Induction of immune responses to bovine herpesvirus type 1 gD in passively immune mice after immunization with a DNA-based vaccine

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The potential for plasmids encoding a secreted form of bovine herpesvirus type 1 (BHV-1) glycoprotein D (gD) to elicit immune responses in passively immune mice following intramuscular immunization was investigated. In these experiments, 6- to 8-week-old female C3H/HeN or C57BL/6 mice were passively immunized with hyperimmune antisera raised against BHV-1 recombinant, truncated (secreted) gD immediately prior to immunization with plasmids. A single immunization of passively immune mice with plasmid encoding the secreted form of BHV-1 gD resulted in rapid development of both cell-mediated immunity and antibody responses. Furthermore, 50% of mice immunized with a suboptimal dose of recombinant gD formulated into an adjuvant developed significant levels of serum antibodies if mice were pre-treated with hyperimmune antisera. The apparent failure of passive polyclonal antisera to suppress the induction of immune responses to pSLRSV may be related to the immunoglobulin subtypes present in the hyperimmune sera.

Introduction

A significant problem facing vaccine development today is the inability to elicit active humoral immunity and reproducible cell-mediated immunity in neonatal animals born to immune mothers (Harte et al., 1983; Bona & Bot, 1997). The failure to induce effective immunity in passively immune neonates is largely the result of two primary and mechanistically distinct problems. The first problem arises as a result of the relative immaturity of the neonatal immune system and the inability of these animals to mount an effective immune response to the vaccine doses normally given to adult animals (Ridge et al., 1996; Sarzotti et al., 1996).

The second reason for failure of neonatal animals to respond efficiently to vaccination is the well-documented, but poorly understood phenomenon where passively acquired maternal antibody mediates suppression of active immunity (Xiang & Ertl, 1992). It appears that maternal antibody specifically inhibits B-cell mediated immunity and indirectly impedes the expansion of a nascent population of primed T-cells. Although maternal antibody is absolutely critical for early protection against many neonatal pathogens, it leads to a situation where there is an unavoidable window of susceptibility to disease following the decline of maternally derived antibody and prior to the development of active humoral responses (MacDonald, 1992). This window of susceptibility occurs because the maternal titre capable of inhibiting the response to vaccination is frequently lower than the titre required for protection. Thus young animals must ‘wait’ until suppressive passive titres have declined to the point where they are no longer able to interfere in the development of an active humoral response to vaccination.

This window of susceptibility to infectious disease can often amount to several weeks and despite nomograms designed to calculate the point at which maternal titres have decayed, many young animals remain unprotected for days or weeks longer than necessary. Frequently multiple immunizations are utilized, with varying degrees of success, to avoid unnecessarily long periods of disease susceptibility in neonates. However, this approach may be more acceptable and easier to implement in humans as opposed to many food animal species.

We propose that features mechanistically unique to the novel DNA-based vaccines offer an approach that has some potential to overcome immune deficiencies characteristic of neonatal animals.

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Genetic immunization, also termed nucleic acid, polynucleotide, somatic transgene or DNA-based vaccination, was first described seven years ago and subsequently shown to be successful against a number of pathogens (Tang et al., 1992; Donnelly et al., 1993; Ertl & Xiang, 1996; Hassett & Whitton, 1996; Ulmer et al., 1996; Gerloni et al., 1997; Wolff et al., 1992; Davis et al., 1996). More recently it has been demonstrated that in situ transfection of dendritic cells and the accompanying adjuvant effects of hypomethylated CpG motifs within the plasmid DNA play a significant, and perhaps critical, role in the efficient development of immunity to these novel vaccines (Pertmer et al., 1996; Condon et al., 1996; Pisetsky, 1996; Carson & Raz, 1997; Roman et al., 1997).

In the present study, we investigated the humoral and T-helper (Th) responses in 6- to 8-week-old C3H/HeN and C57BL/6 mice passively immunized with hyperimmune sera or antigen-specific MAbs prior to immunization with a single dose of a DNA-based vaccine encoding a truncated version of the bovine herpesvirus-1 (BHV-1) glycoprotein D (gD) (Tikoo et al., 1993). This DNA vaccine was designated pSLRSV.SgD and encodes a secreted form of gD that lacks the normal hydrophobic transmembrane anchor that occurs in the authentic gD. We have previously demonstrated that immunization of C3H/HeN mice with pSLRSV.SgD induces a substantial humoral and cell-mediated antigen-specific immune response (Lewis et al., 1997). In this experiment we utilized 6- to 8-week-old mice to eliminate the compounding problem of murine neonatal immunodeficiency and the propensity to tolerate or deviate responses towards Th2-type immunity following conventional immunization in this species. This gave us the opportunity to study the effects of passive antibody titres on the immune responses to DNA vaccination, and to gain some insight on the potential for this vaccine approach to overcome maternal antibody suppression.

Methods

Mice. Female 5- to 6-week-old C3H/HeN and C57BL/6 mice were purchased from Charles River. All mice were acclimatized for 1–2 weeks prior to vaccination with DNA-based or conventional vaccines.

Plasmids. All restriction enzymes and DNA-modifying enzymes as well as markers and plasmids were purchased from Pharmacia or New England Biolabs unless indicated otherwise. Expression cassettes utilizing promoter/enhancer regions from murine cytomegalovirus (MCMV) and Rous sarcoma virus long terminal repeat (RSV-LTR) were constructed. These regulatory elements were placed in the high-copy plasmid pSL301 (Invitrogen) to generate the expression vectors pMCEL.Nul and pSLRSV.Nul. Creation of an expression cassette expressing a secreted or truncated version of BHV-1 gD was described previously (Tikoo et al., 1993). Briefly, vector PRSV1.3gD, which encodes a truncated form of gD, was generated by introduction of a unique XhoI site at the SaclI site immediately adjacent to the transmembrane domain of prSV1.3 encoding the full-length, or authentic, gene for BHV-1 gD. Following restriction digestion and end repair, this unique XhoI site was blunt-end ligated to a short linker sequence encoding the unique Nhel restriction site and three stop codons. Expression of this truncated gene leads to secretion of a glycosylated product of approximately 62 kDa (Tikoo et al., 1993). Vectors pSLRSV.AgD (AgD, authentic or full-length gD) and pSLRSV.SgD (SgD, secreted or truncated gD) were created by ligating the 947 bp Nhel fragment from prSV1.3 and ligating it into Ndel-digested pMCEL.AgD and pMCEL.SgD (not shown). The Nhel fragment from prSV1.3 contains the RSV-LTR promoter/enhancer region and a portion of the 5’ end of the gD gene. Ligation of a 585 bp Ndel/BglII fragment from prSV1.3 ligated into Ndel/BglII-digested pMCEL.Nul generated vector pSLRSV.Nul (Tikoo et al., 1990, 1993). In vitro expression of all constructs was demonstrated by immunoprecipitation of truncated gD from media harvested following stable and transient transfection of L929 (ATCC No. NCTC clone 929) and COS-7 (ATCC No. CRL 1651) cell lines (data not shown).

Passive immunization. Hyperimmune donor mice (C3H/HeN) were prepared by intramuscular immunization with 100 µg pSLRSV.SgD. Seropositive mice were then boosted on days 14 and 24 with recombinant truncated gD (10 µg and 1.5 µg per mouse, respectively) emulsified in VSA3 (Biostar) adjuvant (VSA3/trgD). This immunization regime results in much higher antibody titres than boosting with DNA alone. Sera from hyperimmune donor mice were harvested 10–14 days after the final boost.

All donor mouse hyperimmune blood was collected by cardiac puncture of halothane-anaesthetized mice. Sera were prepared by brief centrifugation of pooled blood and transferred to sterile 15 ml centrifuge tubes (Corning). Recipient mice were physically restrained and intraperitoneally injected with an appropriate amount of hyperimmune antisera to result in passive antibody serum titres in recipient mice of between 10000 and 20000 ELISA units. BHV-1 gD-specific MAbs PB136, 3D9, 2C8 (IgG), and 1G6 (IgG2a) have been described and were diluted and administered as described for hyperimmune polyclonal antisera (van Drunen Littel-van den Hurk et al., 1984; Hughes et al., 1988). All recipient mouse titres were verified after 24 h using a gD-specific ELISA.

Immunization with plasmid and protein vaccines. Inbred 7-week-old female C3H/HeN mice were injected with plasmid DNA, purified with an anion-exchange column (Qiagen), dissolved in normal saline. All injections of DNA were intramuscular whereas injections of purified antigen (authentic or recombinant truncated gD) formulated in VSA3 were subcutaneous at the dorsal midline of the thorax on each mouse. DNA-immunized mice were physically restrained and injected using a 0.5 ml disposable Microject syringe with a 29 gauge needle. All mice received doses of plasmid DNA split between the left and right quadriceps muscle mass. Mice were not boosted unless indicated otherwise. Non-lethal tail bleeds were carried out every 2 weeks until the experiments were terminated. Mice were euthanized by Halothane (MTC Pharmaceuticals) overdose.

ELISA. Immunol 2 microtitre plates (Dynatech Laboratories) were coated with purified recombinant truncated gD (Biostar) at a concentration of 0.050 µg per well. Coating antigen dilutions were done in ELISA coating buffer (0.012 M NaHCO, 0.038 M NaHCO, pH 9.6) and coating was allowed to proceed overnight at 4 °C. Plates were washed five times in PBS (0.137 M NaCl, 0.003 M KCl, 0.008 M NaHPO, 0.001 M NaHPO) with 0.05% Tween 20 (PBST) prior to addition of fourfold dilutions of mouse sera prepared in PBST with 0.5% gelatin (PBST-g). (Bio-Rad). After a 2 h incubation, plates were washed in PBST and an affinity-purified biotinylated goat anti-mouse Ig (Zymed Laboratories) diluted to 1/5000 in PBST-g was added to each plate. After an incubation of 60 min, plates were washed extensively and Streptavidin–alkaline phosphatase (Gibco Life Technologies), diluted to 1/2000 in PBST-g, was added to each plate. Following a 60 min
incubation, plates were washed six times in PBST. Prior to addition of substrate, the plates were washed two additional times in PBS. Development of plates involved the addition of 0·01 M p-nitrophenyl phosphate (Sigma) in substrate buffer [0·104 M diethanolamine (Sigma), 0·5 mM MgCl₂]. Antibody isotyping ELISAs were carried out in a similar fashion except that biotinylated goat anti-murine IgG₁, IgG₂a, IgG₂b, and IgG₃ were used at a dilution of 1/8000 and IgM (Caltag Laboratories) was used at a dilution of 1/1000. Incubation time with these antibodies was 60 min. Addition of Streptavidin–alkaline phosphatase and development of plates was as described for total IgG ELISA.

Spleen and lymph node cell harvests. Mouse splenocytes were prepared by mechanically grinding spleens through a sterile nylon mesh into a sterile Petri dish containing 10–20 ml RPMI 1640 (Sigma). This material was then pipetted into sterile 15 or 50 ml tubes and allowed to sit, on ice, for 10 min. Debris that settled to the bottom was aspirated. Cells were centrifuged at 250 g for 60 s. Lysis was terminated by addition of 14 ml RPMI 1640 at room temperature. Samples were pelleted by centrifugation (250 g, 10 min). Lysates were prepared by mechanically grinding spleens through a sterile nylon mesh in a sterile scalpel in a small volume of CMF solution [0·1 M Hank’s Balanced Salt Solution (HBSS), Ca²⁺- and Mg²⁺-free; 10 mM HEPES, pH 7·2; 25 mM NaHCO₃, pH 7·2; and 2% FBS] and then mixed with 10 ml digestion buffer (1 × HBSS, 10% FBS, 15 mM HEPES, pH 7·2 containing 150 U/ml collagenase (CLS 1; Worthington Biochemical Corporation), and 0·015 mg/ml DNase I (Pharmacia Biotech). Diced nodes in digestion buffer were transferred to 25 ml Erlenmeyer flasks, containing a sterile Teflon-coated stir bar, and incubated at 37 °C, with stirring, for 90 min. After antigen stimulation, cells were pelleted by centrifugation (250 g) and re-suspended in RPMI 1640. Splenocyte numbers were assayed by staining with Trypan blue (Gibco Life Technologies) and counting on a haemocytometer. Splenocytes were centrifuged (250 g) and re-suspended at a final concentration of 1 × 10⁷ cells/ml in RPMI 1640 supplemented with 10% foetal bovine serum (FBS) (Sigma), 100 U/ml penicillin, 100 µg/ml streptomycin (Sigma), 2 mM L-glutamine (Gibco Life Technologies), 1 mM sodium pyruvate (Gibco Life Technologies), 10 mM HEPES (Gibco Life Technologies) and 5 × 10⁻⁴ M 2-mercaptoethanol (Sigma) (complete RPMI-1640). A modification of a method for harvesting Peyer’s patch lymphocytes was utilized to prepare single cell suspensions from lymph nodes (Spalding, 1983). Briefly, iliac lymph nodes were collected into 15 ml tubes containing sterile chilled PBSA (Gibco Life Technologies). Pooled nodes were decanted into a sterile Petri dish and excess PBSA was discarded. Nodes were diced with a sterile scalpel in a small volume of CMF solution [0·1 × Hanks’ Balanced Salt Solution (HBSS), Ca²⁺- and Mg²⁺-free; 10 mM HEPES, pH 7·2; 25 mM NaHCO₃, pH 7·2; and 2% FBS] and then mixed with 10 ml digestion buffer (1 × HBSS, 10% FBS, 15 mM HEPES, pH 7·2) containing 150 U/ml collagenase (CLS 1; Worthington Biochemical Corporation), and 0·015 mg/ml DNase I (Pharmacia Biotech). Diced nodes in digestion buffer were transferred to 25 ml Erlenmeyer flasks, containing a sterile Teflon-coated stir bar, and incubated at 37 °C, with stirring, for 90 min. Samples were harvested into sterile 15 ml plastic tubes and allowed to sit for 10 min. Large pieces of undigested material were returned to the Erlenmeyer flask and incubated for an additional 30–60 min with 5 ml digestion buffer. This material was combined with the initial harvest and cells were centrifuged (250 g) to pellet. Digestion buffer was decanted and the cells were re-suspended in PBSA. Cells were counted using a haemocytometer and centrifuged (250 g) one final time. Cells were re-suspended to a final concentration of 1 × 10⁷ cells/ml.

Cytokine ELISPOT. A cytokine-specific ELISPOT assay was used as described previously (Czerkinsky et al., 1988; Baca-Estrada et al., 1996). Briefly, single cell suspensions isolated from spleens, lymph nodes or bone marrow were stimulated in vitro by incubating in complete RPMI 1640 at 37 °C and 5% CO₂ for 20 h in the presence of recombinant authentic gD (0·1 µg/ml). After antigen stimulation, cells were washed twice in complete RPMI 1640 and diluted to a concentration of 1 × 10⁶ cells/ml for use in the ELISPOT assay. Plates were developed by the addition of 50 µl substrates, BCP and NBT (Moss). Plates were allowed to develop at ambient temperature for 10–30 min, after which time, extensive washing in distilled water stopped reactions. After air drying, spots were counted under a dissecting microscope.

Statistical analysis. Data were entered into a database in the statistical analysis program Instat GraphPad. Differences in antibody titres among vaccine groups were investigated using the non-parametric Mann–Whitney U test.

Results
Humoral immunity following DNA immunization
We have previously demonstrated that naive C3H/HeN (H-2b) mice immunized with a DNA vaccine encoding BHV-1 gD mounted potent humoral and cell-mediated immune responses (Lewis et al., 1997). Data in naive mice immunized intramuscularly with pSLRSV.SgD were consistent with earlier work and showed 80% of animals seroconverting at 2 weeks and 90% seroconverting at 4 weeks after a single immunization. Fig. 1(a,b) demonstrates the extremely rapid development of active antibody titres in passively immunized mice after a single intramuscular injection with pSLRSV.SgD. This rapid development of active titres is evident at 2 weeks and 4 weeks post-immunization in the low (Fig. 1a) and high (Fig. 1b) dose passively immune mice, respectively. The development of active humoral immunity in passively immune mice receiving pSLRSV.SgD and the magnitude of mean serum antibody titres suggests that the presence of pre-existing passive polyclonal antibodies did not prevent the induction of active immune responses to a single immunization with a DNA vaccine.

Passive and active immunization in C57BL/6 mice
It has been known for some time that the genotype of an inbred strain of mouse can influence the immune response to a given antigen (Julia et al., 1996). We repeated the experiment summarized in Fig. 1(a,b) with C57BL/6 (H-2b) mice to determine if DNA-based vaccines could also circumvent pre-existing passive antibodies in C57BL/6 mice as demonstrated for C3H/HeN mice. C57BL/6 mice immunized one time with 100 µg pSLRSV.SgD, in the presence or absence of passive serum antibodies to BHV-1 gD, generated humoral immune responses similar in kinetics and magnitude to those observed in C3H/HeN mice (Fig. 2a,b). We included a conventional vaccine formulation (VSA3/tgD) in this experiment at a dose (0·1 µg per mouse) that primes splenic T-cells but does not induce detectable serum antibodies without boosting (Baca-Estrada et al., 1996). Immunization of naive C57BL/6 mice with VSA3/tgD gave results consistent with previous data (Fig. 2a). However, immunization of passively immune C57BL/6 mice with a suboptimal dose of VSA3/tgD resulted in four out of eight immunized animals developing moderate to high serum antibody levels (Fig. 2c). This data strongly suggests that the hyperimmune serum utilized to passively immunize
6- to 7-week-old C57BL/6 mice enhanced, rather than suppressed, the development of humoral immunity to this subunit vaccine formulation. Splenic cytokine profiles showed that recall responses in mice immunized with pSLRSV.SgD were characteristic of a Th1 type of immunity regardless of the passive immune status of the mice (Fig. 3 a). Immunization of naive and passively immune mice with VSA3/rtgD resulted in a profound reduction in the numbers of splenic IFN-γ-secreting cells relative to mice receiving pSLRSV.SgD (Fig. 3 n). Cytokine profiles for naive mice receiving pSLRSV.SgD and VSA3/rtgD are consistent with earlier experiments (Baca-Estrada et al.,

Fig. 1. Effect of passive immunity on induction of active responses by DNA immunization. Hyperimmune serum for passive transfer to recipient mice was prepared as described in Methods. Hyperimmune serum was injected into the intraperitoneal cavity on day −2 and recipient serum IgG titres were read by ELISA on day −1. Mean serum antibody levels for mice vaccinated with pSLRSV.SgD (○), or low and high doses of hyperimmune antiserum followed by pSLRSV.SgD (△) are depicted. Passive serum antibody decay rates (□) are shown for low (a) and high (b) dose hyperimmune antisera. All experimental groups contained 10 mice. Data are presented as the geometric mean and standard error of the mean for all mice in each group. ELISA values are depicted as $A_{405}$ at a 1/200 serum dilution.

Fig. 2. Effect of passive immunity on the development of immunity in mice. (a) Seronegative C57BL/6 mice were immunized (quadriceps) with 50 µg pSLRSV.SgD (○), 100 µg recombinant produced gD in VSA3 adjuvant (▽) or passively administered mouse anti-gD antibody (□). Normal decay of passively administered antibody and the development of active immunity in seronegative animals administered a DNA or protein vaccine are shown. (b) Immune responses in individual passively immunized mice given a DNA vaccine pSLRSV.SgD 48 h after intraperitoneal injection with antibody. (c) Immune response in individual passively immunized mice given a recombinant passive vaccine (VSA3/rtgD) 48 h after intraperitoneal injection with antibody. In (b) and (c), each symbol represents the results from an individual mouse.
BHV-1 gD DNA vaccination of passively immune mice

Fig. 3. Effect of passive immunity on cytokine and antibody secretion profiles following immunization with pSLRSV.SgD (designated pRSV.SgD in the figure) or VSA3/rtgD. Pooled cells were prepared from spleens (a) and retroperitoneal iliac lymph nodes (b) harvested at 7 weeks post-immunization from seropositive C57BL/6 mice. The figure shows levels of IL-4 (hatched bars) and IFN-γ (white bars) secreting cells per \(1 \times 10^6\) cells following 20 h in vitro re-stimulation with authentic full-length gD. Cytokine ELISPOT results are expressed as the mean of triplicate wells from pooled cells from ten (control passive hyperimmune group, Pass. Ab), nine (pSLRSV.SgD), seven (pSLRSV.SgD + Pass. Ab), five (VSA3/rtgD) and four (VSA3/rtgD + Pass. Ab) mice from each group.

Fig. 4. Immunoglobulin isotype profile of pooled donor hyperimmune antisera. Levels of gD-specific IgM, IgG₁, IgG₂a, IgG₂b and IgG₃ were measured as described in Methods. Values are represented as the reciprocals of the highest dilution resulting in values three standard deviations above background.

Fig. 5. Effect of MAbs on the development of immunity following DNA immunization

Groups of C3H/HeN mice were passively immunized with anti-gD MAbs of different (IgG₁ or IgG₂a) isotypes. Fig. 5 (a, b)
immunization with pSLRSV.SgD are shown in (Fig. 5b) following a single immunization with pSLRSV.SgD. The most striking data obtained was clear and significant (P<0.05) evidence that the isotype of the passive antibody has a direct impact on the induction of active humoral immunity following immunization with a DNA-based vaccine. Pre-treatment of C3H/HeN mice with MAb PB136 (IgG1) suppressed the development of active humoral immunity while pre-treatment with MAb 1G6 (IgG2a) augmented the magnitude and seroconversion efficacy of the humoral immune response. Although individual mouse data are not shown, we were able to demonstrate that 2 weeks after DNA immunization six out of eight mice developed active titres when the pre-existing passively administered anti-gD antibody was a MAb of the IgG2a isotype and that only one out of eight mice immunized with pSLRSV.SgD in the face of pre-existing titres of PB136 (IgG1) seroconverted during the same time period. Furthermore, a mixture of three IgG1 MAbs (PB136, 3D9 and 2C8) profoundly suppressed the development of active humoral immunity to an even greater extent than PB136 alone (Fig. 5a). Indeed, only two out of eight mice from this group seroconverted during the first 8 weeks of the experimental period with only one maintaining measurable active titres at 10 weeks (data not shown).

**Discussion**

In this study, we have evaluated the ability of pSLRSV.SgD, a DNA-based vaccine encoding a secreted version of BHV-1 gD, to elicit active immunity in passively immune C3H/HeN or C57BL/6 mice. Potent active humoral immunity was evident within 2-4 weeks of immunization regardless of the mouse strain or the passive immune status of injected mice (Fig. 1a, b).

At least two recent articles describe the use of DNA-based vaccines to circumvent maternal antibody suppression (Monteil *et al*., 1996; Hassett *et al*., 1997). Monteil *et al*., (1996) utilized 1-day-old piglets from immune and non-immune dams and demonstrated that following intramuscular injection with 400 µg plasmid encoding pseudorabies virus gD, piglets from immune dams did not develop serum antibodies or demonstrate any evidence of priming. Furthermore, piglets from non-immune dams developed moderate serum antibody titres only following a boost at 6 weeks post-partum. Hassett *et al*. (1997) immunized murine neonates from immune and non-immune dams intramuscularly with 50 µg plasmid encoding the nucleoprotein (NP) from lymphochoriomeningitis virus (LCMV). These authors demonstrated that approximately 50% of neonates from immune and non-immune dams developed MHC-restricted, NP-specific CTLs more rapidly than unprimed animals following sublethal challenge. Further, approximately 95% of these immune neonates showed reductions in splenic virus titres following challenge. These authors argued that maternal antibodies did not interfere with the development of NP-specific CTL responses because LCMV NP is an intracellular protein that would not come into direct contact with serum antibody. These authors did not determine if humoral immunity was induced in this model because LCMV-specific CTL have been demonstrated to be adequate and sufficient to confer protection against virus challenge. This particular study is compelling because it suggests that compartmentalization of the antigen may serve to circumvent some of the problems associated with maternal antibody suppression of immunity. However, this approach would be
restricted to pathogens that can be reliably and completely cleared with CTL responses alone (Zinkernagel, 1996). Neither Hassett et al. (1997) nor Monteil et al. (1996) included a conventional vaccine control, such as modified live LCMV or NP formulated with such adjuvants as ISCOMs or liposomes which are known to facilitate potent CTL responses (Cox & Coulter, 1997). Comparisons between conventional vaccine formulations and DNA-based vaccines would provide considerable insight into the efficacy of these novel vaccines in neonates. Several other publications describe the impact of DNA-based vaccines on neonatal immune responses (Bot et al., 1996; Mor et al., 1996; Wang et al., 1997). While two of these publications describe the successful development of T-cell responses in neonatal mice, they did not address the influence of maternal antibody on the development of active humoral immunity or the mechanism whereby passive antibody suppresses active B-cell responses to antigen.

However, a few studies have recently demonstrated the development of T-cell responses in neonatal mice, they did not address the influence of maternal antibody on the development of active humoral immunity or the mechanism whereby passive antibody suppresses active B-cell responses to antigen. The specific mechanism for the B-cell failure to respond to antigen in the presence of antigen-specific maternal or passive antibody in neonates is unknown. However, recent evidence suggests that simultaneous signalling through the B-cell receptor and the low affinity FcγRIIB1 receptor on naive murine B-cells results in a negative signal delivered to the potential antigen specific B-cells that prevents not only secretion of antigen specific IgM but also inhibits the maturation of these B-cells into IgG-secreting plasma cells and presumably inhibits the development of a memory B-cell population (Phillips & Parker, 1983). Our initial premise was that expression of plasmid-encoded antigen from in vivo-transfected cells would persist beyond the point where passive serum antibodies wanted to non-inhibitory levels and thereby lead to induction of immunity. However, the rapid development of active humoral immunity described by our data suggests that longevity of antigen expression following DNA immunization is not an issue in this model. Figs 1(a, b) and 2(b) clearly demonstrate that C3H/HeN and C57BL/6 mice immunized with pSLRSV.SgD develop active humoral immunity regardless of the presence of high or low doses of passive hyperimmune sera. There are a number of possible explanations for this occurrence. Recent evidence suggests that the efficacy of DNA-based vaccines may stem from the proximity of antigen-presenting cells (bearing injected plasmid sequences encoding antigen) and/or antigen expression to naive T- and B-cell populations (Steinman et al., 1997; Tew et al., 1997). This proximity is suggested by several recent studies implying that local and possibly lymph node resident dendritic cells may be of pivotal importance in the efficient development of ensuing immune responses (Pertmer et al., 1996; Condon et al., 1996; Manickan et al., 1997; Roman et al., 1997). Also, it has been demonstrated that passive titres of IgM actually enhance the development of humoral immunity following vaccination (Henry & Jerne, 1968).

Further support for the postulated involvement of IgM in enhancement of humoral immunity comes from studies showing that a syngeneic idiotypic presented in the context of IgM is highly immunogenic whereas presentation of the same idiotypic in the context of IgG isotypes, IgG1, IgG2a and IgG3 leads to antigen-specific tolerance (Reitan & Hannestad, 1995). There is additional evidence that complement fixation by the polymeric IgM may also be involved in the apparent ability of antigen-specific IgM to enhance humoral responses to immunization (Heyman et al., 1988). Indeed, mutation of Ser406 within the third constant domain of IgM ablates the complement fixation capacity and corresponds to a significant reduction in the ability of IgM:antigen complexes to facilitate thymus-dependent humoral immune responses (Shulman et al., 1986; Heyman et al., 1988). The specific mechanism by which IgM drives the development of T-dependent B-cell responses appears to involve direction of the antigen-IgM:C3d (iC3d) complexes to CD21 receptors on follicular dendritic cells and B-cells (up to the centrocyte stage) for subsequent presentation to primed CD4+ T-cells.

Despite the evidence for the potential roles of IgM and complement in circumvention of passive antibody mediated B-cell suppression, we feel that contribution of passive IgM to the development of active humoral immunity is minimal. Fig. 4 shows that there were low titres of IgM in donor sera utilized in these experiments. Furthermore, a 3–5 h serum half-life for this isotype would effectively minimize the amount of available IgM present at the time of immunization (Vieira & Rajewsky, 1988). Conversely, the somewhat higher levels of gD-specific IgG2a in donor sera, in conjunction with a significantly longer serum half-life, could potentially facilitate circumvention of suppressive pre-existing passive titres. The fact that the hyperimmune sera contained significant levels of IgG1, but still did not inhibit the immune response, may be explained by the significant amounts of IgG2a in the hyperimmune sera which may act to counter the suppressive effects of IgG1. Certainly there is evidence suggesting that IgG2a possesses a much greater capacity to fix complement than IgG1 (Klaus et al., 1979; Ishizaka et al., 1995). Indeed, Fig. 5(a, b) clearly demonstrates that MAb 1G6 (IgG2a) does not suppress the development of active humoral immunity while MAb's PB136, 3D9 and 2C8 (IgG1) do. However, there is in vivo data, based on an idiotype switch variant model, which demonstrate that IgG2a, IgG1, and IgG2b failed to enhance the immunogenicity of the presented idiotype (Reitan & Hannestad, 1995). This work suggests that it may not be the complement fixation capacity of IgG2a that is responsible for the augmented humoral immunity observed in Fig. 5(b). It has been suggested that affinity differences in passive antibodies may have a significant impact on the immune outcome following immunization (Keller et al., 1991).
While a plausible mechanism for how differences in passive antibody affinity can determine outcome to immunization is unknown, it has been documented that high affinity MAbs generally, but not exclusively, suppress the development of humoral immunity while lower affinity MAbs have little or no effect (Keller et al., 1991). Affinity differences between IgG1 and IgG2a antibodies described in Fig. 5 (a, b) may also play a role in modulating the immune response. However, the polyclonality of maternal derived antibodies, in the context of maternal immunization regimes of pathogen exposure, make affinity contribution to inhibition or enhancement of responses to neonatal vaccination difficult to accept. We suggest an alternative explanation which involves differences in antibody Fc affinity for the high affinity FcRI (CD84) receptor present on blood-borne professional antigen-presenting cells (Tew et al., 1997). Fc receptor distribution is well documented and there is evidence that targeting to the high affinity receptors on non-phagocytic (maturing) dendritic-like cells by monomeric antibody:antigen complexes can circumvent the ‘normal’ B-cell downregulation upon exposure to antibody:antigen complexes possibly by limiting access of free antibody:antigen complexes to the lower affinity FcRlIBI receptor on naive B-cells (Ravetch & Kinet, 1991; Van de Winkel & Capel, 1993; Fanger et al., 1996; Ravetch, 1997; Tew et al., 1997). There is also evidence suggesting that the affinity of FcRI for IgG2a and IgG1 is much greater than for IgG1 or IgG2b (Van de Winkel & Capel, 1993). These affinity differences might explain the ability of MAb 1G6 (IgG2a) to enhance humoral immunity while MAbs PB136, 3D9 and 2C8 (IgG1) do not.

In summary, our data suggest that mice immunized once with a plasmid encoding a secreted form of BHV-1 gD were able to mount both humoral and cell-mediated immune responses in the face of pre-existing passive antibodies.

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