lines differ genetically. Genetically well-characterized pigs are difficult to obtain. We cannot exclude the possibility that, just as in mice, the genetic background of pigs influences the antibody response.

In addition, Ben-Porat et al. (1a) reported that gE may be susceptible to antigenic drift, which can result in PRV strains missing one or more MAb-binding sites (7) or expressing an altered gE (11). Moreover, the possibility that pigs develop antibodies against other, as yet unidentified antigenic domains cannot be excluded.

In conclusion, a gE test to confirm PRV infection in pigs in which antibodies against all epitopes on gE are measured may therefore have advantages over a gE test in which only antibodies against one or two epitopes are measured.

ACKNOWLEDGMENTS

This study was supported by research contract BAP-0234-NL of the Biotechnology Action Program of the Commission of the European Community.

We thank H. Huipen for statistical support, M. Van Zijl for providing PRV M207, and Euribrid, Boxmeer, The Netherlands for providing pig sera from two breeding lines.

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