

## ASSOCIATION OF RANTES WITH THE REPLICATION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS IN THP-1 CELLS

D. Li<sup>1</sup>, N. Wu<sup>1</sup>, H. Yao<sup>1</sup>, A. Bader<sup>2</sup>, N. H. Brockmeyer<sup>2</sup>, P. Altmeyer<sup>2</sup>

<sup>1</sup>Institute of infectious disease, first affiliated hospital, medical school, Zhejiang University, Hangzhou, China,

<sup>2</sup>Klinik für Dermatologie und Allergologie der Ruhruniversität Bochum, Germany

### Abstract

**Background:** Severe acute respiratory syndrome (SARS) is a novel infectious disease which is characterized by an overaggressive immune response. Chemokines are important inflammatory mediators and regulate disease due to viral infection. In previous study, we found that SARS-CoV has the ability to replicate in mononuclear cells. In present work, we sought to characterize the replication of SARS-CoV at the presence of RANTES in THP-1 cells.

**Methods:** To determine whether RANTES play a role in the process of SARS, THP-1 cells were incubated with heat-inactivated SARS-CoV and ELISA was used to test RANTES levels in the supernants; Then the effect of dexamethasone on the induced secretion was evaluated. Real-time PCR was used to investigate the effect of RANTES on the replication of SARS-CoV *in vitro*. Macrophages, induced by THP-1 cells, were used as cell model.

**Findings:** Inactive SARS-CoV could induce THP-1 cells secrete RANTES and this increase effect could not be suppressed by DXM. RANTES itself could inhibit the replication of SARS-CoV in THP-1 cells when it was added into the culture before or at the same time with the virus; No inhibition effect was shown when RANTES were added into the culture after SARS-CoV infected the cells.

**Key words:** SARS-CoV; RANTES; THP-1 cell; Secretion; Replication

**Abbreviations:** SARS, severe acute respiratory syndrome; CoV, coronavirus; RANTES, Regulated on activation normal T expressed and secreted

### INTRODUCTION

Severe acute respiratory syndrome (SARS) is a newly recognized illness associated with infection from a novel coronavirus (SARS-CoV). The molecular basis of severe acute respiratory syndrome (SARS) coronavirus (CoV) induced pathology is still largely unclear. Many SARS patients show peripheral blood lymphopenia and inflammatory infiltrate acute lung injury, evidenced in a pronounced increase in macrophages in the alveoli and the interstitium of the lung. It is concluded that immunopathology plays a role in tissue damage, and cytokines are responsible for some signs of disease [1-2].

Learning from other coronavirus mediated diseases, such as mouse hepatitis virus (MHV) -induced central nervous system disease, CCR5 signaling accounts for those pathological changes [3]. Another clinical phenomenon is most SARS patients are Asians, which is similar to HIV-1 ethnic susceptibility [4-8]. Many HIV-1 experts suggest that the way SARS-CoV entry into target cells may closely resemble that which occurs in HIV entry [9-10]. Acute respiratory viruses commonly induce inflammatory chemokines such as RANTES, which can amplify inflammatory responses leading to immunopathology [11-12]. Altogether, a hypothesis rise up that RANTES may be of interest in study of SARS.

RANTES (regulated upon activation, normally T-cell expressed and presumably secreted) is a CC chemokine which recruits and activates monocytes, lymphocytes, and eosinophils, which present in the lung inflammatory infiltrate induced by SARS-CoV infection [13]. In previous study, we found, in addition to T and B lymphocytes, macrophages play a key role in this process [14]. In this study, we analyzed the interaction of SARS-CoV and RANTES in THP-1 monocytic cells which could be induced into macrophages by PMA.

### MATERIALS AND METHODS

#### 1. CELL CULTURE AND REAGENTS

Both Vero and THP-1 (human promonocytic leukemic cell line) cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum (HyClone), 100 U/ml penicillin, 100 µg/ml streptomycin (Invitrogen) and maintained at 37°C in a humidified 5% CO<sub>2</sub> atmosphere. THP-1 cells were induced to differentiate into macrophages by treatment with PMA (Sigma) (16nM) for three days.

#### 2. hRANTES

Recombinant human RANTES were stably expressed by CHO cell (constructed by our institute). Briefly, human RANTES gene was inserted into pCDNA3.1(+)/his vector according to the method described previously (30-32) to generate recombinant pCDNA3.1/RANTES. CHO cells were transfected with pCDNA3.1/RANTES and incubated with 500 µg/ml G418 (clontech). The effective high expressed cells

were selected as stock cells. Resultant culture supernatant, which contained hRANTES, was purified from culture supernatants by affinity chromatography on recombinant protein G columns (Gamma Bind Plus; Pharmacia/LKB) according to the manufacturer's instructions, using a Shimadzu automated preparative/ analytical high-performance liquid chromatography system. The protein concentration was calculated by ELISA. MTT assay was carried out to determine the working concentration. In our experiments, we used the concentration at 1000, 500, 100 pg/ml.

### 3. VIRUSES

Primary isolate of SARS-CoV used for these experiments was isolated from the patient (the sequence was indexed in Genbank). The isolate was propagated in Vero cells and had not undergone more than 10 passages in vitro to minimize the effects of culture adaptation on viral tropism.

Cultures of SARS-CoV-infected Vero cells were used as a source of infectious virus. Supernatants were cleared of cellular debris by high-speed centrifugation, and the viral titers were determined to be  $10^{3.5}$  to  $10^{4.5}$  50% tissue culture infective doses (TCID<sub>50</sub>)/ $10^6$  cells. All infectious experiments were done at a multiplicity of infection (MOI) of 100:1 (This viral titer was selected because severe cell damage was not observed with this viral titer).

Heat-inactivated SARS-CoV, prepared by heated at 80 °C for 30-min, was used for induced secretion assays due to safety and convenient reasons.

### 4. RANTES DETECTION BY ELISA.

Supernatants were stored at -20 °C until an assay for RANTES protein using the human RANTES Quantikine kit (R & D) was performed according to the manufacturer's instructions.

### 5. INFECTION OF CULTURED CELLS WITH SARS-CoV AT THE PRESENCE OF hRANTES

Macrophages were plated at  $5 \times 10^4$ /well in 24-well plate and grown overnight in the appropriate medium. Cells were washed with phosphate-buffered saline for three times, then used for the latter experiments. The purified hRANTES at different concentration were added into the cell culture before, or at the same time, or after SARS-CoV attaching macrophages. When RANTES and virus added at the media at the same time, SARS-CoV was preincubated with RANTES for 1 h at 37 °C in a total of 1 ml of 1640-10% FCS. To allowed the complex be adsorbed by the cells, the plates were gentle rocked of every 10 min for 2 h. After 2 h, the cells were washed extensively to remove unadsorbed viral particles and 2 ml of with fresh culture media was added. Infected cells were incubated at 37 °C under 5% CO<sub>2</sub> in humidified air. For all experiments, uninfected cultures served as negative controls. Culture supernatants and infected cells were harvested at two days postinfection and analyzed by real-time PCR.

### 6. REAL-TIME PCR

Real-time PCR was performed by using a GeneAmp 5700 system (Applied Biosystems) with a Platinum quantitative PCR kit (Bosai Co, China) and Sybr green detection. After an initial cycle of 30 min at 55 °C and 30 sec at 95 °C, cycling was performed for 45 cycles of 95 °C for 15 s, 60 °C for 30 sec and 72 °C for 30 sec.

### 7. STATISTICAL ANALYSIS

We used analysis of variance (ANOVA) for two different purposes. All ANOVAs were carried out with SPSS (release 11.0) procedure glm. P values of less than 0.05 were considered significant.

## RESULTS

### 1. CONCENTRATION OF RANTES PROTEIN IN THE MEDIUM OF MACROPHAGES WITH SARS-CoV

RANTES was detected in the culture medium of macrophage cells 24 h after incubation with inactive SARS-CoV, and significant high level of induced RANTES was shown compared with controls. To demonstrate that DXM, commonly used in clinic to manage this disease, is responsible for the observed negative effect on the inflammatory response to macrophages after SARS-CoV infection, different concentrations of dexamethasone (DXM) were added into the cell culture (Fig. 1). Contrasted to our expected, no difference was shown at all concentrations we used.

### 2. RANTES INFLUENCES THE REPLICATION OF SARS-CoV IN MACROPHAGES

RANTES inhibited the SARS-CoV replication in macrophages when it was added into the culture before virus attached the cells, and the maximum inhibition was found at the concentration of 1000ng/ml, while the inhibitory effect did not differ significantly between the concentrations of 100ng/ml and 500ng/ml. A more reduce of SARS-CoV replication could be seen as RANTES and virus added into the cell culture at the same time, although no significant was shown. When macrophages was contacted with virus first, the inhibitory effect of RANTES on the replication of SARS-CoV does not appear (Table 2, Fig. A-D).

## DISCUSSIONS AND CONCLUSIONS

The current data indicate that SARS-CoV could increase RANTES production by macrophages and this increase effect could not be suppressed by DXM. Potentially, the observation may due to the concentration limitation. Also, the negative inhibition effect could explain the prolonged, proinflammatory cell influx to sites of infection in clinic. In addition, the effect that this steroid had no effect on RANTES secretion is consistent with data from other researches which suggested DXM specific reduced cytokines secretion [15]. The mechanism underlying this observation is the subject of ongoing research.

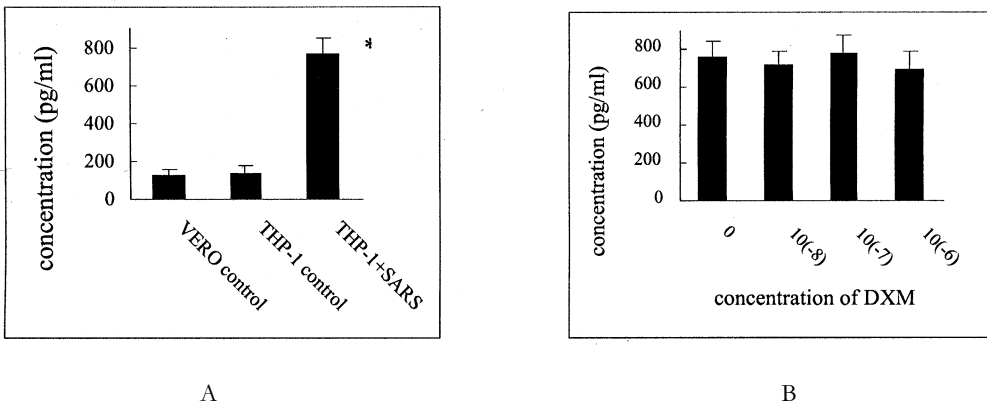
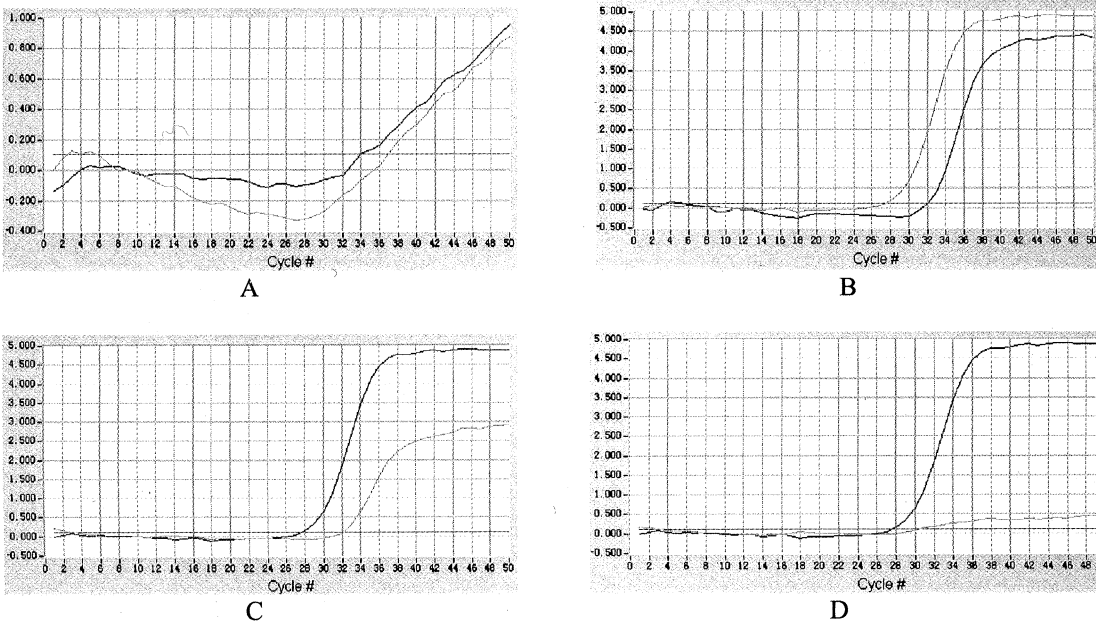


Fig. 1. RANTES induction by SARS-CoV in THP-1 cells with or without DXM. (A) without DXM. Macrophage cells ( $2 \times 10^5$ /ml of 10% DMEM/well), induced by THP-1 cells, in 24-well plates were incubated with heat-inactivated SARS-CoV for 24 h. Supernatants were then assayed for RANTES by ELISA. Supernatant from VERO cells culture was also used as a control. Data represent the mean  $\pm$  SD of triplicate samples from a representative result of 4 experiments. Induction was significant (\* $P < .001$ ) according to the Student 2-tailed t test. (B) with DXM. Cells were first incubated with a serial dilutions of DXM for 1 h, then incubated with heat-inactive SARS-CoV (MOI of 10) for 24 h. DXM has no effect on the chemotaxis of Macrophages induced by inactive SARS-CoV at all concentrations we used.

Table 2. Rantes added into the culture media at different stage, Real-time PCR detection the copies of SARS-CoV in macrophages.

Concentration pg/ml	1000	500	100
stage			
first added RANTES then virus	- *	$6.0_{10^3}$ *	$6.25_{10^3}$ *
added RANTES and virus at the same time	- *	$2.5_{10^3}$ *	$2.5_{10^3}$ *
first added virus then RANTES	$1.0_{10^4}$	$1.0_{10^4}$	$1.0_{10^4}$

note: '-', do not detection SARS-CoV genomic after 45 cycles amplification  
 \* vs positive controls ( $1.2_{10^4}$  copies/ml),  $P < 0.05$



A first added virus then RANTES (100ng/ml)	Black line: positive control
B first added RANTES (100ng/ml) then virus	Green line: tested group
C added RANTES (100ng/ml) and virus at the same time	
D added RANTES (1000ng/ml) and virus at the same time	

Added RANTES into the cell culture before SARS-CoV attacking THP-1 cells, the replication of SARS-CoV was markedly reduced as compared with the controls, especially RANTES at 1000pg/ml. A more reduce of SARS-CoV replication could be seen as RANTES and virus added into the cell culture at the same time, although no significant was shown. Based on the above results, we contribute the more inhibition to the synergistic effects of RANTES which are induced by SARS-CoV. Namely, the virus-induced secretion of RANTES by THP-1 cells helps initiate antiviral response. When THP-1 cells was contacted with virus first, the inhibitory effect of RANTES on the replication of SARS-CoV does not appear. The competitive inhibition, at least to some degree, arouses the hypothesis that RANTES could be used as a protective material.

RANTES is a pro-inflammatory cytokines in the pathogenesis of many diseases[12-12,16]. In this study, we illustrate RANTES itself could inhibit the replication of SARS-CoV in THP-1 cells when it was added into the culture before or at the same time with the virus, although the effect did not show when RANTES were added into the culture after SARS-CoV infected the cells. As for the role of RANTES in vivo, it is hard to elucidate. Since SARS-CoV may cause cytokine cascade, RANTES probably play important roles in amplifying inflammatory response and immunopathology of the disease. So it is more conceivable to screen the levels of RANTES and virus copies in the patients with subclinical or very mild disease.

#### REFERENCES

- Zhou YH, Chen Z. Is the immune system impaired in patients with severe acute respiratory syndrome? *Clin Infect Dis.* 2004;38(6):921-922
- Beijing Group of National Research Project for SARS. Dynamic changes in blood cytokine levels as clinical indicators in severe acute respiratory syndrome. *Chin Med J (Engl).* 2003 Sep;116(9):1283-1287
- Glass WG, Lane TE. Functional expression of chemokine receptor CCR5 on CD4(+) T cells during virus-induced central nervous system disease. *J Virol.* 2003;77(1):191-198
- Yuen KY. The SARS attack on Hong Kong. *Hong Kong Med J.* 2003;9(4):302-303
- Kaw GJ, Tan DY, Leo YS, et al. Chest radiographic findings of a case of severe acute respiratory syndrome (SARS) in Singapore. *Singapore Med J.* 2003;44(4):201-204
- Chan-Yeung M, Xu RH. SARS: epidemiology. *Respirology.* 2003;8 Suppl:S9-S14
- Lam WK, Zhong NS, Tan WC. Overview on SARS in Asia and the world. *Respirology.* 2003;8 Suppl:S2-S5
- Cohen J. Epidemiology. HIV gains foothold in key Asian groups. *Science.* 2001;294(5541):282-283
- Kliger Y, Levanon EY. Cloaked similarity between HIV-1 and SARS-CoV suggests an anti-SARS strategy. *BMC Microbiol.* 2003;3(1):20
- Yamamoto N, Yang R, Yoshinaka Y, et al. HIV protease inhibitor nelfinavir inhibits replication of SARS-associated coronavirus. *Biochem Biophys Res Commun.* 2004;318(3):719-725
- Lampinen M, Carlson M, Hakansson LD, et al. Cytokine-regulated accumulation of eosinophils in inflammatory disease. *Allergy.* 2004;59(8):793-805
- Hizawa N, Yamaguchi E, Konno S, et al. A functional polymorphism in the RANTES gene promoter is associated with the development of late-onset asthma. *Am J Respir Crit Care Med.* 2002;166(5):686-690
- Glass WG, Rosenberg HF, Murphy PM. Chemokine regulation of inflammation during acute viral infection. *Curr Opin Allergy Clin Immunol.* 2003;3(6):467-473
- Li L, Wo J, Shao J, et al. SARS-coronavirus replicates in mononuclear cells of peripheral blood (PBMCs) from SARS patients. *J Clin Virol.* 2003;28(3):239-244
- Thomas LH, Sharland M, Friedland JS. Steroids fail to down-regulate respiratory syncytial virus-induced IL-8 secretion in infants. *Pediatr Res.* 2002;52(3):368-372
- Kedzierska K, Crowe SM. Cytokines and HIV-1: interactions and clinical implications. *Antivir Chem Chemother.* 2001;12(3):133-150

*Received: January 14, 2005 / Accepted: January 21, 2005*

#### *Address foe correspondence:*

Prof. Dr. Norbert Brockmeyer  
 Department of Dermatology  
 Ruhr-University Bochum  
 St. Josef-Hospital  
 Gudrunstr. 56  
 D-44791 Bochum, Germany  
 Phone +49(0)234/5093470  
 Fax +49(0)234/5093472  
 e-mail n.brockmeyer@derma.de