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Molecular mimicry: a herpes virus glycoprotein antigenically related to a cell-surface glycoprotein expressed by macrophages, polymorphonuclear leucocytes, and platelets

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SUMMARY

Bovine herpes virus type 1 (BHV-1) gIII is a major virion glycoprotein with homology to the immunoglobulin superfamily. We have investigated the possibility that gIII is related to host molecules and have identified a gIII-specific monoclonal antibody (mAb) that cross-reacts with normal bovine cells. The cross-reactive entity was expressed mainly on monocyte/macrophages (Mϕ), polymorphonuclear leucocytes (PMN) and platelets, and was identified as a 43,000–63,000 molecular weight (MW) cell-surface glycoprotein. For Mϕ, the glycoprotein appears to be a general lineage marker, rather than a maturation or activation marker, and may be a functional receptor, as evidenced by its endocytosis via coated pits and its involvement in proliferation of mononuclear cells *in vitro*. This novel leucocyte marker was also detected on subsets of human, ovine and canine Mϕ. Competitive binding assays with sera from cattle immunized with BHV-1 or gIII revealed apparent low responsiveness to the cross-reactive epitope. The results suggest that BHV-1 gIII is antigenically related to a novel host leucocyte receptor and that evasion and/or interference with leucocyte function may be a consequence of this molecular mimicry relationship.

INTRODUCTION

Molecular mimicry refers to the existence of homologous features in molecules of both a pathogen and its host, as demonstrated by antigenic cross-reactivity and/or homologous functions (Damian, 1989). Both antigenic and functional molecular mimicry have been described for herpes viruses, e.g. (i) antigenic cross-reactivity of a non-structural herpes simplex virus type-1 protein and keratin (Oldstone & Notkins, 1986), (ii) antigenic cross-reactivity of a human cytomegalovirus immediate early protein and a class II major histocompatibility complex (MHC) antigen (Fujinami *et al.*, 1988), and (iii) functional receptor-binding mimicry between Epstein–Barr virus gp350 and complement component C3d (Nemerow *et al.*, 1989). Antigenic mimicry may affect immune responses, while functional mimicry may affect the tissue tropism and/or pathogenesis of infections (Damian, 1989).

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Abbreviations: BFB, bovine fibroblast; BHV-1, bovine herpes virus type 1; FBS, fetal bovine serum; GBK, Georgia bovine kidney; Ig, immunoglobulin; mAb, monoclonal antibody; MDBK, Madin–Darby bovine kidney; Mϕ, monocytes/macrophages; PBMC, peripheral blood mononuclear cells; PMN, polymorphonuclear leucocytes.

Bovine herpes virus type 1 (BHV-1) is associated with 'shipping fever' pneumonia of cattle in which BHV-1 is thought to play an immunosuppressive role (Babiuk, Lawman & Bielefeldt Ohmann, 1988). The pathogenesis of BHV-1 infection is not well understood; however, it has been reported that the major BHV-1 glycoproteins are involved in the initial attachment, penetration and spread of the virus (Fitzpatrick, Zamb & Babiuk, 1990b; Okazaki *et al.*, 1986), and that BHV-1 can interact with and produce functional abnormalities in bovine polymorphonuclear leucocytes (PMN), monocyte/macrophages (Mϕ) and T lymphocytes (Bielefeldt Ohmann & Babiuk, 1985; Forman & Babiuk, 1982; Griebel *et al.*, 1990). Whether BHV-1 glycoproteins mediate the interactions with leucocytes is at present unknown.

We have recently described the sequence of a major BHV-1 glycoprotein, gIII, which is related to the immunoglobulin (Ig) superfamily (Fitzpatrick, Babiuk & Zamb, 1989). We have therefore investigated the possibility that molecular mimicry, mediated by gIII, may be involved in the interactions of BHV-1 and the bovine immune system. In this report, we describe a gIII-specific monoclonal antibody (mAb) that cross-reacts with a 43,000–63,000 molecular weight (MW) cell-surface glycoprotein expressed in normal bovine cells—predominantly Mϕ, PMN and platelets. The glycoprotein was also detected on subsets of human, ovine and canine Mϕ and may be a novel

leucocyte marker with functional properties. BHV-1-immune cattle demonstrated apparent low-responsiveness to the cross-reactivie gIII epitope. Thus, immunological evasion and/or interference may ensue from this molecular mimicry relationship.

MATERIALS AND METHODS

Cells and tissues

Madin–Darby bovine kidney (MDBK), Georgia bovine kidney (GBK), bovine fibroblast (BFB), BS-C-1, MA-104 and 3T3 cells (all originally from ATCC) were cultured in Eagles minimal essential medium supplemented with 10% fetal bovine serum (FBS). Murine LMTK− cells were cultured in Dulbeccos-modified Eagles medium supplemented with 5% FBS. Peripheral blood mononuclear cells (PBMC), from various species, were isolated by Ficoll–Hypaque (Pharmacia, Dorval, Quebec, Canada) centrifugation of buffy coat cells (Bielefeldt Ohmann & Babiuk, 1985). Bovine alveolar Mϕ were obtained by bronchoalveolar lavage of sedated calves, aged 4–12 months (Bielefeldt Ohmann & Babiuk, 1986a). Enriched populations of blood Mϕ were obtained by incubating PBMC on serum-coated, glass or plastic surface for 2–24 hr, after which non-adherent cells were washed off (Bielefeldt Ohmann *et al.*, 1988b). PMN were prepared from blood samples, after removal of the buffy coat and hypotonic lysis of erythrocytes (Bielefeldt Ohmann & Babiuk, 1985). Normal bovine tissues were obtained from 4–12-month-old calves and from foetuses (Bielefeldt Ohmann *et al.*, 1988b). Cell lines and Mϕ cultures were stimulated with recombinant bovine interferon-gamma (IFN-γ) or tumour necrosis factor-alpha (TNF-α), described in detail elsewhere (Bielefeldt Ohmann *et al.*, 1988a,b). Lymphocyte proliferation studies used PBMC in a tritiated thymidine-based blastogenesis assay (Bielefeldt Ohmann & Babiuk, 1985).

Virus and antibodies

BHV-1 strain P8-2 was grown in MDBK cells and purified on potassium tartrate gradients (Misra, Blumenthal & Babiuk, 1981). BHV-1 gIII-specific mouse mAb, polyclonal rabbit and polyclonal bovine antisera were generated and characterized, as described previously (Babiuk *et al.*, 1987; Van Drunen Littel-van den Hurk & Babiuk, 1985; Van Drunen Littel-van den Hurk *et al.*, 1984). The mAb were purified by caprylic acid and ammonium sulphate precipitation (McKinney & Parkinson, 1987). Bovine leucocyte-specific mAb (Davis *et al.*, 1987) were obtained from Dr W. Davis (Washington State University, Pullman, WA). Other mAb and antisera were obtained from Dimension Laboratories (Mississauga, Ontario, Canada). Table 1 shows the properties of the mAb used.

Immunocytochemistry and flow cytometry

Cell lines were stained for mAb reactivity by an avidin-biotin-enhanced immunoperoxidase method (Fitzpatrick *et al.*, 1988). Cytospin preparations of leucocytes and cryostat sections of tissues were stained for mAb reactivity by an alkaline phosphatase–anti-alkaline phosphatase method (Bielefeldt Ohmann *et al.*, 1988b). For flow cytometry, trypsinized cultured cells or purified leucocytes were stained with mAb and secondary fluorescein isothiocyanate- and/or *R*-phycoerythrin–conjugates (Fitzpatrick *et al.*, 1990b). For each sample, fluorescence and light scatter data for 20,000 cells were collected and analysed with a Coulter Epics C flow cytometer.

Radiolabelling of cells and immunoprecipitation

MDBK cells, enriched bovine Mϕ and BHV-1-infected cells were labelled with ^{35}S-methionine, lysed by detergent treatment and sonication, immunoprecipitated with mAb, separated by SDS–PAGE and fluorographed (Fitzpatrick *et al.*, 1988). Tritiated-glucosamine labelling and pulse–chase studies were performed as described by Van Drunen Littel-van den Hurk & Babiuk (1986).

Immunoelectron microscopy

Cells were labelled in suspension either by direct labelling, using mAb 1C11 conjugated to 10 nm gold particles, or by indirect labelling using unconjugated mAb 1C11 followed by rabbit anti-mouse IgG conjugated to 8 nm gold particles (Bielefeldt Ohmann *et al.*, 1988b, 1990). Specificity controls included (i) unlabelled 1C11, (ii) unlabelled, irrelevant, isotype-matched

Table 1. Properties of the BHV-1-specific or bovine leucocyte antigen-specific mAb used

mAb	Isotype	Specificity	References
1C11	IgG2a	BHV-1 gIII (epitope Ib) and a normal bovine cell surface glycoprotein	Fitzpatrick *et al.* (1990a) Van Drunen Littel-van den Hurk *et al.* (1984) This paper
1D6	IgG2a	BHV-1 gIII (epitope VI)	Van Drunen Littel-van den Hurk *et al.* (1984)
EBM11	IgG1	Intracellular Mϕ protein (pan-lineage for human Mϕ; maturation marker for bovine Mϕ)	Bielefeldt Ohmann *et al.* (1988b) Knapp *et al.* (1989)
MY4	IgG1	CD14 (pan-lineage for human Mϕ; subpopulation-specific for bovine Mϕ)	Goyert *et al.* (1986) Knapp *et al.* (1989) This paper
DH59B	IgG1	Myeloid cell-surface antigen (pan-lineage for bovine Mϕ)	Bielefeldt Ohmann *et al.* (1988b) Davis *et al.* (1987)
TH14B	IgG2a	Class II MHC antigens (DR)	Davis *et al.* (1987)
TH81A	IgG2a	Class II MHC antigens (DQ)	Davis *et al.* (1987)
H42A	IgG2a	Class II MHC antigens (DP)	Davis *et al.* (1987)

mAb, and (iii) gold-labelled, irrelevant mAb–which were used in blocking and/or direct binding assays. Surface expression and internalization studies were conducted as described in detail previously (Bielefeldt Ohmann *et al.*, 1990). Labelled cells were fixed, post-fixed, embedded, thin-sectioned and examined in a Philips transmission electron microscope at 60 KV.

Competitive binding ELISA

Immulon II (Dynatech, Alexandria, PA) plates were coated with 250 ng purified BHV-1 per well by incubation overnight at 4°, washed four times with phosphate-buffered saline (PBS), and blocked by addition of 3% normal goat serum in PBS for 2 hr at 22° (Fitzpatrick *et al.*, 1990a). After decanting the blocking solution, dilutions of BHV-1-specific or gIII-specific bovine antisera were applied for 1 hr at 22°. The bovine antisera were then decanted and various dilutions of mAb were applied for 1 hr at 22°. The plates were then washed as above and biotinylated goat anti-mouse Ig was applied for 1 hr at 22°. Finally, the plates were washed and a streptavidin–horseradish-peroxidase conjugate was applied for 1 hr at 22°. The substrate development and plate reading were conducted as described elsewhere (Fitzpatrick *et al.*, 1988).

RESULTS

Reactivity of mAb 1C11 with bovine cell lines *in vitro*

BHV-1 gIII possesses a 90 amino acid domain with high homology to class II MHC antigen constant domains (Fitzpatrick *et al.*, 1989). We therefore screened a panel of gIII-specific mAb for reactivity with a cloned MDBK cell line which constitutively expresses class II MHC antigens (D. Fitzpatrick, unpublished results). One gIII-specific mAb, 1C11, reacted strongly with this cell line (Fig. 1). Examination of other cell lines revealed that bovine GBK and BFB cells exhibited similar reactivities, whereas primate MA-104 and BS-C-1 cells, and murine 3T3 and LMTK − cells, did not (data not shown). Since GBK and BFB cells do not normally express class II MHC antigens (Bielefeldt Ohmann *et al.*, 1988a), the results implied that the entity reacting with mAb 1C11 was not related to class II MHC antigens. This was verified by analysis of GBK and BFB cells pre- and post-treatment with recombinant bovine IFN-γ (Bielefeldt Ohmann *et al.*, 1988a), which induced expression of class II MHC antigens but not the 1C11 antigen(s) (data not shown).

Verification of the clonality of mAb 1C11

At this point, the dual specificity of mAb 1C11 was verified by limiting dilution of the original hybridoma (Van Drunen Littel-van den Hurk *et al.*, 1984) and testing the subclones for reactivity with BHV-1 and normal MDBK cells. All 31 subclones tested demonstrated the cross-reactivity described above and the same isotype as the original mAb (data not shown).

Reactivity of mAb 1C11 with normal and foetal bovine tissues

The *in vivo* distribution of the 1C11 antigen(s) was examined in a range of tissues from healthy calves and foetuses (Table 2, Fig. 2). In calves, mAb 1C11 specifically labelled cells of the Mϕ

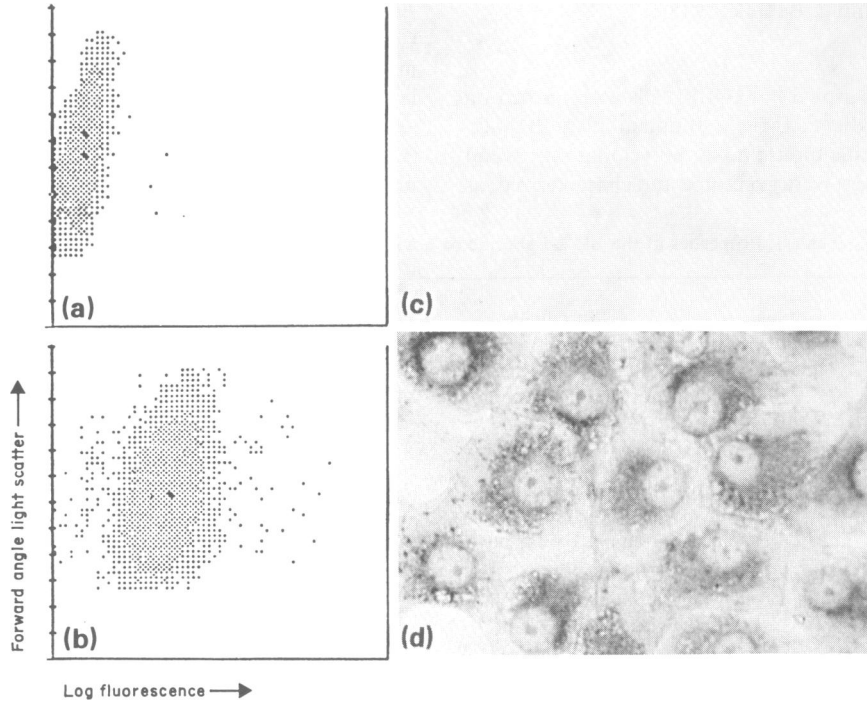

Figure 1. Flow cytometric and immunocytochemical reactivity of mAb 1C11 with a bovine MDBK cell line. (a) Flow cytometry of unfixed, non-permeabilized MDBK cells stained with an irrelevant mAb (1D6). (b) Flow cytometry of unfixed, non-permeabilized MDBK cells stained with mAb 1C11. (c) Immunocytochemistry of methanol-fixed and permeabilized MDBK cells stained with an irrelevant mAb (1D6). (d) Immunocytochemistry of methanol-fixed and permeabilized MDBK cells stained with mAb 1C11.

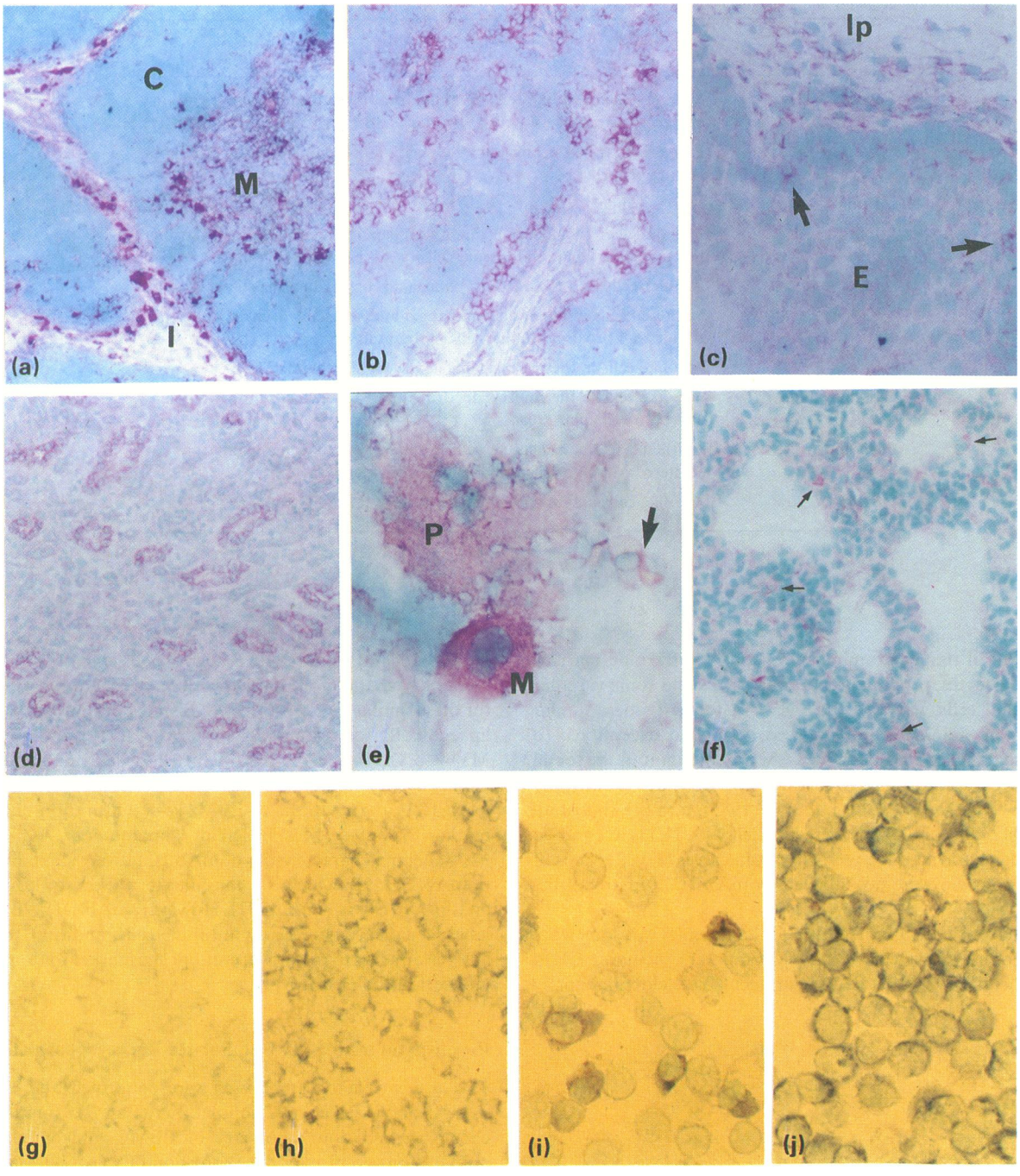

Figure 2. Immunohistochemistry of normal bovine tissues, from 4–12-month-old calves, and isolated bovine leucocytes stained with mAb 1C11 using an alkaline phosphatase–anti-alkaline phosphatase method. (a) Thymus: 1C11-positive cells are localized in the medulla (M) and interlobular interstitia (I) but not the cortex (C) (magnification × 105). (b) Lymph node (medullary region): 1C11-positive cells occur in the sinuses (magnification × 105). (c) Rumen: Mϕ in the lamina propria (lp) and Langerhan's cells (arrowed) in the epithelium (E) stain for the 1C11 antigen (magnification × 280). (d) Kidney: 1C11-positive renal tubule cells (magnification × 210). (e) Bone marrow (cytospin preparation of biopsy): 1C11-positive platelets (P), megakaryocyte (M) and Mϕ (arrow) are indicated (magnification × 336). (f) Lung (slightly collapsed due to euthanasia method): 1C11-positive Mϕ in the alveolar septae are arrowed (magnification × 210). (g) Purified blood PMN stained with a control mAb (EBM11) (magnification × 420). (h) Purified blood PMN stained with mAb 1C11) (magnification × 420). (i) Purified alveolar Mϕ stained for CD14 (magnification × 420). (j) Purified alveolar Mϕ stained for the 1C11 antigen (magnification × 420).

Table 2. Distribution of 1C11 antigen in normal and foetal bovine tissues

Tissues	1C11$^+$ cell types
Skin	Mononuclear Mϕ-like cells in lamina propria and Langerhan's cells in epidermis (strong reactivity); vascular endothelial cells (weak reactivity)
Tongue	Langerhan's cells (strong reactivity) and mononuclear Mϕ-like cells in the lamina propria
Rumen	As for Tongue
Lung	Mononuclear Mϕ-like cells in alveolar septae and alveolar Mϕ (strong reactivity); vascular endothelial cells (weak reactivity)
Liver	Kupffer cells (strong reactivity); megakaryocytes in foetal tissue
Kidney	Scattered cells in glomerular mesangia and segments of proximal tubules; all proximal tubule cells in foetal tissue
Spleen	Mϕ-like cells in the red pulp; megakaryocyte in foetal tissue
Lymph node	Mϕ-like cells in the sinuses; dendritic and Mϕ-like cells in the follicular and para-follicular areas
Thymus	Epithelial stroma/reticulum cells in the medulla (strong reactivity); scattered Mϕ-like cells in the cortex and interstitia
Bone marrow	Mϕ-like cells and megakaryocytes (strong reactivity)

lineage in all tissues examined. Reactivity with bone marrow megakaryocytes, proximal renal tubule cells and some vascular endothelial cells was also observed. In foetal tissues, similar patterns of staining were observed, with two exceptions: (i) positively labelled megakaryocytes were abundant in the foetal liver, spleen and bone marrow, in accordance with the ontogeny of these cells (Noden & de Lahunta, 1985), and (ii) almost all proximal renal tubule cells were labelled with 1C11, in contrast to the segmental staining pattern seen in calves (Fig. 2). Cells of lymphoid origin were not labelled by mAb 1C11 in any of the tissues tested.

The *in vivo* distribution of the 1C11 antigen(s) was somewhat similar to that described for other Mϕ markers, including CD14 (Goyert *et al.*, 1986), the EBM11 antigen (Bielefeldt Ohmann *et al.*, 1988b), and the DH59B antigen (Davis *et al.*, 1987). Parallel sections were therefore stained with mAb 1C11 or mAb specific for these similar markers (Table 1; results not shown). More Mϕ lineage cells were stained with mAb 1C11 than with mAb MY4—a CD14-specific mAb which labelled only a subset of bovine Mϕ. Similarly, more Mϕ lineage cells were stained with mAb 1C11 than with mAb EBM11—a maturation-dependent marker for bovine Mϕ (Bielefeldt Ohmann *et al.*, 1988b). The Mϕ-labelling characteristics of mAb 1C11 were similar to those of mAb DH59B, which is a pan-lineage marker for bovine Mϕ and PMN (Davis *et al.*, 1987). However, mAb DH59B did not label megakaryocytes or renal tubule cells, and thus the 1C11 antigen appears to be a novel pan-lineage marker for bovine Mϕ and certain other cell types.

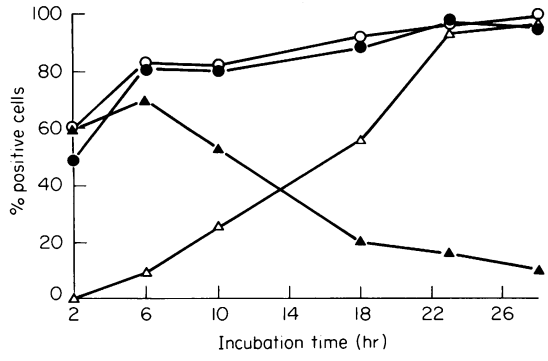

Figure 3. Kinetics of expression of the 1C11 antigen and other bovine Mϕ markers by bovine Mϕ following *in vitro* culture. Enriched bovine peripheral blood Mϕ were cultured *in vitro* in lipopolysaccharide-free media for varying periods of time, fixed and permeabilized with acetone, and immunocytochemically stained for expression of the 1C11 antigen (○), class II MHC antigens (●), CD14 (▲), or the EBM11 antigen (△).

Reactivity of mAb 1C11 with isolated leucocyte populations

The *in vivo* reactivity analyses were confirmed and extended using isolated leucocyte populations (Fig. 2). Immunocytochemistry and flow cytometry of PBMC and enriched Mϕ preparations confirmed the expression of the 1C11 antigen on Mϕ. Immunocytochemistry of purified platelet preparations revealed the presence of the 1C11 antigen on these cells—verifying the reactivity detected for megakaryocytes. Immunocytochemistry of PMN also revealed intracellular expression of the 1C11 antigen in neutrophils.

The kinetics of expression of the 1C11 antigen by blood Mϕ following activation and/or maturation *in vitro* was examined and compared to the expression of CD14, the EBM11 antigen and class II MHC antigens (Fig. 3). Mϕ cultured *in vitro*, in endotoxin-free media, spontaneously increased expression of the 1C11, EBM11 and class II MHC antigens, but lost expression of CD14—with the increase in 1C11 antigen expression parallel to that of class II MHC antigens rather than

the EBM11 antigen. However, expression of the 1C11 antigen differed from that of class II MHC antigens following *in vitro* stimulation of peripheral blood Mϕ cultures with recombinant bovine TNF-α or recombinant bovine IFN-γ (Bielefeldt Ohmann *et al.*, 1988a,b). Both cytokines increased the intensity of class II MHC antigen expression on Mϕ but did not significantly affect expression of the 1C11 antigen (data not shown).

Thus, the 1C11 antigen appears to be a general myeloid and platelet lineage marker, rather than a specific maturation or activation marker for a subset of bovine Mϕ.

Biochemical characterization of the 1C11 antigen

Radioimmunoprecipitation of uninfected and BHV-1-infected MDBK cells demonstrated the dual specificity of mAb 1C11 and identified the cross-reactive entity in uninfected cells as two broad ^{35}S-methionine-labelled bands of 34,000–38,000 and 43,000–63,000 MW, which were present under both non-reducing (data not shown) and reducing conditions (Fig. 4a). The 43,000–63,000 MW band also incorporated tritiated-glucosamine (Fig. 4a, lane 5), indicating that this band contains a glycoprotein(s). Analysis of alveolar Mϕ yielded similar results, except that the high MW band ranged from 43,000 to 69,000 MW (data not shown). Supernatants from radiolabelled MDBK and Mϕ cultures contained no 1C11-reactive antigens (data not shown). Pulse-chase labelling of alveolar Mϕ revealed a single pulse precursor of 27,000 MW (Fig. 4b). It was concluded that the 43,000–63,000 MW band may be a single heterogeneous glycoprotein derived from the 27,000 MW pulse precursor, and that the 34,000–38,000 MW band may be an under-glycosylated form and/or a degradation product of the 43,000–63,000 MW band.

Immunoelectron microscopy

To examine expression of the 1C11 antigen on myeloid cells, purified alveolar Mϕ and blood PMN were gold-labelled with mAb 1C11 (Fig. 5). Surface expression of the 1C11 antigen on alveolar Mϕ varied from cell to cell, in accordance with the heterogeneity of this Mϕ population (Bielefeldt Ohmann & Babiuk, 1986a), and was unevenly distributed—even in cells where capping and endocytosis were inhibited by incubation in sodium azide and at a temperature of 4°. When labelled alveolar Mϕ were warmed to 37°, the 1C11 antigen was internalized, via coated pits and an endosomal pathway, with accumulation of gold particles in phagolysosomes (Hubbard, 1989). There was no evidence of (i) transport of gold label to the nuclear membrane, (ii) recycling of gold label to the plasma membrane, or (iii) exocytosis of phagolysosomally accumulated gold label. However, these experiments do not exclude the possibility that the intracellular gold particles were dissociated from the 1C11 mAb, the secondary conjugate, and/or the 1C11 antigen, once in the acidic environment of endosomes and phagolysosomes. Examination of PMN in a parallel series of experiments revealed weak surface expression of the 1C11 antigen but no evidence of endocytosis (data not shown).

Stimulation of mononuclear cell proliferation by mAb 1C11

In view of the immunoelectron microscopy results, which suggested that the 1C11 antigen may be a functional receptor, bovine PBMC were incubated in the presence or absence of various doses of mAb 1C11 and/or the mitogens phytohaemagglutinin A and concanavalin A. In the absence of mitogens, mAb 1C11 consistently stimulated low levels of proliferation of PBMC (mAb 1C11 mean c.p.m., 13,603; control mAb, mean c.p.m., 1577; results representative of two batches of mAb 1C11, two control mAb, and three 10-fold dilutions of each mAb; means from four animals with standard errors less than 10% of the means). Responses to mitogens were not significantly affected by mAb 1C11—even at suboptimal mitogen doses (data not shown).

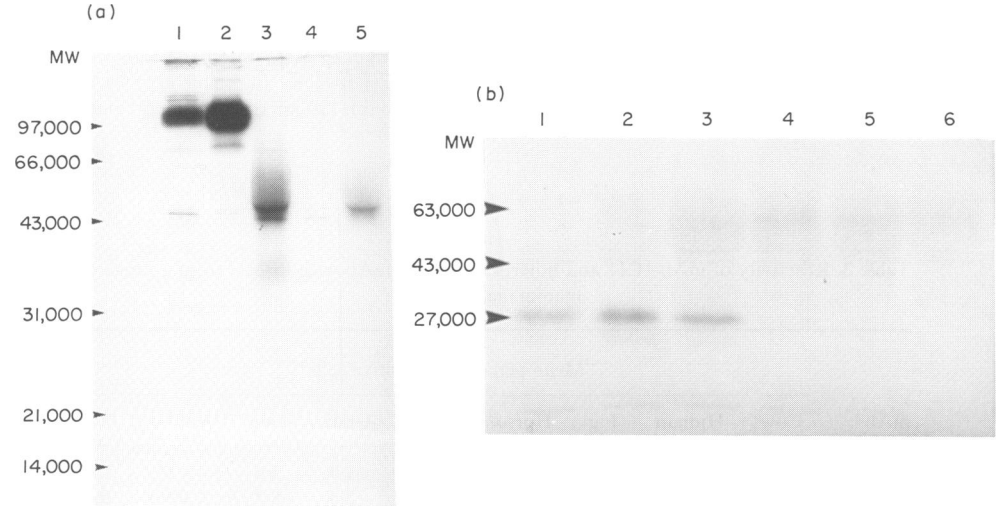

Figure 4. Radioimmunoprecipitation of normal or BHV-1-infected MDBK cells or alveolar Mϕ with mAb 1C11. (a)^{35}S-methionine-labelled, BHV-1-infected MDBK cells precipitated with mAb 1C11 (lane 1); ^{35}S-methionine-labelled, BHV-1-infected MDBK cells precipitated with mAb 1D6 (lane 2); ^{35}S-methionine-labelled MDBK cells precipitated with mAb 1C11 (lane 3); ^{35}S-methionine-labelled MDBK cells precipitated with mAb 1D6 (lane 4); ^{3}H-glucosamine-labelled MDBK cells precipitated with mAb 1C11 (lane 5). The 99,000 MW glycoprotein (lanes 1 and 2) is BHV-1 gIII. (b) Alveolar Mϕ ^{35}S-methionine pulse-labelled for 15 min, cold chased for 0 (lane 1), 15 (lane 2), 30 (lane 3), 45 (lane 4), 60 (lane 5), or 120 (lane 6) min, and precipitated with mAb 1C11.

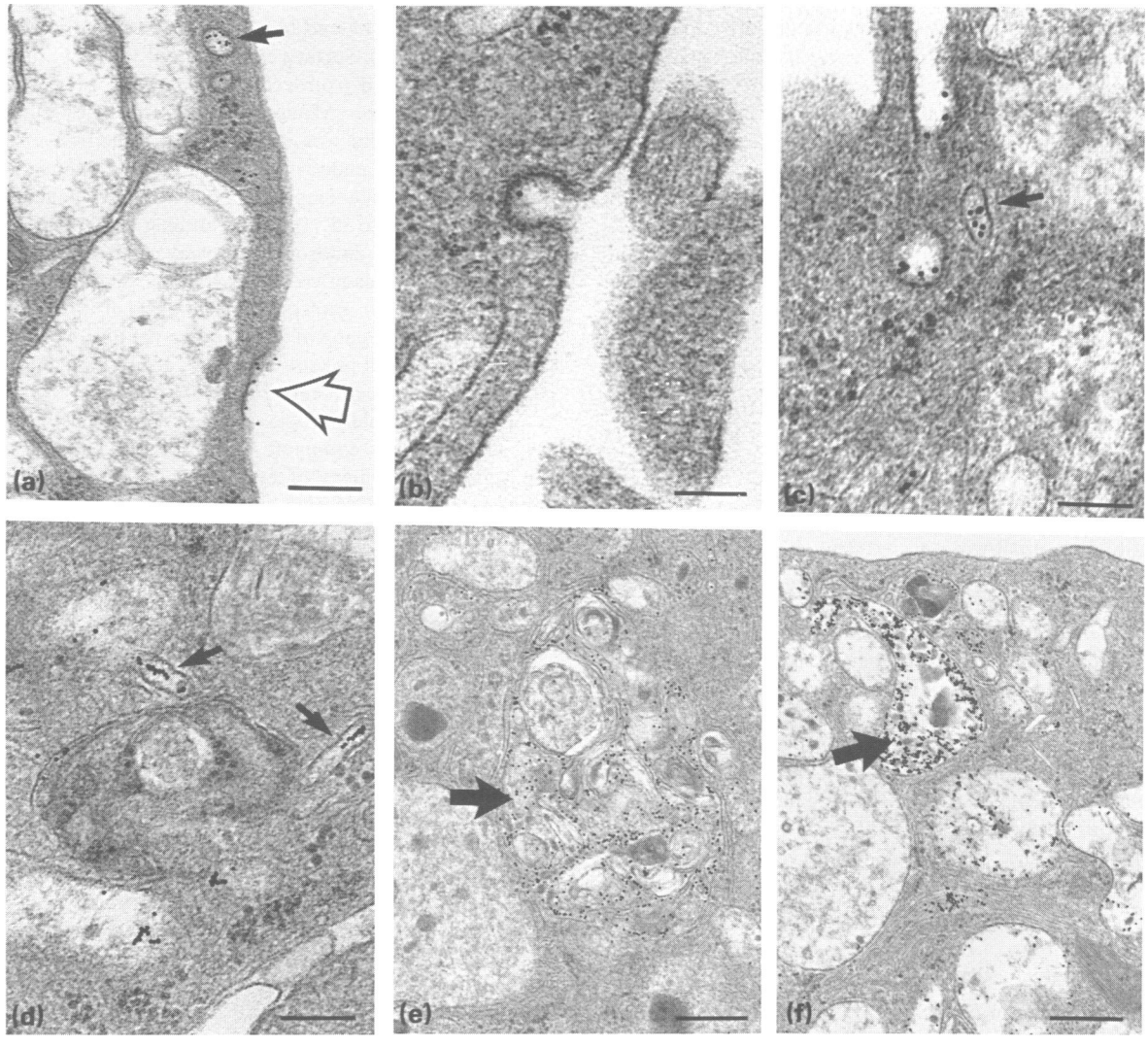

Figure 5. Immunoelectron microscopy of the surface expression and internalization of the 1C11 antigen by alveolar Mϕ. Binding of mAb 1C11 reveals antigen localization over specialized membrane areas (a, large open arrow). Internalization occurs via coated pits (b, c) and small receptosomes (a, c, small arrow), followed by transport via endosomes (d, small arrows) and accumulation in phagolysosomes (e, f, medium arrows). Bars = 375 nm (a), 176 nm (b), 150 nm (c), 190 nm (d), 300 nm (e) and 420 nm (f).

Table 3. Reactivity of mAb 1C11 and other Mϕ-specific mAb with PBMC from a range of mammalian species

	Mean percentage positive cells*								
mAb	Cow	Human	Pig	Horse	Sheep	Goat	Dog	Rabbit	Rat
1C11	17·3	5·0	3·5	2·7	6·3	1·9	5·6	0·2	0·0
MY4	12·0	14·1	7·1	3·1	14·1	12·8	8·2	3·9	0·0
EBM11	0·2	0·0	0·1	0·1	0·2	0·0	0·9	0·1	0·0

* Mean reactivity determined by flow cytometry (all mAb) and/or immunocytochemistry (mAb 1C11, MY4) of PBMC from three or four different individuals. Standard errors did not exceed 5% of the means. mAb EBM11 recognizes an intracellular Mϕ antigen and served as a negative control for these surface labelling studies.

Expression of the 1C11 antigen on Mϕ of other species

In view of the novel distribution, biochemical, and functional properties of the 1C11 antigen, we tested human, porcine, caprine, ovine, equine, canine, rabbit and rat PBMC for expression of antigenically related surface molecules by immunocytochemistry and flow cytometry. PBMC from several species exhibited low but significant levels of reactivity with mAb 1C11—particularly human, ovine, and canine PBMC (Table 3). Enrichment of Mϕ from these three species demonstrated a parallel enrichment for reactivity with mAb 1C11 (data not shown).

Low serological responses to the 1C11 epitope in BHV-1 gIII-immune cattle

Sera from cattle immunized with purified gIII were tested for reactivity against the 1C11 epitope on BHV-1. Competitive-binding ELISA results revealed low responsiveness to the 1C11 epitope (maximum inhibition at a dilution of $10^{-1}=27\%$; mean of four animals with standard errors less than 5% of the mean), in spite of high responses to other gIII epitopes (maximum inhibition at a dilution of $10^{-1}=68->95\%$; mean of the same four animals with standard errors less than 14% of the means). Similar results were obtained using sera from four animals which were naturally infected with BHV-1 (data not shown).

DISCUSSION

The above studies provide preliminary evidence for three distinct but inter-related phenomena: (i) that an antigenic relationship exists between an epitope of BHV-1 and an epitope of a cellular glycoprotein of the host of this virus, (ii) that the host cell glycoprotein is a novel, potentially functional, leucocyte marker for myeloid cells and platelets—in the bovine and other species including humans, and (iii) that the BHV-1-host mimicry may affect the immune response during BHV-1 infections in cattle.

The existence of an antigenic relationship between BHV-1 gIII and a host cell molecule supports reports that antiviral mAb with cross-reactivity to host cells are not uncommon (Oldstone & Notkins, 1986). We also initially considered that this finding supported our studies which identified Ig-related domains in gIII (Fitzpatrick *et al.*, 1989). However, epitope mapping has determined that the 1C11 epitope lies within an amino-terminal domain of gIII and not within any of the carboxy-terminal, Ig-related domains of gIII (Fitzpatrick *et al.*, 1990a). Moreover, the Ig-related domains of gIII appear to be recognized relatively poorly by mouse, rabbit and bovine anti-gIII antisera (D. Fitzpatrick, unpublished data). Thus, more than one type of antigenic relationship may affect the responses to different domains of gIII, and a complex form of molecular mimicry may exist. This is corroborated by recent reports (Ayers *et al.*, 1989; Israel *et al.*, 1988), where sera from BHV-1-immune cattle showed poor or variable responses to several gIII epitopes, despite strong responses to other gIII epitopes and BHV-1 proteins.

The host cell glycoprotein bearing the 1C11 epitope possesses a set of tissue distribution and biochemical characteristics which appears to distinguish it from all other well-characterized leucocyte antigens (Horejsi & Bazil, 1988; Knapp *et al.*, 1989). In particular, expression of the 1C11 antigen on platelets as well as Mϕ and PMN, its lack of expression on lymphocytes, and its MW of 43,000–63,000 with a 27,000 MW pulse precursor, appears to be a unique combination of properties. The functional potential of the 1C11 antigen, as demonstrated by its receptor-like trafficking through coated pits (Hubbard, 1989) and its stimulation of the proliferation of PBMC, suggest that further investigation of the structure, expression and function of this molecule is warranted—preferably employing a wider range of immunological reagents. The expression of a 1C11-like antigen on a subset of human Mϕ (Table 3) increases the potential value of such studies.

The significance of antigenic molecular mimicry is dependent upon the closeness of the mimicry and the nature of the immunological response to the shared epitopes (Damian, 1989). For BHV-1 gIII, the low responsiveness to the 1C11 epitope in immune bovine sera suggests that the antigenic relationship between the gIII determinant and the host cell glycoprotein determinant is very close such that partial immunological tolerance may be involved. Alternatively, the 1C11 epitope on gIII may elicit cross-reactive antibody responses, but these may not be readily detectable in serum samples due to adsorption to the host 1C11 antigen and/or immune complex formation (Oldstone, 1984). These possibilities may be tested by precise mapping of the 1C11 epitope of gIII followed by analysis, in cattle, of the relative immunogenicity and immune complex-generating ability of sequential synthetic derivatives of the epitope. An additional possibility, that the 1C11 epitope of gIII may functionally mimic and compete with the activities of the host 1C11 antigen, may be tested using similar reagents in *in vitro* bioassays.

The above study exemplifies the complex interactions between herpes viruses and the immune systems of their hosts, as well as the value of studies of viral infections in natural animal models for dissection of viral pathogenesis and immunity (Bielefeldt Ohmann & Babiuk, 1986b).

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