Veterinary Immunology and Immunopathology 159 (2014) 58–73

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Generation by self re-fusion of bovine^3 × murine^2 heterohybridomas secreting virus-neutralizing bovine monoclonal antibodies to bovine herpesvirus 1 glycoproteins gB, gC, and gD

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ARTICLE INFO

Article history:
Received 2 May 2013
Received in revised form
26 December 2013
Accepted 7 February 2014

Keywords:
Heterohybridoma
Bovine monoclonal antibody
Bovine herpesvirus 1
Virus glycoprotein
Virus neutralization
Passive immunity

ABSTRACT

Seventy-eight heterohybridomas (HH) stably secreting bovine monoclonal antibodies (BomAb) to *Bovine herpesvirus 1* (BHV1) were produced by fusing lymph node cells from a BHV1 hyperimmunized calf with 3 types of non-secreting fusion partners. Seven were generated through fusion with the murine × murine (murine^2) hybridoma SP2/0, 3 through fusion with bovine–murine^2 HH previously generated using cells from the same calf, and 68 through fusion with bovine^2-murine^2 HH previously generated by sequential fusions using cells from the same calf. The chromosome number of example HH increased with increasing numbers of input fusions. A variety of indirect fluorescent antibody assay patterns was observed using the BomAb, suggesting diverse antigen specificity. Three bovine^3–murine^2 HH secreted IgG1 BomAb neutralizing BHV1 without complement, and were chosen for further characterization. SDS-PAGE of detergent-solubilized BHV1 proteins bound to the 3 neutralizing BomAb demonstrated their individual specificities for BHV1 envelope glycoproteins gB, gC, and gD, the major neutralization targets for BHV1. The 3 HH stably secreted the BomAb in culture for over one year, and pilot-scale production of the BomAb was accomplished by *in vivo* and *in vitro* methods. A cocktail of the 3 BomAb was administered intravenously (i.v.) to a 6-month-old calf and its serum neutralization activity decreased with a half-life consistent with non-immune clearance, suggesting that BomAb may be useful for passive immune treatment of disease in cattle. Rabbits were passively protected by i.v. injection with each of the anti-gB and anti-gD BomAb when challenged i.v. with

Abbreviations: AS, antiserum; b, bovine origin; BHV1, bovine herpesvirus 1; BomAb, bovine monoclonal antibody; Chr, chromosome; dpc, days post challenge; dpi, days post inoculation; gB, BHV1 glycoprotein B; gC, BHV1 glycoprotein C; gD, BHV1 glycoprotein D; gp, glycoprotein; HH, heterohybridoma; IFA, indirect fluorescent antibody; LC, lymphocyte; LN, lymph node; m, murine origin; MumAb, murine monoclonal antibody; MW, molecular weight; VN, virus neutralization.

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http://dx.doi.org/10.1016/j.vetimm.2014.02.004
0165-2427/Published by Elsevier B.V.

BHV1 24 h later. Self re-fusion was shown to be advantageous for efficiently producing HH stably secreting host monoclonal antibodies. The BomAb described should prove useful in studies of the host immune response to BHV1, as reagents, and as sources of bovine immunoglobulin sequences.

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1. Introduction

Bovine herpesvirus 1 (BHV1) causes diseases of global economic significance in cattle and is the subject of national control and eradication programs (reviewed in Levings and Roth, 2013a). BHV1 is a member of the family Herpesviridae, subfamily Alphaherpesvirinae, genus Varicellovirus (Davison, 2010).

Laboratory rabbits have been infected with BHV1 by a variety of routes, resulting in lesions and disease that were often route-specific (Bindrich, 1960; Armstrong et al., 1961; Persechino et al., 1965; Bwangamoi and Kaminjolo, 1973; Kelly, 1977; Lupton et al., 1980; Rock and Reed, 1982; Brown and Field, 1990). Rabbit infection and disease systems (including those in neonatal rabbits) have been suggested as models for bovine systems, such as vaccine efficacy evaluation (Kelly, 1977; Lupton et al., 1980; Rock and Reed, 1982; Valera et al., 2008).

Alphaherpesvirus envelope glycoproteins (gp) function in the virion envelope's attachment to and fusion with the host cell membrane, and are the targets of protective cellular and humoral host immune responses (reviewed in Levings and Roth, 2013b). BHV1 gps B, C, and D (gB, gC, and gD) are the primary inducers and targets of virus neutralizing (VN+) Ab (Collins et al., 1985; van Drunen Littel-van den Hurk and Babiuk, 1986; Turin et al., 1999) and gB, gC, and gD subunit vaccines were each protective (Babiuk et al., 1987). BHV1 gp epitopes have primarily been characterized using murine mAb (MumAb) (Collins et al., 1984; van Drunen Littel-van den Hurk and Babiuk, 1985; Marshall et al., 1988; Hughes et al., 1988; Ayers et al., 1989).

Host species mAb may offer advantages over MumAb in identifying epitopes important in natural infection (Trkola et al., 1996), including protective and immunodominant (Perryman et al., 1990) epitopes, useful for vaccine and diagnostic assay design. They may also be useful for passive protection (Sandborn et al., 2012), and as diagnostic or research reagents. Human mAb have been generated using human-human hybridomas, human-mouse heterohybridomas (HH) and in a variety of other ways (reviewed in Wang, 2011; Sullivan et al., 2011), but the methods other than HH have not generally been available for or employed in other species. In addition, some alternative techniques do not generate mAb representative of the immune response, or may result in mAb limited in quantity or restricted in Ig class (Jessup et al., 2000). Transforming lymphocytes (LC) with species-specific viruses (Epstein-Barr virus for humans, bovine leukemia virus for cattle) is one example where an alternative strategy has been useful for non-murine, non-human species, although the transformed bovine LC usually produce bovine IgM mAb.

HH, also called heteromyelomas, inter-species hybridomas, or xenohybridomas have been and continue to be successfully employed as a source of host mAb for a variety of non-murine laboratory animal (Raman et al., 1994; Ukaji et al., 2011) and veterinary (Flynn et al., 1989; Greenlee et al., 1990; Keggan, 2013) species, including cattle (reviewed in Groves and Tucker, 1989; Groves and Morris, 2000).

Fusions between bovine LC (b) and murine myelomas (m) or murine hybridomas (m^2) have produced HH stably secreting bovine Ig of unknown specificity (Srikumaran et al., 1983, 1984), Ig specific for the non-infectious agent immunogen (Anderson et al., 1986), and Ig specific for the infectious agent immunogen (Raybould et al., 1985; Guidry et al., 1986; Anderson et al., 1987; Kennedy et al., 1988). Srikumaran et al. (1990) produced $b \times m^2$ HH secreting non-neutralizing bovine mAb (BomAb) specific for BHV1 gC. Fusions of non-murine LC with murine myeloma cells have often resulted in loss of secretion as a result of rapid chromosome (Chr) loss. The best studied HH, human-murine HH, lose human Chr preferentially (Crocem et al., 1980; Wollweber et al., 2000). 'Re-fusion' or the use of HH as fusion partners yielded better results in a variety of species (Yang et al., 1990; Wang, 2011), including in cattle. It has been used to increase the number of non-murine Chr in product HH, increasing the likelihood of retaining Chr coding for Ig (Crocem et al., 1980), those key to survival (Wang et al., 1998), and those associated with Ig production and secretion but not coding for Ig (Raison et al., 1982). In one report (Tucker et al., 1984) the re-fused lines carried two to three times the number of non-murine (bovine) Chr as the single-fused HH.

Both $b \times m^x$ (Tucker et al., 1984; Anderson et al., 1986, 1987; Groves et al., 1987; Kennedy et al., 1988; Kemp et al., 1990) and $b^2 \times m^x$ aminopterin-sensitive HH (Anderson et al., 1986, 1987; Tucker et al., 1987; Groves et al., 1988) have been used for re-fusion with bovine LC. In some cases the selected HH fusion partners themselves secreted Ig, but in most cases clones not secreting Ig were chosen for use. In each case the fusion partners and LC were derived from different individuals of the same outbred species.

The aims of this study were: (1) to examine the effect on HH generation rates of increasing degrees of re-fusion with LC from the same individual and stimulated with the same agent; (2) to exploit the increased rate of productive HH generation after re-fusion to derive HH producing VN+ BomAb to BHV1 envelope gp; and (3) to conduct basic characterization of the VN+ BomAb *in vitro* and *in vivo*.