HCoV-229E INFECTS AND ACTIVATES MONOCYTES

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1. INTRODUCTION

Human coronaviruses (HCoV) are respiratory pathogens with neurotropic and neuroinvasive properties. Indeed, cell lines of neural origin[1,2] and human primary cultures from the central nervous system (CNS)[3] are susceptible to infection by HCoV-OC43, and RNA was found to persist in human brain.[4] HCoV-OC43 can also cause a vacuolating encephalitis in mice[5] and RNA can persist for up to 1 year.[5a] HCoV-OC43 uses the neural route via the olfactory bulb to gain access to the CNS,[6] but no such pathway has been described for HCoV-229E as it does not infect mice, probably because of lack of an adequate receptor. A possible alternative neuroinvasive pathway would consist in passage through the blood-brain barrier (BBB) by infection or passage through brain endothelial cells and/or transport by infected leukocytes. Human immunodeficiency virus type 1 (HIV-1) is a good case in point, where brain infiltration of infected T lymphocytes[7] or monocytes[8,9] is crucial in initiating the neuropathology known as AIDS-dementia. It was previously reported that both OC43 and 229E could productively infect primary human monocytes/macrophages.[10] However, the results presented here rather suggest that only HCoV-229E productively infects human monocytic cells, while HCoV-OC43 infection is highly restricted. Moreover, the result obtained with the THP-1 cell line, which represents an excellent model to study the interaction between HCoV-229E and human monocytic cells, suggested that monocytes could be activated by infection and could serve as a reservoir and vector into the CNS for neuroinvasive HCoV-229E *in vivo*.

2. MATERIALS AND METHODS

2.1. Viruses and Cell Lines

HCoV strains (229E and OC43) were obtained from ATCC, plaque-purified and grown on L132 cells (229E) or HRT-18 cells (OC43). Human cell line THP-1 (gifts from Daniel Oth, INRS-Institut Armand-Frappier), were cultured in RPMI 1640 supplemented

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with 10 mM HEPES, 1 mM pyruvate sodium, MEM non essential amino acids, 100 U/mL penicillin, 100 µg/mL streptomycin, 2-mercaptoethanol 2 x 10^{-5} M (Invitrogen), and 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Wysent).

Leukocytes were isolated through Ficoll-Hypaque (Amersham) and PBMC were prepared at 2.5 x 10^6/mL. Monocytes were adsorbed onto 24-well plastic plates (Corning) for 90 minutes at 37°C in complete RPMI 1640 supplemented with the same components as for THP-1 cells except that 10% (v/v) heat-inactivated autologous serum was used. Cultures were then washed to harvest lymphocytes while adsorbed cells were fed with new medium. Part of these monocytes were cultured with 2% (v/v) autologous serum and infected the next day. The other fractions were induced to differentiate into macrophages (7 days in 10% (v/v) autologous serum in RPMI) before infection.

2.2. Infection and Activation of Cells and Titration of Infectious Virus Production

Cells were infected at a multiplicity of infection (MOI) of 1.0 and incubated 4 hours at 37°C. They were then washed with RPMI 1640 w/o serum and grown in RPMI 1640 supplemented with 10% (v/v) FBS (THP-1 cells) or 2% (v/v) (primary monocytes) or 10% (v/v) (primary macrophages) autologous serum. Infection was carried out for up to 7 days. Samples were taken at different times post-infection for evaluation of infectious virus production using an immunoperoxidase assay. [11]

To evaluate whether the THP-1 cells were activated following infection by HCoV, metalloproteinases (MMP) and TNF-α secretion were measured. Zymography on SDS-PAGE was performed to evaluate MMP production and TNF-α production was evaluated using the Quantikine system (R&D Systems).

3. RESULTS

Primary human monocytes/macrophages cells were reported to be susceptible to a productive infection by HCoV-OC43 and 229E. However, our results suggest that 229E[10] productively infects human monocytic cells, while OC43 infection is highly restricted. As shown in Table 1, monocytes and macrophages from most donors were susceptible to a productive infection, while lymphocytes appeared restrictive to infection. On the other hand, infectious OC43 virions were never detected in any leukocytic cell types (data not shown).

As the amount of HCoV-229E infectious virus detected dropped rapidly (Table 1), a short-term kinetics of virus production was performed on two independent donors. Results showed that the production of virus was transient (Figure 1A). Moreover, the THP-1 cell line, which represents an excellent model to study the interaction between HCoV-229E and human monocytic cells, appeared equally susceptible to infection by HCoV-229E (Figure 1B). Further investigation showed that these cells could be activated following infection as shown by an increased production of MMP-9 (Figure 1B) and by the release of TNF-α in the medium (Table 2).

Table 1. Susceptibility of human PBMC to infection by HCoV-229E at 37°C (MOI 1). Virus titers, log $TCID_{50}$/mL in cell culture medium.

Donor		1	2	3	4	5	6	7	8	9	10
Day pi											
1	Mono	3 ±0.6	≤0.5	3±0.2	3±0.5	3±0.2	3±0.5	4±0.7	4±0.2	3±0.0	4±0.4
	Macro	4 ±0.4	≤0.5	3±0.3	≤0.5	5±0.2	5±0.2	≤0.5	4±0.5	3±0.4	4±0.2
	Lympho	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
3	Mono	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
	Macro	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
	Lympho	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
5	Mono	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
	Macro	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
	Lympho	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
			≤0.5								
7	Mono	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
	Macro	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
	Lympho	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5

4. DISCUSSION

Because HCoV possess neuroinvasive properties, it is of great interest to investigate the possibilty that these ubiquitous human respiratory viruses can use leukocytes as a vehicle to gain access to the CNS. The study presented here provides interesting insights into a possible route taken by human coronaviruses to reach the CNS. Indeed, as human monocytic cells are susceptible to infection by HCoV-229E and as they are activated following this infection, they could have an important role in helping HCoV gain access to the CNS. Monocytic cells can on the one hand support virus replication, at least transiently, and they also produce MMP-9 and TNF-α. Therefore, they represent a suitable vector for viral transport to the CNS, as virally-activated monocytic cells can

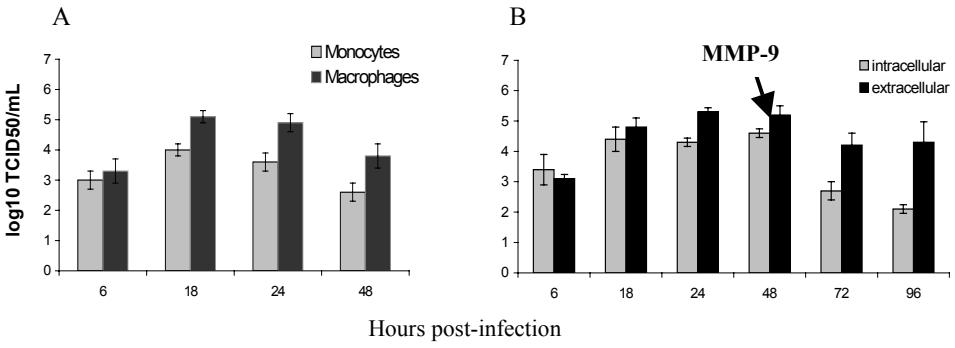

Figure 1. Primary human monocytic cells and the human monocytic cell line THP-1 are equally susceptible only to HCoV-229E. (A) Kinetics of infectious virus production in primary human monocytes and macrophages. (B) Kinetics of infectious virus production in THP-1 cells. The arrow indicates an increased MMP-9 activity at 48 hours postinfection.

Table 2. Production of TNF-α by THP-1 cells following infection by HCoV-229E (pg/mL).

Hours pi	**16**	**24**	**42**	**68**
Mock	0.0	0.0	0.0	0.0
HCoV-229E	0.0	29.2 ± 8	90.1 ± 5	104.6 ± 5

increase secretion of proMMP-9 (Figure 1B), therefore directly contributing to BBB breakdown[12] and TNF-α, which can facilitate the passage of monocytes through the BBB by upregulating the expression of ICAM-1.[13] Moreover, after gaining access to the CNS, the monocytes could release neurotoxic factors, therefore contributing to neurodegeneration.

5. REFERENCES

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