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Lymphocyte Proliferative Responses to Separated Bovine Herpesvirus 1 Proteins in Immune Cattle†

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The immune response to bovine herpesvirus 1 (BHV-1) infection can protect cattle from subsequent challenge with the virus. This protection involves a variety of defensive strategies, and the activation of most of these defenses requires the recognition of viral proteins by the cellular immune system. To identify some of the BHV-1 proteins recognized by T lymphocytes, we measured in vitro proliferative responses to individual proteins. Viral proteins were separated by gel electrophoresis followed by Western immunoblotting, and immunoblots were evaluated for serological reactions. Unstained blotted fractions were processed into antigen-bearing particles for analysis in blastogenesis assays. Purified BHV-1 proteins obtained by immunoadsorbent chromatography were processed and included for comparison in both enzyme-linked immunosorbent and proliferation assays. The tegument protein VP8 and the glycoprotein gIV appeared to be the antigens which most consistently stimulated the proliferation of lymphocytes from BHV-1-immunized animals. Positive blastogenic responses were also detected to gI, gIII, and to one or more uncharacterized, low-molecular-weight proteins in some of the cattle tested. These results indicate that T-lymphocyte proliferative responses to BHV-1 proteins are detectable in immune cattle and may be important in protection from BHV-1 infection.

Bovine herpesvirus 1 (BHV-1) contains a number of well-characterized proteins and glycoproteins (29). Glycoprotein gI includes the gIa protein (130 kilodaltons [kDa], the greater portion of which is cleaved into gIb (74 kDa) and gIc (55 kDa). Glycoprotein gIII (91 kDa) of BHV-1 is also found as a dimer (180 kDa), as is gIV (71 kDa, dimer of 140 kDa) (29, 32). The tegument protein, VP8, has an apparent molecular size of 96 kDa (28).

The specificity of the antibody response to BHV-1 infection in cattle has been evaluated by a number of investigators (7, 18, 19, 27, 28). Among the proteins recognized serologically by BHV-1-infected animals, the most prominent ones are glycoproteins gI, gIII, and gIV (28). The tegument protein VP8 is weakly recognized by immune bovine sera after a primary infection, but antibody levels increase after subsequent exposures to live BHV-1 (28). The antibody response to BHV-1 is supported by T lymphocytes (3, 22, 26, 33), but little is known about the identity of viral proteins which are recognized by the cellular immune response in cattle.

The purpose of the present study was to identify some of the BHV-1 proteins which are recognized by lymphocytes from immune cattle. This recognition was detected by measuring the proliferative response in vitro of lymphocytes cultured in the presence of separated BHV-1 proteins (25). The present results indicate that although immune recognition of all the major glycoproteins was demonstrated in both serological and proliferative responses, the presence of a blastogenic response to gIV seemed to be more consistent than the response to gI or gIII. The tegument protein VP8 stimulated a good cellular immune response in BHV-1-immune cattle but stimulated only a weak humoral immune response. One or more proteins of low apparent molecular weight, which were not identified by monoclonal antibodies to VP8, gI, gIII, or gIV, also triggered lymphocyte proliferative responses in most of the BHV-1-immune cattle tested.

MATERIALS AND METHODS

Cells and virus. Strain P8-2 of BHV-1 was propagated in Madin Darby bovine kidney cells grown in Eagle minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (GIBCO). Confluent monolayers in 150-cm^2 flasks (Corning Glass Works, Corning, N.Y.) were infected at a multiplicity of infection of 1. When extensive cytopathology was observed, the supernatant was collected and the cell debris was removed by centrifugation at 800 × *g* for 10 min. For some blastogenesis assays, this BHV-1 preparation was inactivated by placing 5 ml in a 100-mm petri dish and irradiating for 2 min at a distance of 11 cm from two General Electric G875 UV bulbs. The virus was then pelleted and purified on potassium tartrate gradients as described previously (4, 20). For the blastogenesis assays, RPMI 1640 medium (RPMI) (GIBCO) was used, supplemented with 10% fetal bovine serum, 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and 5×10^{-5} M 2-mercaptoethanol. BHV-1 strain 108, cultured in Georgia bovine kidney cells or syngeneic bovine fibroblasts by the method described above, was used for the first two immunizations of one of each set of the twin cattle, as described below.

Animals and immunization. Three pairs of syngeneic Hereford twin calves were derived by embryo splitting by using microsurgery (34) by staff at the Western College of Veterinary Medicine, University of Saskatchewan. Three unrelated Hereford cattle were used in proliferation assays with affinity-purified BHV-1 proteins VP8, gI, gIII, and gIV. One of each set of the twins (25, 71, and 107) was challenged intranasally with BHV-1 strain 108 approximately 1 year before these experiments, as described in detail previously

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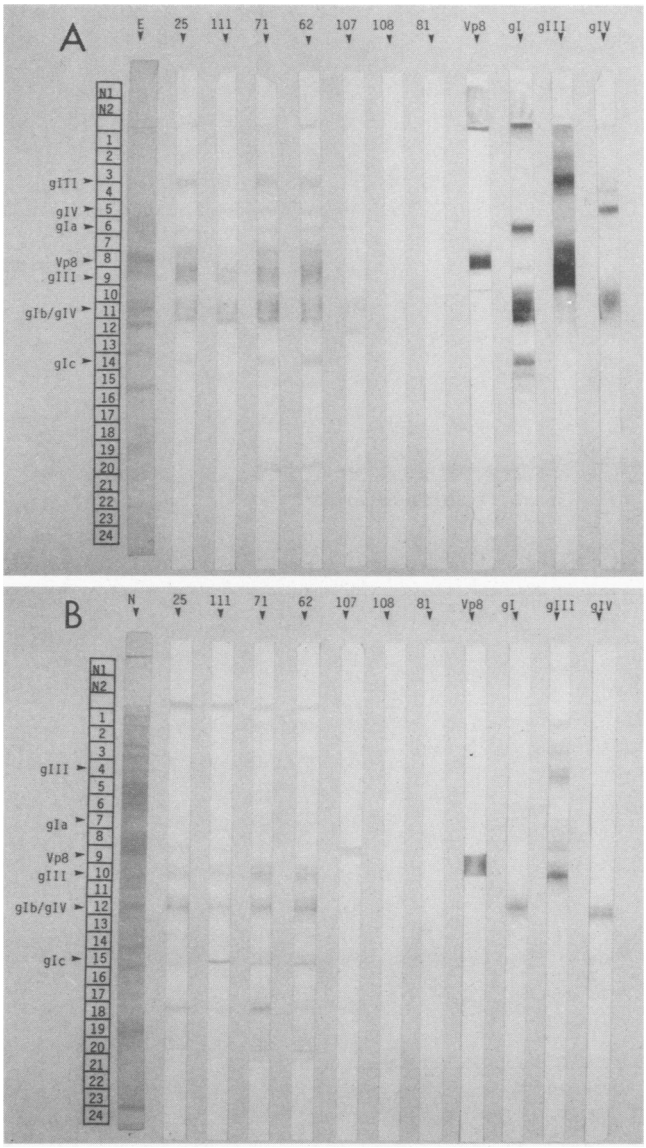

FIG. 1. Western blot analysis of serological reactivity. SDS-polyacrylamide (7.5%) gels were run under reducing conditions with Nonidet P-40-extracted BHV-1 proteins (A) (envelope proteins [E]) analyzed separately from the remaining nonsoluble pellet (B) (nucleocapsid-enriched protein [N]). The scale on the left corresponds to the 5-mm^2 blots used in the blastogenesis assays, and the approximate location of the characterized viral proteins is shown in the left margin. The first lane is stained with amido black, the next seven lanes are immunoblots of the bovine sera as labeled, and the last four lanes are stained with monoclonal antibodies specific for four major BHV-1 proteins as indicated.

(10). The corresponding twins (111, 62, and 108) seroconverted to BHV-1 from contact about 1 month later. The intranasally challenged twins were immunized a second time, 6 months before beginning these experiments, with an intramuscular injection of 5×10^7 syngeneic bovine fibroblasts infected with BHV-1 strain 108. All BHV-1-immunized cattle were injected intramuscularly with 10 ml of supernatant containing 10^7 PFU of live BHV-1 strain P8-2 per ml. This was done 1 and 3 weeks before performing the assays. One or more BHV-1-negative cattle of various breeds were included as controls in all proliferation assays.

TABLE 1. Reciprocal of antibody titer of cattle used in assays

Animal no.	Twin set[a]	SN[b]	ELISA titer[c]				
			BHV-1	gI	gIII	gIV	VP8
25	1	1,024	40,960	2,560	10,240	10,240	160
111	1	512	10,240	2,560	2,560	10,240	160
71	2	1,024	10,240	10,240	10,240	10,240	40
62	2	2,048	40,960	10,240	40,960	40,960	160
107	3	256	2,560	640	640	2,560	40
108	3	256	2,560	2,560	640	2,560	10
81		<4	<10	10	<10	10	<10

[a] Cattle 25 and 111, 71 and 62, and 107 and 108 were respective sets of identical twins; 81 was a control that was not immune to BHV-1.
[b] SN is the serum neutralization titer.
[c] ELISA titers are against BHV-1-, gI-, gIII-, gIV-, or VP8-coated plates as indicated.

SDS-polyacrylamide gel electrophoresis. To enrich for envelope proteins, we suspended purified BHV-1 in TNE (0.01 M Tris hydrochloride, 0.15 M NaCl, 0.001 M EDTA, pH 7.5) containing 1% Nonidet P-40. This suspension was left on ice for 15 min before sonication for 15 s at a setting of 100 on a sonifier cell disrupter (model 1510; Braunsonic, Braun, Melsungen AG, Federal Republic of Germany). Extracted proteins (referred to as the envelope fraction) were separated from insoluble material (nucleoprotein fraction) by ultracentrifugation as previously described (29). The pellet was suspended in electrophoresis sample buffer (0.0625 M Tris hydrochloride [pH 6.8], 1.25% sodium dodecyl sulfate [SDS], 12.5% glycerol, 0.15 M 2-mercaptoethanol, 0.00125% bromophenol blue) and boiled for 1 min before analysis under reducing conditions. The solubilized viral proteins in the supernatant were also separated by gel electrophoresis under reducing conditions. SDS-polyacrylamide gel electrophoresis was performed in 7.5% polyacrylamide discontinuous gels (14) as described previously (31).

Western immunoblot analysis. After electrophoresis, viral proteins were transferred to nitrocellulose in a Bio-Rad transblot cell (Bio-Rad Laboratories, Mississauga, Ontario, Canada) at 30 V for 3 h with an electrode solution of 25 mM sodium phosphate buffer (pH 6.8). The nitrocellulose was then cut into 6-mm-wide strips and assayed for reactivity with sera by using the instructions supplied with the Bio-Rad immunoblot assay kit (28). Antibodies used included sera from BHV-1-immune and nonimmune cattle tested in the blastogenesis assays, a monoclonal antibody that is specific for the BHV-1 protein VP8, and mixtures of monoclonal antibodies specific for BHV-1 glycoprotein gI, gIII, or gIV.

Western blot processing. The nitrocellulose blots were washed for 2 h with three changes of phosphate-buffered saline (0.1 M, pH 7.4) containing 0.3% Tween 20 (1). The blots were then rinsed with distilled water, partially dried between sheets of filter paper, and then stored at −20°C between sheets of Parafilm wrapped in aluminum foil. On the day before the blastogenesis assays were done, the nitrocellulose blots were cut into 5-mm-wide strips. These strips were then further divided into individual pieces 5 mm long, giving each fraction a total surface area of 25 mm^2. The fractions were then placed into individual sterile glass tubes and dissolved for 1 h in 300 μl of dimethyl sulfoxide (Fisher Scientific Co., Fair Lawn, N.J.). An equal volume of 0.05 M carbonate-bicarbonate buffer (pH 9.6) (1) was then added dropwise while vigorously vortexing the sample. The solution was transferred to a sterilized Eppendorf tube and pelleted at 10,000 × g for 10 min. The pelleted antigen-bearing particles (1, 35) were washed once in RPMI and

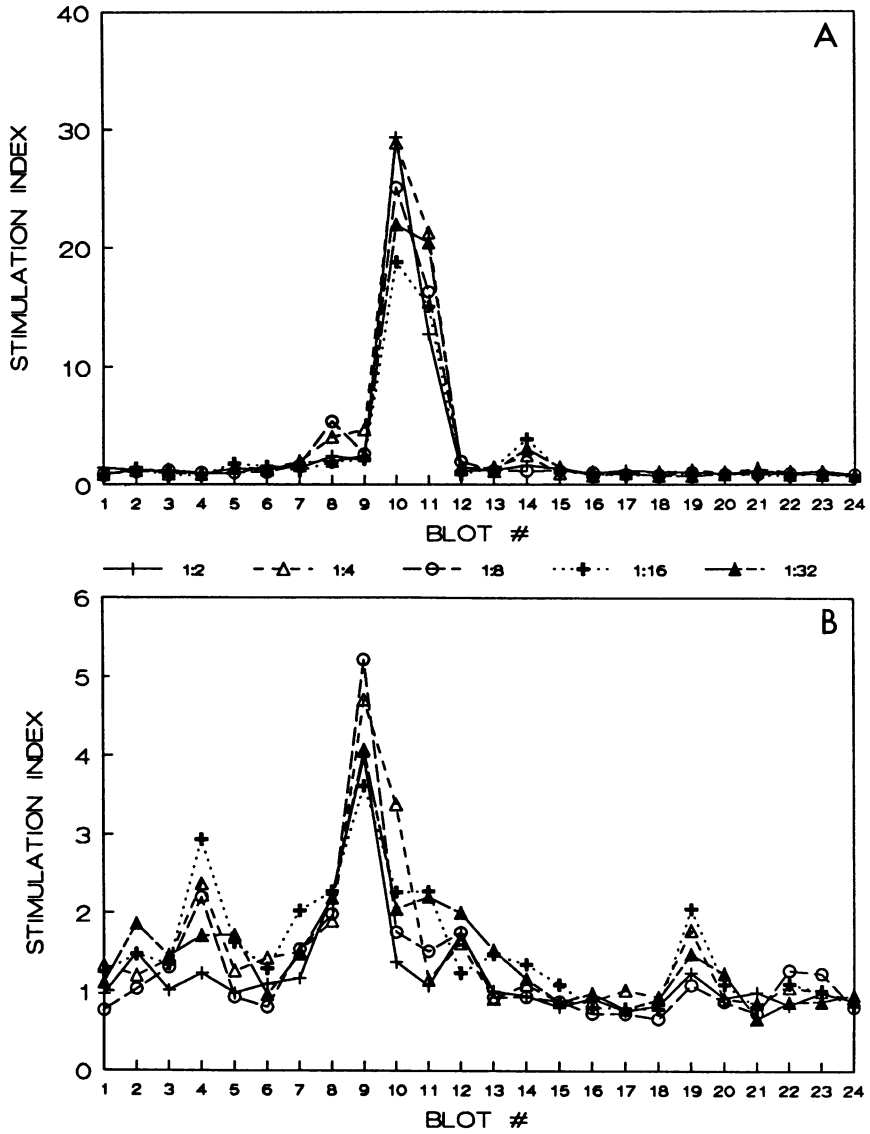

FIG. 2. Blastogenesis results expressed as stimulation indices of assays with five dilutions of the blotted proteins. The envelope (A) and nucleocapsid-enriched (B) blot fractions shown in Fig. 1 were assayed at the dilutions shown with lymphocytes from immune animal 62. Standard deviations of the means of triplicate wells were typically less than 20% and have been omitted for clarity.

then resuspended in 1 ml of RPMI. In some assays, affinity-purified proteins were blotted onto nitrocellulose directly by applying samples of less than 20 μl to 25-mm^2 fractions until the required amount was added (10 μg total per blot fraction) and allowing them to dry for 1 h between applications and again before processing. In one assay, 50 μl of UV-inactivated BHV-1 was applied to, and allowed to dry on, a 25-mm^2 section of nitrocellulose and processed as described above.

Immunoadsorbent chromatography. For comparison with the results obtained with the SDS-polyacrylamide gel electrophoresis-separated proteins, affinity-purified VP8, gI, gIII, and gIV proteins (2, 30) were used in proliferation assays in their native form as well as in a denatured form, prepared by adding 0.15 M 2-mercaptoethanol and 1.25% SDS to the protein and boiling for 1 min.

ELISA. Serum obtained from the twin cattle 2 weeks after immunization was tested by enzyme-linked immunosorbent assay (ELISA) for serological recognition of BHV-1 proteins. Purified BHV-1 (10 μg/ml) or affinity-purified VP8, gI, gIII, and gIV (0.25 μg/ml) were used to coat polystyrene microdilution plates (Immulon 2; Dynatech Laboratories, Inc., Alexandria, Va.), using 200 μl per well. The ELISA was performed essentially as described previously (31) except that affinity-purified, peroxidase-conjugated, rabbit anti-bovine immunoglobulin G (Dimension Laboratory, Mississauga, Ontario, Canada) was used at a dilution of 1:3,000 as the detecting antibody. The A_{450} was measured in a Bio-Rad microplate reader (model 3550).

Virus neutralization assay. The neutralization titers (SN) of the bovine sera were determined as described previously (25). The titer was expressed as the reciprocal of the serum dilution resulting in a 50% reduction of BHV-1 plaques relative to the virus control.

Isolation of PBML. Bovine blood was collected into citrate-dextran and centrifuged for 30 min at 1,000 × *g*. Buffy

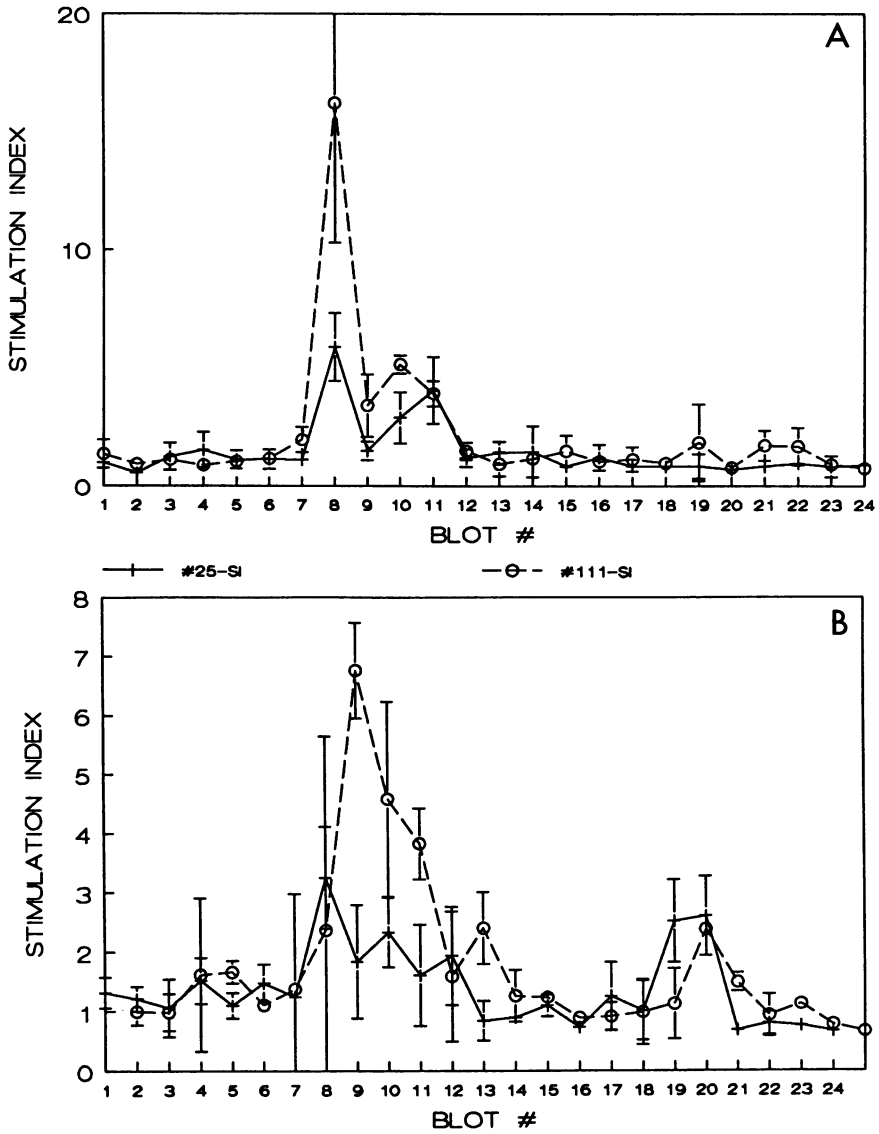

FIG. 3. Blastogenesis results with lymphocytes from the immune twins 25 and 111, expressed as stimulation indices of assays with a 1:4 dilution of antigen-bearing particles from envelope (A) and nucleocapsid-enriched (B) blot fractions. Error bars show the standard deviation of the mean of triplicate wells.

coat cells were aspirated, diluted with an equal volume of RPMI, and layered onto Ficoll-Paque (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) for density gradient separation. The cells were centrifuged at 1,000 × *g* for 40 min, and the band of cells at the interface was aspirated, pelleted, and washed three times in RPMI. Viability, as assessed by trypan blue exclusion, was greater than 95%, and yields of peripheral blood mononuclear leukocytes (PBML) from 50 ml of citrated blood were routinely between 1×10^8 and 2×10^8 cells.

Blastogenesis assays. The antigen-bearing particles were suspended by vortexing immediately before being added to U-bottom 96-well tissue culture-treated plates (Corning). Final culture conditions for the assays consisted of 10^6 PBML per ml in RPMI–10% fetal bovine serum, in a volume of 200 μl per well. The blastogenesis assays were incubated for 5 days in 5% CO_2 at 37°C. Proliferation was measured by adding 0.4 μCi of [*methyl*-^{3}H]thymidine (5.0 Ci/mmol; Amersham International, Amersham, United Kingdom) per well 18 h before harvesting onto glass fiber strips. Thymidine uptake was measured with a liquid scintillation counter, and means and standard deviations were calculated for the triplicate or quadruplicate values. Results are expressed as a stimulation index (mean counts per minute with antigen/mean counts per minute without antigen) with a similar dilution of processed nitrocellulose blots from an area adjacent to the stacking gel being used as negative controls. In the proliferation assays with the soluble or dot-blotted purified proteins, a similar dilution of processed nitrocellulose or medium was used as a control.

RESULTS

Western blot analysis. SDS-polyacrylamide gels were run under reducing conditions with the detergent-extracted envelope proteins (Fig. 1A) or the insoluble pelleted nucleo-

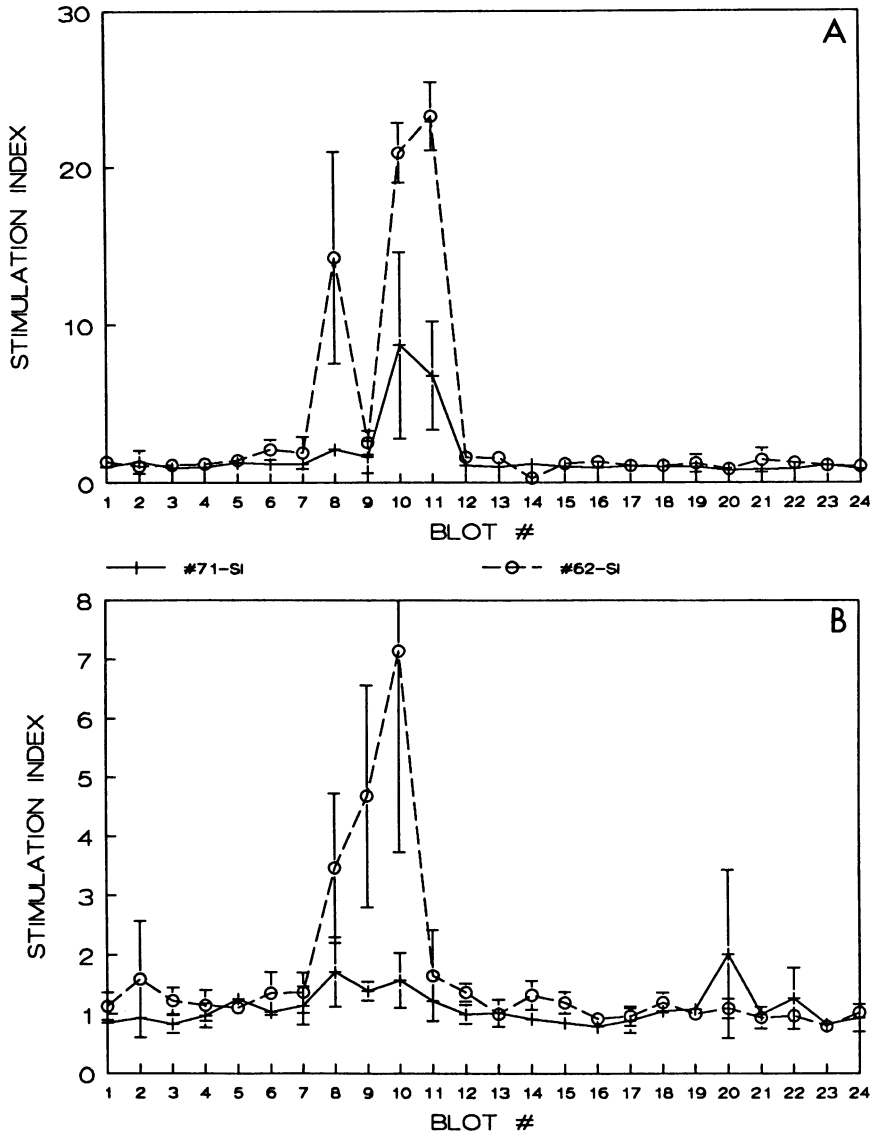

FIG. 4. Blastogenesis results with lymphocytes from immune twins 71 and 62, expressed as stimulation indices of assays with a 1:4 dilution of antigen-bearing particles from envelope (A) and nucleocapsid-enriched (B) blot fractions. Error bars show the standard deviation of the mean of triplicate wells.

capsid proteins (Fig. 1B), and immunoblots were used to identify the BHV-1 proteins present in each fraction. The first lane of Fig. 1 is a strip of the blotted nitrocellulose stained with amido black to nonspecifically detect viral protein bands. This strip could not be aligned precisely with the immunoblots due to approximately 5% shrinkage during the destaining procedure. To better localize specific proteins in the blot, we used monoclonal antibodies to identify glycoproteins gI, gIII, and gIV as well as tegument protein VP8 (shown in the lanes on the right of Fig. 1). The protein labels on the scale at the left show the midpoint of the stained regions from the control lanes.

The immunoblots showed that two of the three sets of BHV-1-immune twins had strong serological reactions to the major glycoproteins (gI, gIII, and gIV) but that all reacted weakly to the band corresponding to the tegument protein VP8. An uncharacterized protein of low apparent molecular weight in the envelope blot was also serologically recognized by one set of twins (Fig. 1A, fraction 20). Two proteins of low molecular weight in the nucleocapsid blot gave visible bands in two of the sets of twins (Fig. 1B, fractions 18 and 20).

ELISA and SN titers. To quantitate the antibody response of the cattle used in these assays, we conducted ELISA and SN tests. The results were similar to the qualitative assessment of the Western blot (Table 1). The major BHV-1 glycoproteins were recognized by all the immune cattle, with one set of twins (107 and 108) having lower titers than the other twins in all the serological assays. The SN results of each pair of twins were within a twofold range, whereas the SN titers for the group varied up to eightfold. Similarly, the ELISA titer of each pair of twins was within a 4-fold dilution range, whereas the range for the entire immune group was between 16- and 64-fold for the ELISA titers for the various antigens. The ELISA against VP8-coated plates showed only a weak serological response with all the immune cattle.

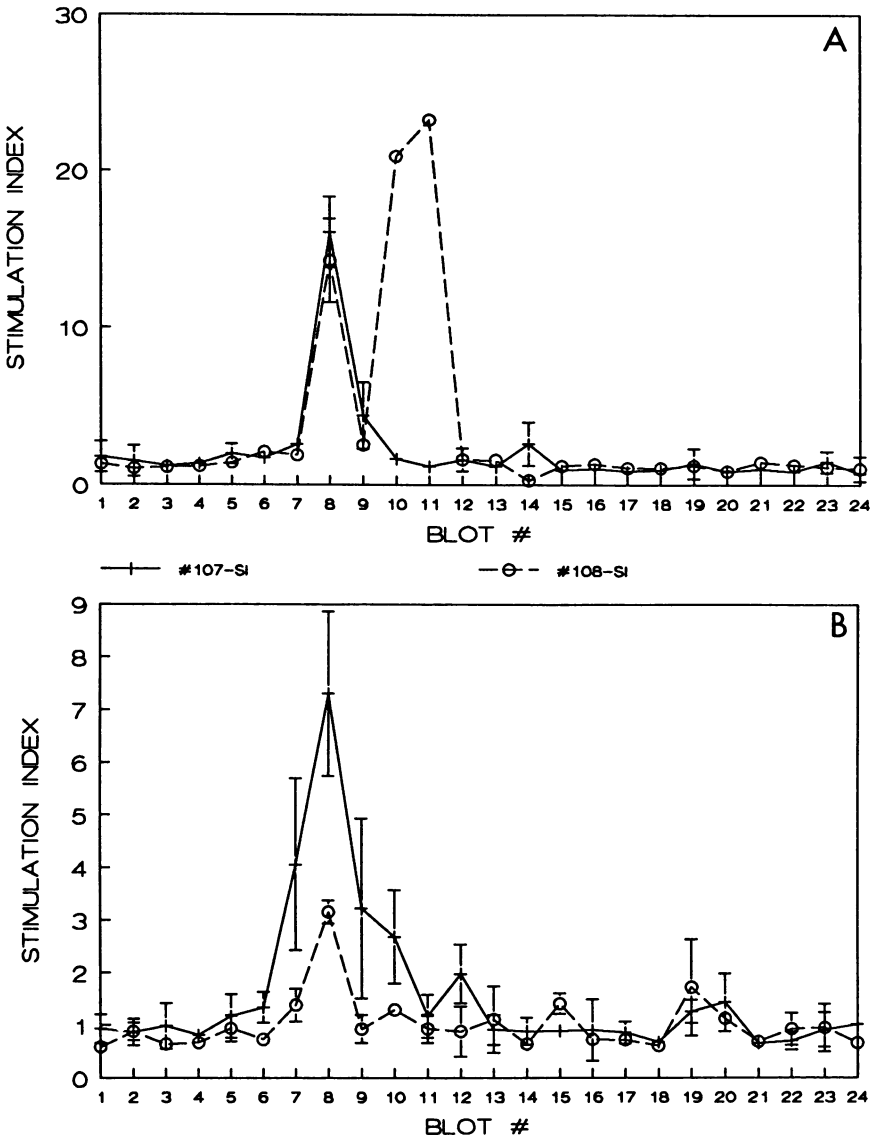

FIG. 5. Blastogenesis results with lymphocytes from immune twins 107 and 108, expressed as stimulation indices of assays with a 1:4 dilution of antigen-bearing particles from envelope (A) and nucleocapsid-enriched (B) blot fractions. Error bars show the standard deviation of the mean of triplicate wells.

These results suggested that the genetic background is more important than the immunization protocol in determining both the level and specificity of the antibody response and that VP8 is weakly recognized serologically.

Blastogenesis assays with antigen-bearing particles derived from Western blots. To determine whether lymphocytes could recognize and respond to any BHV-1 proteins, and to compare this response with serological reactivity, strips of blotted proteins were cut into 24 equal fractions as indicated in Fig. 1 and processed into antigen-bearing particles. To investigate the effect of antigen concentration on the magnitude of the proliferative response, we obtained a profile of the response to each fraction initially using a range of dilutions as indicated in Fig. 2. The stimulation index observed in reacting fractions was similar at all the dilutions tested, indicating that for these fractions the available antigen concentration was not a limiting factor in detecting the response. The dilution of 1:4 was selected for use in subsequent proliferation studies shown in Fig. 3, 4, and 5. Background proliferation in all assays was typically between 2,000 and 5,000 cpm. BHV-1-negative cattle were included in all proliferation assays, and the stimulation index for each of the antigens tested was less than two in all cases (data not shown).

The blastogenesis results to characterized BHV-1 proteins were assessed based on their fraction locations, which were identified by using the immunoblots shown in Fig. 1. The tegument protein VP8 was located at fraction 8 in the envelope blots and at fraction 9 in the nucleocapsid blots. Five of six of the immune cattle had positive proliferative responses to blot fractions containing the tegument protein VP8 (Fig. 3 to 5). Fractions 10 and 11 of the envelope blots, which contained the proteins gIb and gIV, also gave strong proliferative responses in five of six of the immune cattle (Fig. 3A, 4A, and 5A).

The positive proliferative responses to other fractions

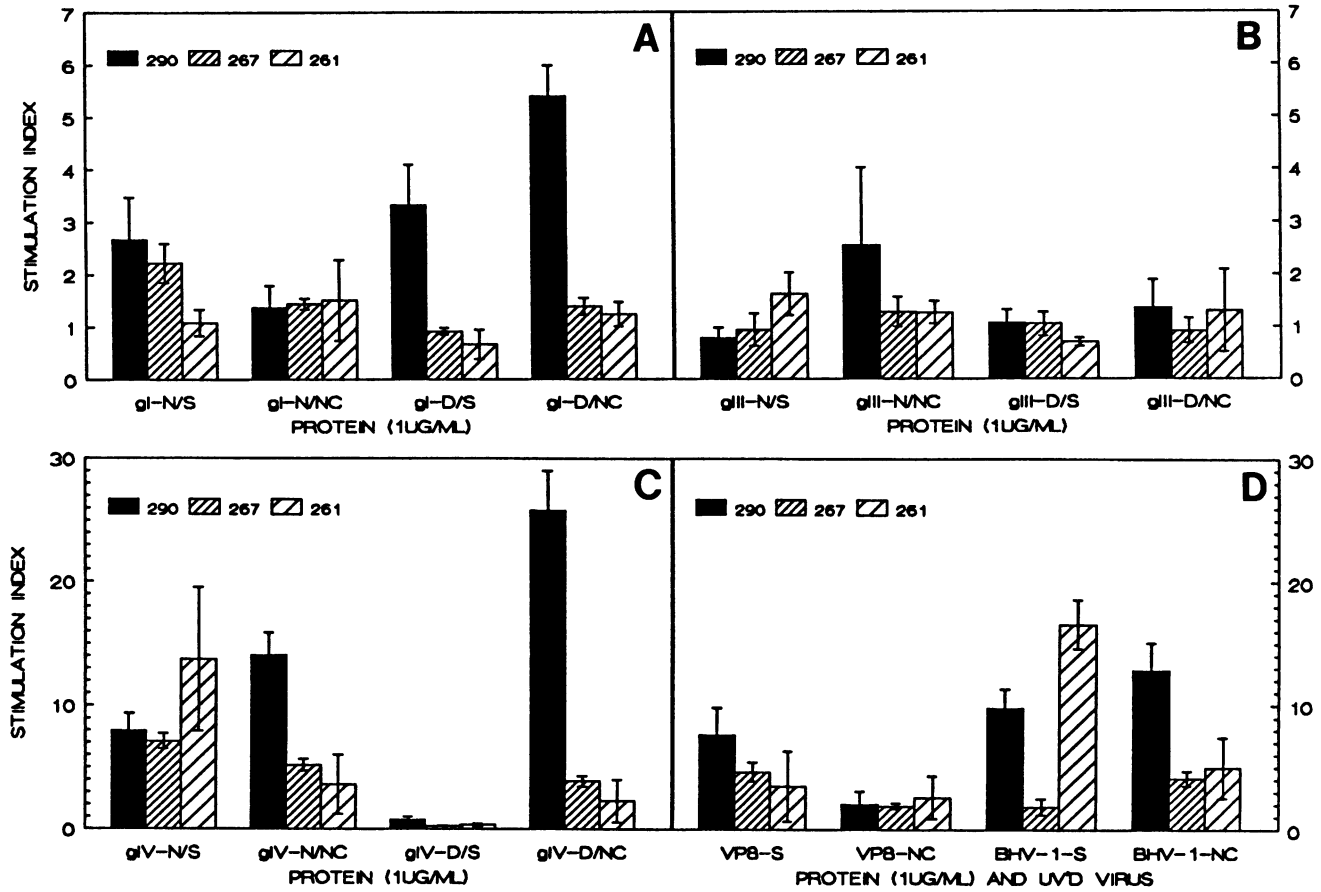

FIG. 6. Stimulation index of blastogenic assays with affinity-purified viral proteins. The proliferative responses to glycoproteins gI (A), gIII (B), and gIV (C) as well as the tegument protein VP8 (D) were assayed at a final dilution of 1.0 μg/ml with lymphocytes from three BHV-1-immune cattle (290, 267, and 261). UV-inactivated BHV-1 (3×10^4 PFU/ml) was used in both soluble and nitrocellulose-bound forms. The first set of bars in panels A, B, and C represents the native soluble glycoprotein (N/S), the second set represents the native nitrocellulose-bound form (N/NC), the third set represents denatured soluble glycoprotein (D/S), and the fourth set represents the denatured nitrocellulose-bound form (D/NC). In panel D, the responses to native VP8 and BHV-1 were assayed in both soluble (S) and nitrocellulose-bound (NC) forms. Error bars show the standard deviation of the mean of quadruplicate wells.

were less prominent and were seen with fewer animals. The gIII monomer present in fraction 9 of the envelope blots and fraction 10 of the nucleocapsid blots (Fig. 2, 3, and 5) gave a weak proliferative response with twins 11, 62, and 107. The dimeric form of gIII migrated in fraction 4 of the nucleocapsid blot and gave a positive response in the assay with twin 62 (Fig. 2B). The glycoprotein gIc in fraction 14 of the envelope blot gave a weakly positive response in an assay with twins 107 (Fig. 5A) and 62 (Fig. 2A). One or more unidentified proteins of low apparent molecular weight gave a positive response in nucleocapsid fractions 19 (25 in Fig. 3B, 108 in Fig. 5B, and 62 in Fig. 2B) and 20 (25 and 111 in Fig. 3B and 71 in Fig. 4B). Thus, a positive proliferative response was obtained in these assays with fractions containing VP8, the glycoproteins gI, gIII, and gIV, as well as unidentified proteins of low apparent molecular weight.

Blastogenesis assays with affinity-purified BHV-1 proteins. To confirm the identities of the blotted proteins involved in stimulating blastogenic responses, we prepared affinity-purified BHV-1 proteins and used them in the assay. The purity of these preparations was identical to that previously published (2, 30). The stimulation index to VP8, added in soluble form at a final dilution of 1.0 μg/ml, ranged from 3.5 to 7.6 in the BHV-1-immunized cattle (Fig. 6D). In contrast, the response to nitrocellulose-blotted VP8 was lower, ranging from 2.0 to 2.6 in stimulation index. UV-inactivated BHV-1 was included as a control, and the stimulation index was higher with the nitrocellulose-blotted virus in two animals but lower in the third when compared with soluble virus (Fig. 6D). One animal (290) had a good proliferative response to gI (Fig. 6A), especially the denatured protein bound to nitrocellulose. This animal also had a positive response to the native nitrocellulose-bound form of gIII (Fig. 6B). Thus, although the denaturation and nitrocellulose binding of proteins had some effects on the magnitude of the proliferative responses measured, these effects were variable between animals and between proteins.

The highest proliferative responses (Fig. 6C) were to preparations containing the gIV protein, with the exception of denatured gIV added in a soluble form. Positive proliferative responses to gIV were still present in wells containing as little as 0.1 μg/ml in two of the cattle (290 and 267), with stimulation indices above three for all four forms of the protein, including denatured soluble gIV (data not shown). This result suggests that the stimulation observed previously

with the fractions containing both gIb and gIV was mainly due to the presence of gIV.

DISCUSSION

Antibody responses specific for BHV-1 were measured in calves immunized with live virus. Despite different immunization protocols, identical twins had very similar responses to the virus. These results support the importance of genetics in determining the level and specificity of antibody responses to virus. Antigen-specific responses of PBML from BHV-1-immune cattle were also analyzed in proliferation assays, using BHV-1 proteins separated by gel electrophoresis and processed into antigen-bearing particles. These studies and other proliferation assays with affinity-purified BHV-1 proteins suggest that the tegument protein VP8 and the glycoprotein gIV are major antigens recognized by the cellular immune response.

Two approaches have been used most frequently to identify antigens with potential as subunit vaccines. Monospecific and monoclonal antibodies have proved to be very valuable in identifying which antigens induce neutralizing antibody responses (17). However, cell-mediated immunity (CMI) is known to be crucial in aiding recovery from many virus infections including herpesviruses (16, 22–26). To date, the most successful technique used to identify specific polypeptides involved in inducing CMI responses and more specifically cytotoxic T-cell (CTL) responses is the cloning and expression of putative antigens and measuring their ability to be recognized by CTLs (5, 8). Using this technology, a number of herpesvirus glycoproteins have been shown to act as potential targets for CTL responses (5, 8). Unfortunately, this procedure is labor intensive, and it would be advantageous if specific target antigens could be easily identified. The recent discovery that various proteins including nonstructural proteins can be involved in inducing CMI responses and are important in inducing resistance to and recovery from infection (9) demonstrates the need for a rapid method to detect CMI-inducing proteins. The uncharacterized proteins in fractions 19 and 20 are a clear indication that proteins other than those that have been focused on in the past may be important in some aspects of protection from BHV-1 infection. Thus, a method is needed by which one could rapidly identify antigens with the potential to stimulate CMI. The approach described in this report provides a method for just that purpose. Once identified, these antigens could then be cloned and expressed and tested for their ability to induce various cytokines which may be involved in stimulating lymphokine-activated killer cells (6) or in inducing CTLs. Such a procedure would expedite the development of efficacious vaccines in humans and animals.

The present results indicate that there is little correlation between the presence of a serological reaction to individual proteins and the magnitude of the stimulation index obtained in the blastogenesis assays. For example, the processed blot fractions which corresponded to the location of the tegument protein VP8 had a high stimulation index, even though this band was only weakly stained in the immunoblots. Although denaturation of VP8 may have altered antibody binding, as has been observed with gIV (11, 12), the ELISA results indicate that the cattle also have a weak antibody response to the native form of VP8. Recent studies with influenza virus demonstrate that mice immunized with a hybrid protein of influenza virus HA2 and a portion of the NS1 protein developed a CTL response which was protective even in the absence of antibody (13). These results combined with our present study clearly indicate that different antigens may induce either cellular or humoral responses independently. Therefore, if one depends only on antibody responses to identify putative vaccine candidates, the most crucial antigen may be overlooked. This difference in protein recognition between T- and B-cell repertoires has also been detected in bacterial systems with *Mycobacterium tuberculosis* immunoblots (15). The absence of B cells specific for a given protein, or the lack of B-cell binding of a denatured soluble protein, could lead to erroneous conclusions about the presence of T cells specific for that protein, since antigen processing and presentation by B cells would be inefficient (33). The use of nitrocellulose as a matrix to insolubilize the protein of interest avoids some of this B-cell bias, since it appears to enhance antigen uptake by cells of the macrophage-monocyte lineage (1). If B cells specific for the protein are present, the contribution by B cells to soluble antigen processing and presentation could actually enhance the proliferative response of the PBML, leading to the variable results of nitrocellulose binding and denaturation observed in Fig. 6.

In the development of subunit vaccines, it is important to ensure that the specific protein is recognized by a variety of individuals in an outbred population. Recent studies clearly indicate that some antigens on eptiopes will only induce an immune response in animals of a certain major histocompatibility complex haplotype (21). The present technique allows one to test a variety of different animals within the species to determine the frequency of unresponsiveness. Studies conducted with twins as well as with unrelated animals (Fig. 6) clearly indicated that such testing is feasible in that one could rapidly identify the most broadly reactive antigen in a variety of different animals within the species.

The use of processed immunoblots in investigating the T-cell repertoire generated in response to viral infection provides useful information about proteins which stimulate T lymphocytes in BHV-1-immune individuals. The analysis of immunodominant antigens in the cellular immune response to BHV-1 in cattle populations should be of assistance in designing appropriate vaccines.

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