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Differential permissivity to measles virus infection of human and CD46-transgenic murine lymphocytes

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Analysis of measles virus (MV) pathogenesis requires the development of an adequate small animal model of MV infection. In this study, permissivity to MV infection was compared in human and transgenic murine T lymphocytes, expressing different levels of the human MV receptor, CD46. Whereas MV binding and entry correlated with CD46 expression, higher levels of MV replication were always observed in human T lymphocytes. This suggests the existence of intracellular factors, acting posterior to virus entry, that could limit MV replication in murine lymphocytes and should be considered when creating new animal models of MV infection.

Measles virus (MV) is a morbillivirus that belongs to the order *Mononegavirales*. MV infection strongly affects the human immune system, leading to impaired lymphocyte function, and is still the major cause of infant mortality in developing countries (Murray & Lopez, 1997). Since humans are the only natural hosts for MV, the study of MV pathogenesis has been hampered by the lack of a convenient animal model. The identification of human CD46, the regulator of complement activation, as a cellular receptor for MV (Naniche *et al.*, 1993; Dorig *et al.*, 1993) has led to the development of CD46-transgenic animals as a possible animal model (Rall *et al.*, 1997; Horvat *et al.*, 1996; Niewiesk *et al.*, 1997; Mrkic *et al.*, 1998; Blixenkrone-Moller *et al.*, 1998; Oldstone *et al.*, 1999; Evlashev *et al.*, 2000), where the major target has been to generate transgenic rodents that express the MV receptor at a level resembling expression in human tissues. Although intracranial injection of MV was efficient in inducing specific pathology in some of these models, peripheral administration of the virus was linked to much lower permissivity and was not associated with any clinical symptom of measles.

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In order to investigate the importance of the level of expression of the MV receptor and to analyse the nature of the different sensitivity to MV infection of human and murine cells, we have compared distinct steps of the MV (Hallé strain) life-cycle in activated human peripheral blood lymphocytes (PBL) and activated CD46-transgenic murine splenocytes obtained from three transgenic lines expressing different levels of CD46: MCP-3, MCP-7 (Horvat *et al.*, 1996; Evlashev *et al.*, 2000) and MCP-B (Thorley *et al.*, 1997).

Firstly, we analysed CD46 expression on murine and human lymphocytes. Human PBL were obtained from donor blood after Ficoll and Percoll density centrifugation and activated for 2 days with 5 μg/ml anti-CD3 MAb (OKT3) and human recombinant IL-2 (5 U/ml) in RPMI 1640, 10% FCS. Splenocytes from wild-type and CD46-transgenic murine lines were purified and activated by mixed lymphocyte reaction or by anti-CD3 MAb and IL-2 and cultured as described previously (Horvat *et al.*, 1996). These activation procedures preferentially activate and expand T lymphocytes, which represent a predominant cell population used in all our experiments. Two days after activation, cells were stained for CD46 expression (Fig. 1A). The level of CD46 on activated human PBL was equivalent to the expression detected on the MCP-B line. MCP-7 and MCP-3 expressed approximately 5 and 50 times less CD46 than did MCP-B, corresponding to what has been described previously with non-activated lymphocytes (Horvat *et al.*, 1996; Thorley *et al.*, 1997).

Binding of MV was then analysed on activated murine lymphocytes and human PBL incubated for 3 h with MV (Hallé strain, 1 p.f.u. per cell). After extensive washing, cells were stained for MV haemagglutinin (H) protein as described previously (Naniche *et al.*, 1993). MV binding was detected on all cells tested and was proportional to levels of initial CD46 expression for each line (Fig. 1 B), being virtually equal in the human PBL and the murine MCP-B line.

We next measured virus entry into lymphocytes 3 h after infection by real-time PCR (LightCycler, Roche). Activated cells, infected as above, were additionally subjected to 30 min

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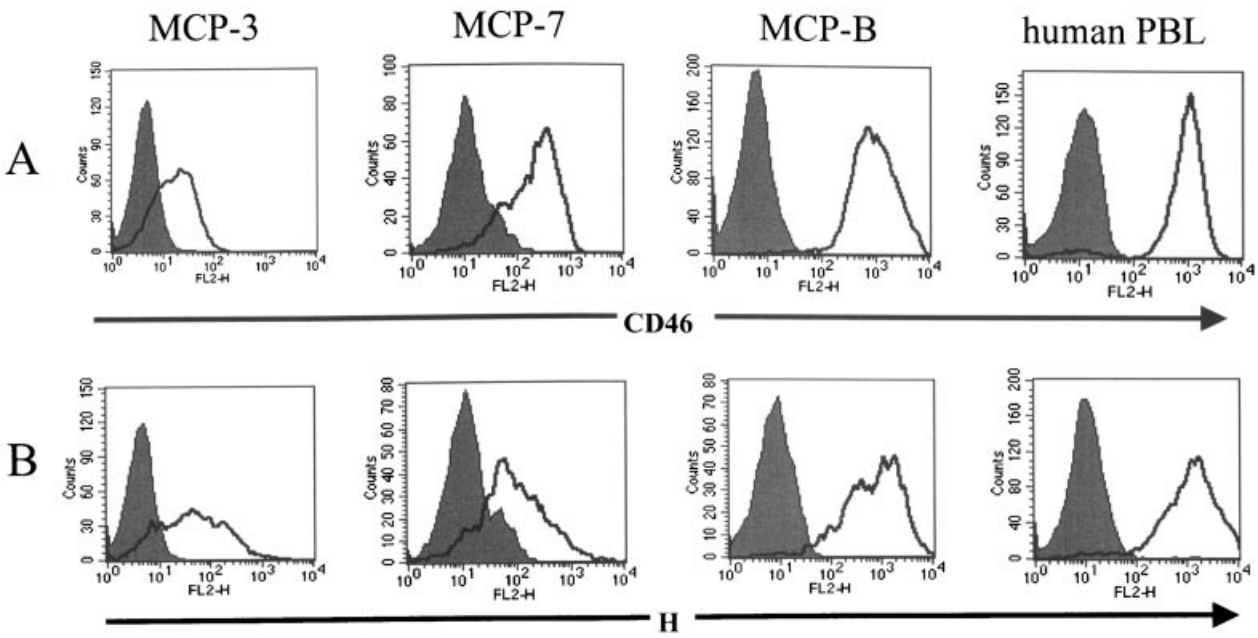

Fig. 1. CD46 expression on transgenic murine and human lymphocytes correlates with MV binding. (A) Lymphocytes from three CD46-transgenic murine lines (MCP-3, MCP-7 and MCP-B) and human PBL were activated and analysed by cytofluorometry for CD46, with anti-CD46 biotinylated MAb MCI20.6, followed by streptavidin–PE. Thick lines show anti-CD46 staining; the shaded histograms show isotype-control staining. (B) Alternatively, cells were incubated with either MV or a mock preparation (containing virus-free supernatant from uninfected Vero cells prepared under the same conditions) for 3 h, washed thoroughly and analysed for H by staining with biotinylated anti-H MAb cl55 followed by streptavidin–PE. Thick lines show MV-exposed cells; shaded histograms show mock-treated cells.

Pronase digestion to ensure elimination of virus from the cell surface, which was confirmed by virus-binding assay (Naniche *et al*., 1992). Total RNA was isolated and cDNA was prepared using 3′ primers specific for glyceraldehyde-3-phosphate dehydrogenase (G3PDH), as described previously (Horvat *et al*., 1996), and for the MV F gene (5′ GCTTCCCTCTGGCC-GAACAATATCG 3′) in the same reaction. In order to amplify genomic viral RNA, cDNA was analysed by PCR using CYBR green I fluorescent dye and primers specific for the H gene (Horvat *et al*., 1996), which is located downstream of F in the viral genome. We performed PCR specific for H and G3PDH in MV-infected human PBL, non-transgenic BALB/c lymphocytes or lymphocytes obtained from the three CD46-transgenic lines. The cycle number when the PCR product was first detected (crossing point), being inversely proportional to the relative abundance of the target sequence, was determined in two different experiments. The specificity of the amplified product was confirmed by melting-curve analysis, which demonstrated the presence of a single peak for each of the two PCRs, and by gel electrophoresis, where bands of the expected sizes (377 bp for H and 529 bp for G3PDH) were detected (data not shown). Our results indicate that the abundance of MV-specific template correlated with the CD46 expression level: similar quantities of the H product (crossing points 25·6 and 25·7, respectively) were detected in human cells and in the MCP-B transgenic line, suggesting that virus entry was equivalent in the two cell types. Lower amplification of H was detected in MCP-7 (28·0), MCP-3 (29·8) and non-transgenic (33·6) lymphocytes. Finally, the G3PDH target sequence was present in similar amounts in all samples tested (crossing point between 12·9 and 13·9), indicating similar conditions for cDNA synthesis in all samples tested. Altogether, these results suggest that the first steps of MV infection in MCP-B murine lymphocytes, expressing the highest level of CD46, are as efficient as in human lymphocytes.

Viral mRNA production in infected cells was studied by Northern blot hybridization. Cells activated as above were harvested 48 h after MV infection and 15 μg total RNA was resolved on a formaldehyde–agarose gel, transferred to a Hybond-N membrane, hybridized with a ^{32}P-labelled, nucleoprotein (NP)-specific DNA probe, stripped and finally re-hybridized with a *β*-actin-specific probe (Horvat *et al*., 1996), which cross-reacts with murine and human mRNA. Blots were exposed to a PhosphorImager screen (Molecular Dynamics) and the resulting images were quantified by using the ImageQuant software (Fig. 2A). NP mRNA was observed in all CD46-transgenic murine lines. However, the relative level of NP after normalization for *β*-actin mRNA expression in all three transgenic lines (0·05–0·07) was much lower than in human cells (5). Similar results were observed for H-specific mRNA (data not shown). Although the level of NP expression followed the initial level of CD46 expression in murine cells

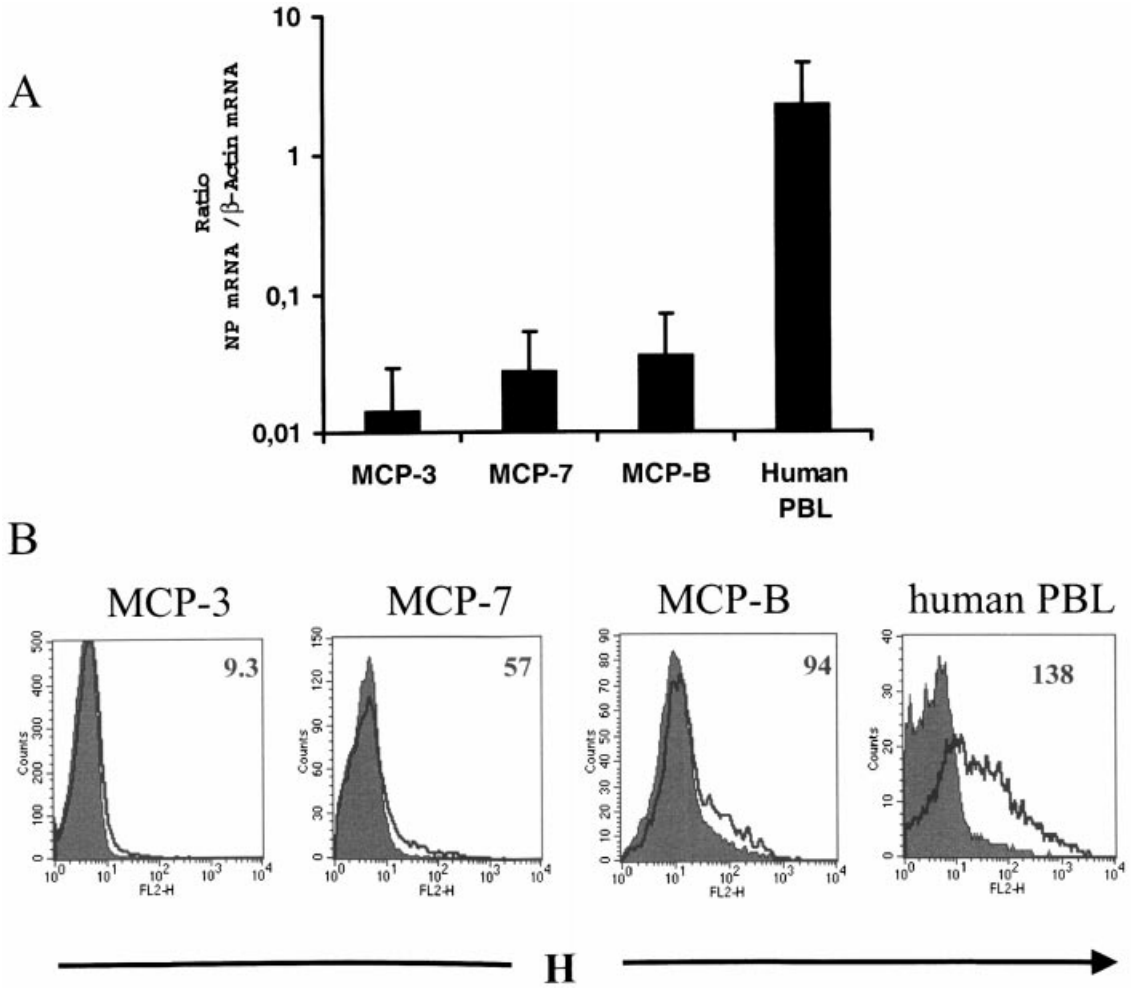

Fig. 2. Expression of MV proteins in infected transgenic murine and human lymphocytes. Activated lymphocytes were infected with MV and analysed by Northern blot and cytofluorometry. (A) Relative abundance of MV NP mRNA, calculated as the NP mRNA/β-actin mRNA band volume ratio after Northern blot hybridization with specific NP and β-actin probes, detected by phosphorimager. Results are expressed as means $\pm$ SD from two experiments. (B) Expression of H protein on MV-infected murine CD46-transgenic and human lymphocytes. Activated murine and human lymphocytes were infected with MV and analysed by flow cytometry for expression of MV H protein (using biotinylated anti-H MAb cI55 followed by streptavidin–PE). Dead cells were excluded from further analysis by propidium iodide staining. Thick lines show MV-infected cells; shaded histograms show cells incubated with UV-inactivated MV. Numbers in the upper-right corners of histograms are the mean fluorescence intensities of the H-positive cell populations. Results are representative of three experiments.

(Fig. 1), it was much lower compared with human lymphocytes, suggesting the existence of murine factors that limit/inhibit MV replication after virus entry.

MV protein expression on the surface of activated murine and human lymphocytes was analysed by cytofluorometry, 48 h after infection (Fig. 2B). As was observed from Northern blot analysis, H expression was much higher in human cells than in murine transgenic lymphocytes. These results were confirmed for the other time-points after MV infection and similar data were obtained when murine T lymphocytes were activated either by anti-CD3 MAb and IL-2 or by mixed lymphocyte reaction (data not shown). F protein expression was also detected in MCP-7 and MCP-B lines, but was generally much lower than that of H protein (data not shown).

Finally, production of infectious particles was analysed as described previously (Horvat *et al.*, 1996). Briefly, T lymphocytes infected as above were harvested at different time-points after MV infection and serial dilutions of lymphocytes were co-cultured with highly permissive Vero cells. Cytopathic effect resulting in formation of lytic plaques (p.f.u.) was determined 4 days later. For all three CD46 murine lines, production of infectious particles peaked at 48 h after infection and was at least 10 times lower than that for human PBL (Fig. 3). These results showed that the initial difference between the transgenic lines, related to the level of CD46 expression, was lost in the later steps of MV replication, arguing against the critical importance of high expression of the MV receptor in obtaining efficient MV replication in transgenic murine

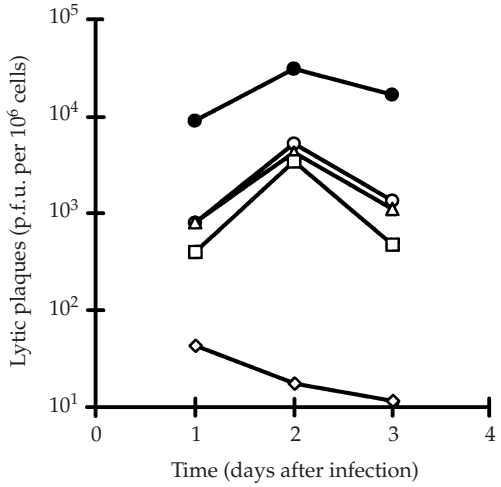

Fig. 3. Production of infectious particles from MV-infected murine MCP-B (○), MCP-7 (△) and MCP-3 (□) CD46-transgenic and human (●) lymphocytes. Lymphocytes from non-transgenic BALB/c mice (◇) were included as negative controls. Activated lymphocytes were infected with MV (1 p.f.u. per cell) and, at different times after infection, cells were incubated with Vero fibroblasts and the production of lytic plaques was determined 4 days later. The results are expressed as numbers of p.f.u. derived from 10^6 infected lymphocytes and are representative of three different experiments.

lymphocytes. However, the species difference was greatly increased, correlating with greater production of MV-specific mRNA and viral proteins in human compared with murine lymphocytes.

Taken together, these results suggest that, although MV binding and entry into murine transgenic T cells can be equivalent to that in human lymphocytes, additional factors limit later steps of MV replication in murine cells. This may reflect a different activation level, less suitable for MV replication, in murine T lymphocytes, which does not approach that of human T cells, regardless of the different activation procedures we tested. The production of and/or sensitivity to IFN type I may be different between human and murine lymphocytes, since CD46-transgenic mice are more permissive to MV infection if crossed into an IFNα/β receptor-deficient background (Mrkic *et al.*, 1998). Less susceptible intracellular conditions could be related to inefficient MV budding, as has been demonstrated recently for a murine fibroblast line (Vincent *et al.*, 1999), but could implicate some other factors, specific for murine lymphocytes, as different cell proteins were shown to be required for MV replication (Moyer *et al.*, 1990). Although the barriers to MV replication seen in CD46-transgenic lymphocytes do not seem to be as drastic as has been shown in the case of human immunodeficiency virus (HIV) replication in CD4- and CCR5-transgenic lymphocytes, where HIV could not be cultured at all (Browning *et al.*, 1997), they may still hinder the creation of an adequate transgenic murine model of MV pathogenesis. Whether MV binding to the recently identified human MV receptor SLAM (CDw150), expressed specifically on activated lymphocytes (Tatsuo *et al.*, 2000), could modulate the intracellular environment in lymphocytes, making them more suitable for MV replication, needs to be determined.

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