

YL Lau 劉宇隆
JSM Peiris 裴偉士
HKW Law 羅嘉慧

Role of dendritic cells in SARS coronavirus infection

Key Messages

1. Severe acute respiratory syndrome coronavirus (SARS-CoV) entered and replicated in monocyte-derived dendritic cells (DCs), but virus replication was incomplete. In addition, SARS-CoV did not induce apoptosis or maturation of infected DCs.
2. SARS-CoV infected DCs showed low expression of antiviral cytokines (IFN- α , IFN- β , IFN- γ , and IL-12p40), moderate up-regulation of proinflammatory cytokines (TNF- α and IL-6), and significant up-regulation of inflammatory chemokines (MIP-1 α , RANTES, IP-10, and MCP-1).
3. SARS-CoV did not modulate gene expression of Toll-like receptors (TLR-1 to 10) but induced significant up-regulation of chemokine receptors (CCR-1, CCR-3, CCR-5).
4. SARS-CoV induced high expression of TRAIL but not FasL gene expression in DCs.
5. The characteristic phenotype of SARS-CoV infected DCs suggested some possible mechanisms of immune escape and amplification of immunopathology in SARS.

Introduction

In 2003, the severe acute respiratory syndrome coronavirus (SARS-CoV) caused severe, rapidly progressive atypical pneumonia with fever, myalgia, and diarrhoea.^{1,2} Viruses were detected in the respiratory tract, stool, and urine of patients indicating that SARS was a systemic disease. White pulp atrophy was noted in the spleen, and lymphoid depletion was noted in lymph nodes. Lymphopaenia and increasing viral load in the first 10 days of disease strongly suggested an evasion of the immune system by SARS-CoV.^{3,4}

Based on the function of dendritic cells (DCs) in immune surveillance, priming, and tolerance, DCs play an important role in the immunopathology of SARS. These cells are professional antigen-presenting cells linking innate and adaptive immunity. Immature DCs reside in the respiratory tract for immune surveillance and respond dynamically to local tissue inflammation in the airways and the distal lung. They express a wide range of c-type lectin receptors and Toll-like receptors (TLRs) for recognition of conserved pathogen patterns and induction of subsequent immune responses.

Some TLRs are expressed on the cell surface (TLR-1, TLR-2, TLR-4, TLR-5, TLR-6, TLR-10), whereas others are expressed in intracellular compartments (TLR-3, TLR-7, TLR-8, TLR-9). They are differentially expressed in different DC subsets and are modulated in response to a variety of stimuli. Viral proteins bind to TLR-2 or TLR-4; single stranded RNA binds to TLR-7 and TLR-8; double stranded RNA binds to TLR-3; and viral DNA binds to TLR-9. The binding of ligands to TLRs triggers the downstream signalling pathways that are involved in the cytokine release during primary induction of inflammation and secondary activation of anti-inflammatory mechanisms. Cross talks between TLRs are common, and the formation of TLR heterodimers allows a higher level of complexity in ligand-receptor binding and subsequent signalling.

Migration of DCs from peripheral tissues to lymph nodes is essential for antigen presentation and triggering of adaptive immune responses. The trafficking of DCs is regulated by chemokines in their microenvironment and their expression of chemokine receptors (CCRs). Differential expressions of CCRs are observed during DC maturation, and some viruses (such as herpes simplex virus) can block CCR expressions on DCs to alter their migratory properties.

Chemokines can be classified as homeostatic (constitutively expressed) or inflammatory (induced/augmented) according to their immune functions. Respiratory viruses commonly induce inflammatory chemokines (such as MIP-1 α /CCL3, RANTES/CCL-5, IP-10/CXCL10, and MCP-1/CCL2) in local tissues and DCs. There are redundancies in the interactions between chemokines and CCRs, as many different ligands bind the same receptor and many receptors bind the same ligand. For example, RANTES binds to CCR-1, CCR-3 and CCR-5, whereas MIP-1 α binds to CCR-1 and CCR-5.

Death receptors and their ligands also play important roles in innate and adaptive immune responses by regulating cell death and survival. Well-characterised death receptor ligands include TNF- α , FasL, and TRAIL/Apo2L. Several viruses (including measles virus, human immunodeficiency virus,

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The University of Hong Kong:
Department of Paediatrics and Adolescent
Medicine
YL Lau, HKW Law
Department of Microbiology
JSM Peiris

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Principal applicant and corresponding author:
Prof Yu Lung Lau
Department of Paediatrics and Adolescent
Medicine, Queen Mary Hospital, Li Ka Shing
Faculty of Medicine, The University of Hong
Kong, Pokfulam, Hong Kong SAR, China
Tel: (852) 2855 4482
Fax: (852) 2855 1523
Email: lauylung@hkucc.hku.hk

cytomegalovirus, and herpes simplex virus) have been shown to induce TRAIL expression on DCs. These 'killer DCs' may be involved in the killing of virus-infected cells or bystander lymphocytes and natural killer cells. Therefore, we aimed to determine if the expression of death receptor ligands on DCs can also be modulated by SARS-CoV.

Clinically, manifestation of SARS was less severe in children than adults. We hypothesised that the developmental status of the host immune system may affect the severity of acute respiratory diseases. We compared the effect of SARS-CoV on adult and cord blood DCs. SARS-CoV could enter DCs and alter their expression of cytokines, chemokines, TLRs, CCRs, and DLRs. There were possible mechanisms of immune escape and amplification of immunopathology in SARS.

Methods

This study was conducted from August 2005 to July 2007. We studied the effects of SARS-CoV on human monocyte-derived DC maturation, apoptosis, cytokines/chemokines expression, receptors expression, and death receptor ligands expression by flow cytometry and real time quantitative polymerase chain reaction (PCR).^{5,6}

Real-time quantitative PCR was used to analyse some immune-related genes based on the advantages that:

- (1) Less DCs are needed for total RNA extraction (0.25-0.5x10⁶ DCs per sample for real-time quantitative PCR versus 2-5x10⁶ DCs per sample for microarray analysis).
- (2) Point 1 above translated to the requirement of less SARS-CoV for infection. This is important for the researchers' safety when performing the experiments.
- (3) Even with the microarray data, the findings need

to be substantiated by increasing the sample size and comparing the gene expression by real-time quantitative PCR.

Results

As evident by electron microscopy and immunofluorescence staining, SARS-CoV could enter both immature and mature human monocyte-derived DCs. Viral replication in DCs was suggested by the detection of negative strands of SARS-CoV RNA. However, there was no increase in viral RNA over time. Using cytopathic assays, SARS-CoV was not detected in DCs and cell culture supernatant. This confirmed that virus replication was incomplete. SARS-CoV did not induce apoptosis or maturation of DCs. SARS-CoV-infected DCs showed low expression of antiviral cytokines (IFN- α , IFN- β , IFN- γ , and IL-12p40), moderate up-regulation of proinflammatory cytokines (TNF- α and IL-6), and significant up-regulation of inflammatory chemokines (MIP-1 α , RANTES, IP-10, and MCP-1).⁵ SARS-CoV did not modulate Toll-like receptors (TLR-1 to 10) gene expression, but induced significant up-regulation of chemokine receptors (CCR-1, CCR-3, CCR-5). There was strong induction of TRAIL but not FasL gene expressions in SARS-CoV-infected DCs.

Discussion

The role of immune evasion in the severity and immunopathology of SARS has been supported.²⁻⁴ In this study, DCs, which are the key antigen-presenting cells, played crucial roles in anti-viral immune responses and may have involved in some immune escape mechanisms specific for SARS-CoV (Table). Particularly, up-regulation of chemokines and death receptor ligands may have contributed to infiltration of cells into the lungs

Table. Possible mechanism of immune evasion in SARS patients

Parameter	Observations from this study ^{5,6}	Possible mechanism of immune evasion ^{3,4}	Reported pathology in SARS patient ^{1,2}
Permissiveness	SARS-CoV can enter DCs	Use DCs as vehicle or 'Trojan horse'	Dissemination of virus to multiple organs
Viral replication	SARS-CoV replication is incomplete	-	-
Dendritic cell (DC) survival	SARS-CoV did not induce DCs apoptosis	-	-
DC maturation	SARS-CoV did not induce maturation	Escape from adaptive immune response; induction of regulatory DC and regulatory T cells	Low antibody production in the first 10 days
Anti-viral cytokines	Low interferons and IL-12	Block anti-viral responses	Low antibody production in the first 10 days of SARS
Proinflammatory cytokines	Moderate expression of TNF- α and IL-6	-	-
Inflammatory chemokines	$\uparrow\uparrow\uparrow$ MIP-1 α , RANTES, IP-10, and MCP-1	Increased cell trafficking and DC trafficking	$\uparrow\uparrow$ mononuclear inflammatory cell infiltration in lungs
Toll-like receptors	No induction	-	-
Chemokine receptors	\uparrow CCR-1 & CCR-5; $\uparrow\uparrow$ CCR-3; no change in CCR-7	Increase DC trafficking; suppress activated T cells or induce regulatory T cells	Reduced immune responses
Death receptor ligands	Low FasL; strongly up-regulated TRAIL	Killing cells at the site of infection and in lymphoid organs	Lymphopaenia; white pulp atrophy in spleen and lymphoid depletion in lymph nodes

and lymphopaenia, respectively. Further studies into the mechanisms of newly emerged viruses to evade the innate immune responses are necessary.

There was significant induction of RANTES and its expression of corresponding receptors CCR-1, CCR-3, and CCR-5 mRNA in SARS-CoV-infected DCs. Our previous gene association study has shown a higher death rate in SARS patients who have inherited the high-production gene allele of RANTES.⁷ Further investigations into the therapeutic strategies that can reduce RANTES production are warranted.

The up-regulation of TRAIL gene expression in both adult and cord blood DCs after SARS-CoV infection represented a killer DC phenotype. This up-regulation is similar to our observation in macrophages infected by avian influenza virus. Further investigation is needed to confirm the cytotoxic function of SARS-CoV-infected DCs on immune cells, and to help design therapeutic strategies that reduce TRAIL gene expression, neutralise TRAIL, or block signalling of TRAIL receptors, so as to reduce lymphopaenia.

Comparing adult and cord blood DCs, both the basal and SAR-CoV-induced gene expression levels of chemokines and CCR genes were significantly higher in the latter. Based on the function of chemokines on cell trafficking, more severe infiltration of cells into the lungs was expected in children. On the contrary, SARS was less severe in children than adults. The age-dependency of SARS severity merits further studies to elucidate the underlying mechanisms. As the information on the chemokine and CCR expression and function in children is scanty, further studies are warranted.

Conclusions

Dendritic cells played an important role in the pathogenesis of SARS. The lack of antiviral cytokine response, intense chemokine up-regulation, induction of CCR expression and strong expression of TRAIL observed in SARS-CoV-infected DCs suggested possible mechanisms of immune escape and amplification of immunopathology in SARS.³

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