

# PREFERENTIAL INFECTION OF MATURE DENDRITIC CELLS BY THE JHM STRAIN OF MOUSE HEPATITIS VIRUS

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## 1. INTRODUCTION

The JHM strain of mouse hepatitis virus (MHV-JHM) causes acute encephalitis and acute and chronic demyelinating diseases in mice.<sup>1</sup> After infection, viruses are largely cleared by T cells; however, demyelination is induced as a consequence of this process. CD4 and CD8 T-cell responses, as well as an anti-viral antibody response are induced in infected mice, and they are critical to control virus replication and recrudescence.<sup>2,3</sup> Although an adaptive immune response is induced in infected mice, the anti-MHV CD8 T-cell response is reduced in mice infected with a virulent strain of MHV (named MHV-4 or JHM.SD<sup>4</sup>) when compared with mice infected with the A59 strain.<sup>5</sup> This might indicate suboptimal DC function during infection with JHM.SD. Because of the critical role DCs play in the host immune response to viral pathogens, viruses have developed strategies to depress the function of these cells.

Dendritic cells are readily infected with A59 *in vitro* and form syncytia. However, the antigen-specific T-cell response is very robust *in vivo*, suggesting that dendritic cells are still functional in this setting.<sup>5,6</sup> Previous work has focused on the effect of MHV infection on bone marrow (BM)-derived DCs after culture *in vitro*. These cultures usually include a mixture of mature and immature DCs. In general, only myeloid DCs are present in these cultures. In order to resolve the apparent contradiction between the *in vitro* and *in vivo* results, we assessed the extent to which mature and immature DCs are infected by MHV.

## 2. RESULTS

BM-derived DCs were prepared from B6 mice and cultured *in vitro* as previously described.<sup>7</sup> After 6–7 days in culture, cells were infected with the JHM strain of MHV. After 7 hr, extensive syncytia formation was observed. BM-derived cultures include cells

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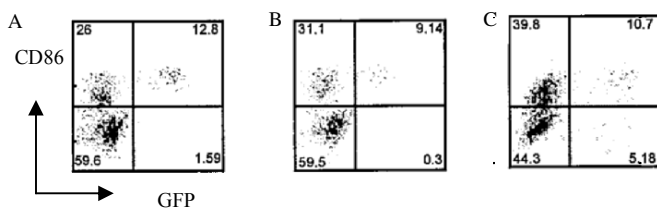
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that are positive and others that are negative for the DC marker, CD11c. By confocal microscopy, we showed that virus antigen was present in CD11c<sup>+</sup> cells. Infection was productive with increases in virus titers observed by 8 hr p.i. (data not shown). Thus, JHM, like A59, readily infects DCs *in vitro*.

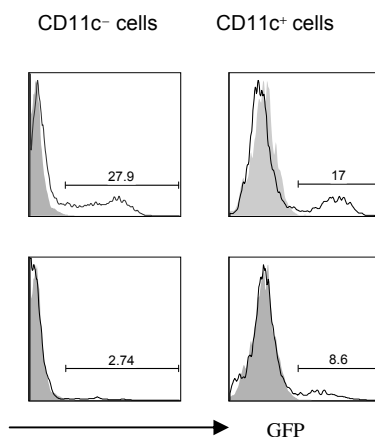
Immature DCs are critical for antigen uptake whereas mature DCs present antigen to T cells and orchestrate the innate and adaptive immune responses. One explanation for the apparent contradiction between the *in vivo* and *in vitro* results described above is that MHV preferentially infects either mature or immature DCs. For this purpose, we infected unfractionated BM-derived DCs with a recombinant JHM that expresses GFP (rJHM.GFP) and analyzed cells by FACS at 9 hr p.i. In this virus, GFP was inserted by targeted recombination into gene 4 of the virus because this gene is not necessary for growth in tissue culture cells or mice.<sup>8</sup> The majority of infected CD11c<sup>+</sup> cells exhibited high expression of MHC class I and II antigen (data not shown) and of the costimulatory molecule, CD86 (Figure 1), consistent with a mature phenotype. An attenuated strain of JHM, J2.2-V-1,<sup>9</sup> and the A59 strain also preferentially infected mature DCs. However, compared with JHM, A59 infected immature DCs to a greater extent (Figure 1). Further, CD11c<sup>-</sup> precursor cells present in the DC culture were about 10 times more susceptible to infection with A59 compared with JHM (Figure 2).

In order to further determine if JHM directly infects mature DCs or JHM infects immature DCs and induces their subsequent maturation, we separated CD86<sup>hi</sup> and CD86<sup>lo</sup> DCs by flow cytometric sorting prior to infection with rJHM.GFP. As shown in Figure 3A, CD86<sup>hi</sup> cells were about 5–10 times more susceptible to JHM infection than were immature DCs. We also quantified the proportion of GFP<sup>+</sup> cells, including syncytia, by fluorescent microscopy. Again, the percentage of GFP<sup>+</sup> cells was approximately 5–10 times higher in CD86<sup>hi</sup> than in CD86<sup>lo</sup> DCs (data not shown). We also found that CD86<sup>hi</sup> DCs produced 10 times more JHM than CD86<sup>lo</sup> DCs at the peak of the infection (Figure 3B).

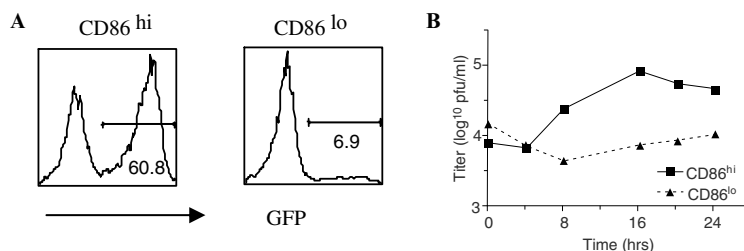
MHV receptor CEACAM1a is expressed on DCs,<sup>10</sup> and infection of DCs *in vitro* is CEACAM1a-dependent.<sup>6</sup> One explanation for the differential infection of mature and immature DCs is that CEACAM1a is expressed at higher levels on CD86<sup>hi</sup> cells. However, using anti-CEACAM1a mAb (kindly provided by Dr. K Holmes), we observed equivalent expression of CEACAM-1a on CD86<sup>hi</sup> and CD86<sup>lo</sup>CD11c<sup>+</sup>DCs by FACS analysis (data not shown).



**Figure 1.** rJHM.GFP, rJ2.2-V-1.GFP, or rA59.GFP-infected DCs are mostly CD86<sup>high</sup>. Shown are samples after gating on CD11c<sup>+</sup> cells. The percentage of infected cells was determined by GFP expression. Cells were harvested 9 hr after infection with rJHM.GFP (A) or rJ2.2-V-1.GFP (B) at an m.o.i of 10, or 7 hr after infection with rA59.GFP (C).



**Figure 2.** CD11c<sup>-</sup> cells are more susceptible to infection with A59 than JHM. DCs were infected with either rA59.GFP top or rJHM.GFP bottom at an m.o.i. of 10. Cells were harvested 7 hr after infection with rA59.GFP, and 9 hr after infection with rJHM.GFP.



**Figure 3.** rJHM.GFP preferentially infects mature DCs. A. CD86<sup>hi</sup> or CD86<sup>lo</sup> DCs were separated using a flow cytometer prior to infection with rJHM.GFP (m.o.i. of 10). Cells were harvested at 9 hr p.i., and the percentage of GFP<sup>+</sup> cells in each population was assessed by FACS. B. CD86<sup>hi</sup> or CD86<sup>lo</sup> DCs were infected with JHM at an m.o.i. of 10 and samples were harvested for titers at the indicated times.

### 3. DISCUSSION

Our results show that JHM infects cultured BM-derived DCs, with extensive syncytia formation. However, we could not detect a significant number of infected DCs in infected mice (data not shown). Mature DCs are the major population susceptible to JHM infection. However, the majority of DCs in naïve or infected animals are immature. This preferential infection of mature DCs may explain the low percentage of infected DCs that we observed in infected animals. Some other viruses also preferentially infect mature DCs. For example, mature blood-derived human DCs were infected at a higher level by RSV than were immature cells.<sup>11</sup> Although human CMV preferentially infects immature blood-borne DCs, recent results show that the virus has a tropism for mature Langerhans cells.<sup>12</sup>

Although MHV receptor CEACAM1a is expressed on DCs,<sup>6</sup> JHM does not infect cultured DCs efficiently: at an m.o.i. of 100, only 70% of mature DCs are infected with the virus (data not shown). Furthermore, immature DCs are more refractory to infection, even though levels of receptor are similar on the two types of cells. The essential role of

CEACAM1a in MHV infection of DCs was shown in a previous study, in which the presence of anti-CEACAM1a antibody blocked the process completely.<sup>6</sup> However, JHM may require a second host factor for efficient infection of some cells, as previous studies showed that JHM infected some cell lines less efficiently than A59.<sup>13</sup> Our data also show that A59 more readily infects CD11c<sup>-</sup> precursor cells and CD86<sup>lo</sup> DCs than does JHM. These results suggest that JHM, compared with A59, might be more dependent upon the presence of this putative second factor for infection. This factor might be expressed at low levels on CD86<sup>hi</sup> DCs, and even lower level on CD11c<sup>-</sup> cells and CD86<sup>lo</sup> DCs.

Collectively, our results suggest that JHM entry into DCs or uncoating after entry might be dependent on a cofactor; endosomal proteases have been implicated in SARS-CoV entry, and MHV entry might also require a similar enzyme.

#### 4. REFERENCES

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