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Replication of murine cytomegalovirus in mast cells

Brief Report

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Summary. Mast cells purified from the peritoneal cell population and mast cells derived in culture from bone marrow cells were examined for their sensitivity to murine cytomegalovirus (MCMV) infection in vitro. While up to 70% of mast cells expressed viral antigens, less than 12% of the cells produced infectious virus. Transmission electron microscopy demonstrated nucleocapsids in the nuclei and in association with the cisternal elements of the Golgi apparatus. Some complete virions were found within small cytoplasmic vacuoles. In contrast with previous studies of macrophages and fibroblasts, the susceptibility of mast cells to MCMV infection in vitro was not influenced by the H-2 or non-H-2 genotype of the donor.

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Cytomegalovirus (CMV) infections result in significant morbidity and mortality in neonates [23] and immunocompromised individuals, including allograft recipients [4, 21] and AIDS patients [2]. Although a CMV-induced immune response can limit the infection, many manifestations of the disease are immunologically-mediated [6]. Previous studies have suggested that mast cells may contribute to the pathogenesis of several viral diseases including respiratory syncytial [14], parainfluenza [5], herpes simplex [11], and Sendai [19] virus infections. However, the contribution of mast cells to CMV-induced immunopathologic processes following the production of infectious virus or viral antigens or via the release of pharmacological agents from infected cells has not been examined. This study examines the capacity of mast cells in the peritoneal cavity or derived from bone marrow cell preparations to support replication of murine cytomegalovirus (MCMV) in vitro. Previous studies have established that the infection of murine macrophages and fibroblasts with

MCMV in vitro is determined by the H-2 and non-H-2 genes of the donor mouse strain and correlates with in vivo resistance to lethal MCMV infection [9, 17, 18]. Accordingly, the influence of host genetic constitution on the susceptibility of mast cells to MCMV infection was assessed in this study.

Specific pathogen-free BALB/c, BALB.B, BALB.K, C57BL/10 (B10), B10.D2, and B10.BR mice obtained from the Animal Resources Centre (Murdoch, Western Australia) were used for the collection of cells required in this study. The mice were housed under minimal disease conditions in the Department of Microbiology at the University of Western Australia. Serological screening established that the colony remained free of MCMV, mouse hepatitis virus, Sendai virus, and *Mycoplasma pulmonis*.

MCMV derived from the Smith strain by Dr June Osborn was maintained by salivary gland passage in weanling female BALB/c mice [1]. Tissue culture-derived MCMV was produced by infection of confluent mouse embryo fibroblast (MEF) cultures with salivary gland-passaged virus under centrifugation at 800 **g** for 30 min [16]. The stock used for experiments in this study contained 2.65×10^6 plaque forming units (pfu)/ml.

Mast cells derived from bone marrow cells were prepared as described previously [3]. Briefly, bone marrow cells collected from the femurs and tibias of mice by aspiration with RPMI 1640 (GIBCO, U.S.A.) via a bent 26 gauge needle were treated with 0.184 M NH_4Cl to lyse erythrocytes, washed in fresh medium and resuspended in culture medium [HEPES-buffered RPMI 1640 supplemented with 30% WEHI 3-conditioned medium, 10% heat inactivated foetal calf serum (FCS; 56 °C, 60 min) 2 mM L-glutamine, 0.1 mM non-essential amino acids (Commonwealth Serum Laboratories, Australia) 1 mM sodium pyruvate and 0.5 mM 2-mercaptoethanol]. The cells were seeded into tissue culture flasks at 8×10^6 cells/flask in culture medium and incubated for three weeks at 37 °C in 8% CO_2 in air with weekly medium changes. The cultures were then incubated for an additional four days in medium supplemented with 1 mM sodium butyrate to induce granulation of mast cells and prevent further cell replication. The cells were washed and suspended in fresh sodium butyrate-containing medium during the second day of this incubation.

Peritoneal cells were collected by lavage with phosphate-buffered saline (PBS; pH 7.2, 333 mOsmol) supplemented with 5% FCS. The cells were washed and suspended in RPMI 1640 containing 2% FCS (RPMI/2% FCS) at 5×10^6 cells/ml. Mast cells were concentrated from the peritoneal cell population by centrifugation of 2×10^7 cells through 5 ml of Percoll (1.075 g/ml; Pharmacia, Sweden) in HEPES-buffered RPMI 1640 supplemented with 10% FCS (RPMI/10% FCS) at 400 **g** for 30 min. The mast cells were collected from the cell pellet, washed in RPMI/2% FCS, resuspended and counted. The resulting suspensions contained >95% mast cells as determined by morphological examination of Giemsa stained preparations.

For the infection of cells, 0.25 ml of MCMV was added to an equal volume of peritoneal or bone marrow-derived mast cells adjusted to 2×10^7 cells/ml

in RPMI/2% FCS in FCS-precoated wells of 24-well tissue culture plates and centrifuged at 800 **g** for 30 min at room temperature. Mock-infected cultures were prepared using equal volumes of cell suspensions and medium without MCMV. Following incubation at 37 °C in 8% CO_2 in air for 90 min, the cells were resuspended by pipetting with warm medium, washed and dispensed at 5×10^5 cells/well into 24-well tissue culture trays containing sterile glass coverslips for incubation at 37 °C in 8% CO_2 in air.

For electron microscopy studies, peritoneal cell preparations infected as described above were maintained for 24 or 48 h in FCS-precoated 6-well tissue culture trays. The cells were dislodged with a rubber scraper, fixed overnight in cold 2.5% gluteraldehyde in 0.1 M cacodylate buffer (pH 7.4) and pelleted by centrifugation. Cell pellets were suspended in bovine serum in 1.5 ml tubes and the mixture clotted by the addition of 2.5% gluteraldehyde to fill the tubes. The clots were cut into 1 mm^3 segments, post-fixed in 1% osmium tetroxide in cacodylate buffer, treated with uranyl acetate, dehydrated in graded solutions of ethanol and embedded in Araldite. Sections were cut using an LKB ultramicrotome, stained with lead citrate and examined on a Phillips 410 electron microscope.

Mast cells expressing viral antigens three days after infection were enumerated following immunoperoxidase staining of acetone-fixed coverslip preparations (85% acetone; 0 °C, 10 min). Hyperimmune anti-MCMV sera used in the detection of viral antigens were collected from BALB/c mice inoculated intraperitoneally (i.p.) with 2.5×10^4 pfu of MCMV and rechallenged with 3.7×10^4 pfu of MCMV i.p. four weeks later. Sera were collected 18 days after the final challenge, pooled and stored at −20 °C until required. The fixed mast cell preparations were incubated with the hyperimmune antiserum, followed by biotinylated sheep anti-mouse immunoglobulin, streptavidin biotinylated horseradish peroxidase complex (Amersham, U.K.) and 3′3′ diaminobenzidene tetrahydrochloride substrate (Sigma, U.S.A.). The cells were lightly counterstained with haematoxylin, dried and mounted in DePeX (BDH Chemicals, Australia) for microscopic examination. In all assays, at least 150 cells were counted from several regions on two separate coverslips and subjected to chi-square (χ^2) analysis. Hyperimmune antiserum was not found to react with mock-infected mast cells. In addition, mast cells in infected and uninfected preparations did not react with normal mouse serum.

Productively infected peritoneal mast cells were enumerated using an infectious centre assay as described previously [22]. Briefly, serial two-fold dilutions of mast cell suspensions were prepared in RPMI/10% FCS in 24-well tissue culture trays and incubated for 1 h at 37 °C in 8% CO_2 in air. The supernatant was removed and 2×10^5 MEFs in RPMI/10% FCS were added to each well for a further 1 h incubation at 37 °C in 8% CO_2 in air. The supernatant was replaced with 2 ml of 2% methylcellulose in Eagle's minimal essential medium (GIBCO, U.S.A.) with 2% FCS and the trays incubated for 5 days prior to staining with 1% methylene blue containing 10% formalin.

The mast cells which reside in the peritoneal cavity belong to the connective tissue mast cell lineage. These are phenotypically different from the interleukin 3-dependent cultured mast cells which resemble histochemically the mucosal mast cells found in vivo (reviewed by Stevens and Austin [19]). Mast cells of both lineages were examined for their ability to support replication of MCMV and their subsequent expression of viral antigens is discussed. In addition, the release of infectious MCMV was determined following infection of peritoneal mast cells. The enrichment of peritoneal mast cells by centrifugation through Percoll removed cells of low density including fibroblasts and macrophages which would otherwise have interfered with the detection of virus release from MCMV-infected mast cells. The fractionation of cells on discontinuous Percoll density gradients has been described previously [10]. Infectious centre assays were not performed with MCMV-infected, bone marrow-derived mast cells since the preparations were found to contain cells expressing the macrophage phenotypic marker, Mac-1.

Peritoneal mast cells from BALB/c mice were examined by electron microscopy 24 and 48 h after infection (Fig. 1). A proportion of mast cells showed evidence of viral replication and the structural changes that were seen were generally similar at these times. Nuclei were slightly enlarged and possessed an irregular outline with a few projections and flaps punctuating the nuclear periphery. Euchromatin was increased, nucleoli were slightly enlarged and fibrillogranular material occupied the central parts of the nucleoplasm. Nucleocapsids were scattered at the periphery of the fibrillogranular material but the number was never large. The nucleocapsids measured 95–100 nm in diameter and some contained a nucleoid some 50–55 nm in diameter. Cytoplasmic fibrillogranular material was not detected but a few nucleocapsids were seen in close apposition to the cisternal elements of the Golgi apparatus. Occasionally, complete virions were found within small cytoplasmic vacuoles.

Expression of MCMV-induced antigens by peritoneal and cultured mast cells revealed that these cells were susceptible to infection with the virus under the conditions employed in this study (Tables 1 and 2). However, infected cells did not appear cytomegalic and intranuclear and cytoplasmic inclusions were not visible microscopically. The percentage of peritoneal mast cells which released infectious virus particles was assessed by infectious centre assay and found to be lower than the percentage which were expressing MCMV antigens (Table 2). These findings are consistent with electron microscopy studies demonstrating few complete virions present in infected mast cells. The efficiency of infectious virus production by MCMV-infected mast cells was similar to that

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Fig. 1. a Mast cell 48 h after infection with MCMV. Nucleocapsids (arrows) are present in its enlarged nucleus. Magnification × 22,000. **b** Mast cell 48 h after infection with MCMV. Nucleocapsids are seen in the nucleus (thick arrows) and a few are present in the vicinity of the Golgi apparatus (arrows). Magnification × 37,000

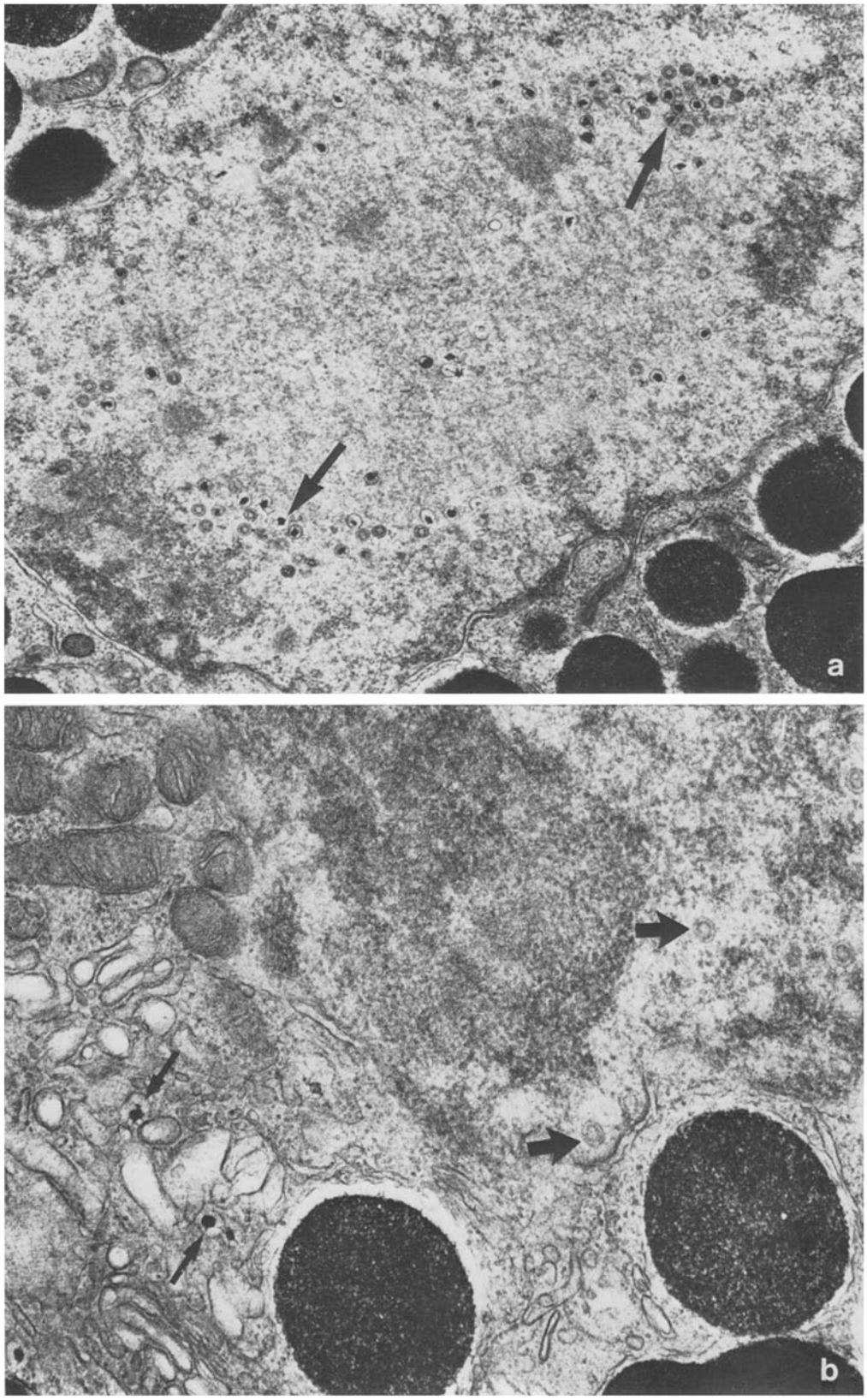
a
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Table 1. Percentage of culture-grown, bone marrow-derived mast cells infected with MCMV

Donor strain	H-2 Haplotype	Relative LD_{50}[a]	% of cells expressing MCMV antigens[b]
BALB/c	d	1	70
BALB.B	b	1	83
BALB.K	k	10	52
B10.D2	d	2–4	65
B10	b	2–4	58
B10.BR	k	20–30	68

[a] Relative LD_{50} refers to the ratio of the LD_{50} dose for a particular strain relative to that of BALB/c [7]

[b] MCMV antigen expression determined three days post-infection by immunoperoxidase staining

Table 2. Percentage of peritoneal mast cells infected with MCMV

Donor strain	Experiment 1		Experiment 2	
	% cells expressing MCMV antigens[a]	% cells releasing infectious MCMV[b]	% cells expressing MCMV antigens	% cells releasing infectious MCMV
BALB/c	ND	ND	61	9
BALB.B	43	4	ND	ND
BALB.K	40	6	62	6
B10	49	8	59	ND
B10.BR	35	12	70	9

[a] MCMV antigen expression determined three days post-infection by immunoperoxidase staining

[b] Release of infectious MCMV determined by infectious centre assay

ND Not done

previously recorded for macrophages but lower than the efficiency of virus production in MEFs where as many as 60% of cells expressing viral antigens also release infectious MCMV [18]. Studies by Muller and Hudson demonstrated that fibroblasts were most susceptible to productive MCMV infection during the cellular DNA synthetic phases [15], suggesting that events associated with the host S-phase are required for the initiation of viral DNA synthesis. Since peritoneal mast cells and macrophages are terminally differentiated, their inefficiency in producing infectious MCMV may relate to the lack of cellular mitotic processes. The results of this study suggest that if mast cells are infected with CMV in vivo, they are unlikely to contribute to the host's total virus load but may contribute to the concentration of virus or viral antigens at localized sites.

Statistical analysis of viral antigen expression and virus release in MCMV-infected mast cell preparations showed no consistent pattern of genetically-determined susceptibility to infection among the strains examined. For example, peritoneal mast cells from genetically-resistant B 10.BR and BALB.K mice did not differ significantly from cells of susceptible B 10 and BALB/c mice in their sensitivity to MCMV infection. Although bone marrow-derived BALB/c and BALB.B mast cells were more sensitive to infection than BALB.K (χ^2-test; $p < 0.05$) this pattern of susceptibility was not evident in the B 10 congenic strains. Therefore, H-2 genes did not influence the sensitivity of mast cells to MCMV infection under the conditions of this study. This result differs from that obtained with macrophages where sensitivity of the cells from different strains was determined by the H-2 genotype of the donor animal [17, 18]. Such studies demonstrated a 10-fold greater resistance to infection of H-2^k-derived macrophages in comparison with those from H-2^d mice, where 90–100% of H-2^d cells expressed MCMV antigens following infection under the conditions employed in the present study. Macrophages with the H-2^b haplotype showed intermediate sensitivity to infection. Similar strain-related patterns of susceptibility to MCMV infection have been obtained for fibroblasts in vitro, although sensitivity to infection was also influenced by non-H-2 genes [9]. Non-H-2 genes did not influence the susceptibility of peritoneal or bone marrow-derived mast cells to MCMV infection (χ^2-test; $p > 0.05$).

There is evidence indicating that the MHC antigens may serve as a cellular receptor for human CMV [8] via the binding of virion-bound β-2 microglobulin (β-2 m) to the heavy chains of the Class 1 antigens. In the murine model of CMV infection, H-2-determined differences in the susceptibility of individual cells to infection with MCMV may then be related to the varying affinities of β-2 m-bound MCMV for Class 1 molecules of the H-2 complex in different mouse strains. However, recent studies in our laboratory show that β-2 m-deficient MCMV has residual infectivity (Wykes, in prep.). Furthermore, Keay et al. [13] demonstrated the binding of an 86 kDa envelope glycoprotein of HCMV (gp86) to a cell surface receptor on human fibroblasts with an approximate molecular size of 92.5 kDa which was not related to the interaction of β-2 m with HCMV. These findings suggest there may be an alternative receptor for cytomegaloviruses which may be relevant to the infection of mast cells. Enhanced infection of peritoneal macrophages by salivary gland-passaged MCMV containing MCMV-specific antibodies has been reported and is believed to be mediated by the Fc portion of the antibody [12]. However, MCMV infection of mast cells was performed with tissue culture-derived virus which contained no MCMV-specific antibodies to ensure that infection by this mechanism did not occur.

In conclusion, this study has demonstrated that mast cells are capable of supporting replication of MCMV in vitro. Infected mast cell preparations showed evidence of viral replication by the expression of MCMV antigens and the presence of nucleocapsids in the nucleus and in association with the cisternal

elements of the Golgi apparatus. A small proportion of peritoneal mast cells released infectious MCMV. These results indicate that the contribution of mast cells to CMV-induced immunopathologic disease warrants further investigation.

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