Interaction of severe acute respiratory syndrome-associated coronavirus with dendritic cells

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Severe acute respiratory syndrome (SARS) of humans is caused by a novel coronavirus of zoonotic origin termed SARS-associated coronavirus (SARS-CoV). The virus induces severe injury of lung tissue, as well as lymphopenia and destruction of the architecture of lymphatic tissue by as-yet-unknown mechanisms. In this study, the interaction of SARS-CoV with dendritic cells (DCs), the key regulators of immune responses, was analysed. Monocyte-derived DCs were infected with SARS-CoV and analysed for viability, surface-marker expression and alpha interferon (IFN-α) induction. SARS-CoV infection was monitored by quantitative RT-PCR, immunofluorescence analysis and recovery experiments. SARS-CoV infected both immature and mature DCs, although replication efficiency was low. Immature DCs were activated by SARS-CoV infection and by UV-inactivated SARS-CoV. Infected DCs were still viable on day 6 post-infection, but major histocompatibility complex class I upregulation was missing, indicating that DC function was impaired. Additionally, SARS-CoV infection induced a delayed activation of IFN-α expression. Therefore, it is concluded that SARS-CoV has the ability to circumvent both the innate and the adaptive immune systems.

INTRODUCTION

A new form of life-threatening, atypical pneumonia called severe acute respiratory syndrome (SARS) has emerged recently as a human disease involving over 8000 cases and 774 deaths in 30 countries, mainly in the Republic of China (WHO, 2004). A novel virus most probably of zoonotic origin, termed SARS-associated coronavirus (SARS-CoV), was isolated from patients and identified as the aetiological agent (Drosten et al., 2003; Fouchier et al., 2003; Ksiazek et al., 2003; Kuiken et al., 2003; Peiris et al., 2003a). Until then, human coronaviruses had been found to be associated with mild upper respiratory-tract infections. SARS-CoV, however, leads to a severe clinical outcome and has been isolated from stool and urine samples, indicating systemic infection (Peiris et al., 2003a).

Autopsies of patients who died of SARS-CoV infection revealed severe alveolar damage of the lungs and heavy injury of the lymphatic tissue (Ding et al., 2003, 2004; Lang et al., 2003; Nicholls et al., 2003). The latter includes massive necrosis in the white pulps and the marginal sinus, destruction of germinal centres and apoptosis of lymphocytes, accompanied by an infiltration of monocytic cells. These changes are strong evidence that immunopathogenesis is driving the severe outcome of the disease.

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Furthermore, infection with SARS-CoV of iDCs and the fibroblast cell line 293 leads to a delayed expression of alpha interferon (IFN-α), indicating that SARS-CoV circumvents the activation of the innate immune system.

Encountering virulent or UV-inactivated SARS-CoV, DCs were activated, but lacked major histocompatibility complex (MHC) class I upregulation. This indicates that mechanisms to escape the adaptive immune system are involved in the pathogenesis of SARS-CoV infection.

METHODS

Cells and viruses. Vero E6 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) plus 10% fetal calf serum (FCS) supplemented with 100 IU penicillin and 100 μg streptomycin ml⁻¹.

For virus-stock generation, Vero E6 cells were grown in cell-culture flasks until they reached 80% confluence. The growth medium was removed and the cells were inoculated with 0.01 m.o.i. SARS-CoV strain FFM-1 in 5 ml infection medium (DMEM, 2% FCS, 20 mM HEPES). After incubation for 1 h at 37 °C, the virus inoculum was removed and replaced by regular growth medium. At 72 h post-infection, the virus supernatants were harvested and cell debris was removed by centrifugation (3000 g for 5 min at 4 °C). Virus stocks were stored at −80 °C and thawed immediately before use. Virus titres were determined by a standard plaque assay as described previously (Spiegel et al., 2004).

Generation and infection of iDCs and mDCs. DCs were prepared from peripheral blood mononuclear cells (PBMCs) of healthy individuals as described by Sallusto & Lanzavecchia (1994). PBMCs were purified by Ficoll gradients (Pharmacia). The adherent-cell fraction was further purified by using anti-CD2 and anti-CD19 immunomagnetic beads (Dynal). iDCs were produced by culturing 5 × 10⁵ cells ml⁻¹ in 90% RPMI 1640 medium, 10% FCS, 2 mM glutamine, 100 IU penicillin ml⁻¹ and 100 μg streptomycin ml⁻¹ for 7 days in the presence of 50 ng granulocyte–macrophage colony-stimulating factor ml⁻¹ (Leukomax; Novartis Pharma) and 500 U IL-4 (Glenillex). For maturation, a mixture of 10 ng IL-1α (Leukomax; Novartis Pharma) and 1000 U IL-6 ml⁻¹ (Shandon) was added to the culture medium.

iDCs and mDCs were infected with SARS-CoV (m.o.i. of 5) by adding infectious supernatant of SARS-CoV-infected Vero cells to their growth medium.

RNA extraction, quantitative SARS-CoV Taqman RT-PCR, IFN-α and γ-actin RT-PCR. For RNA extraction, SARS-CoV-infected, mock-infected and UV-inactivated SARS-CoV-incubated iDCs, mDCs and 293 cells were collected at the indicated time points after infection and RNA was isolated by TRIzol extraction (Invitrogen).

SARS-CoV Taqman RT-PCR was used to determine the viral load and to measure the increase of viral RNA. All Taqman assays were performed on 5 μl RNA extract, with 15 pmol primers (NCCORFP, 5'-TGGCTCTGCTCCCTTGGGA-3'; NCCORRP, 5'-TAATGCAGGATCTTCCG-3') and 10 pmol probe (NCCORRP, FAM-5'-CAGCCATTGCGATGAGTGCA-3'-AMRA) (TIB MolBiol) in a final volume of 20 μl using a Lightcycler RNA Master Hybridization Probes kit (Roche). The Taqman RT-PCR was performed at 61 °C for 20 min, 95 °C for 5 min and 45 cycles of 95 °C for 15 s and 60 °C for 30 s (Weidmann et al., 2004).

For IFN-α and γ-actin RT-PCR, 1 μg RNA of each sample was subjected to treatment with DNase I (MBI Fermentas) followed by reverse transcription with SuperScript II (Invitrogen) using random-hexamer primers (Amersham Pharmacia Biotech). Amplification reactions were performed with 4 μl aliquots of each reverse transcription reaction with 10 pmol primers specific for IFN-α (forward primer, 5'-TCCATGGAAGTGCAGCAGC-3'; reverse primer, 5'-ATTGCCT-TCTGCAACCTCCC-3') detecting the multiple subtypes of IFN-α (Larrea et al., 2001) or primers specific for γ-actin (forward primer, 5'-GCCGTGCAATGGAGAAAAGA-3'; reverse primer, 5'-CATGG-CGCGGTTGTTGAAGGT-3') (Sigma-Ark). The mixture reactions were subjected to an initial denaturation step for 2 min at 94 °C. Then, 0.25 U recombinant Taq polymerase (Eppendorf) was added and 35 cycles of denaturation (94 °C for 30 s), annealing (56 °C for 1 min) and extension (72 °C for 1 min) were performed, followed by a final extension step at 72 °C for 10 min. The amplification products were separated on a 2% agarose gel containing 50 ng ethidium bromide ml⁻¹ and visualized by UV transillumination in a Chemidoc XRS imager (Bio-Rad).

Immunofluorescence microscopy. DCs (5 × 10⁵) were harvested at different time points post-infection, washed in 5 ml PBS (Ca²⁺⁻⁻ and Mg²⁺⁻⁻ free) and fixed in 5% paraformaldehyde for 10 min. Then, the cells were resuspended in 100 μl PBS and attached to SuperFrost Plus microscope slides (Shandon) by centrifugation for 2 min at 900 r.p.m. at high acceleration in a Cytospin 2 centrifuge (Shandon). Mouse mAb CMRF-56 (kindly provided by Derek Hart, Mater Medical Research Institute, Brisbane, Australia) was used for staining of DCs. For detection of viral nucleoprotein, cells were incubated with 1:1000-diluted anti-SARS-CoV N rabbit polyclonal antibody (Spiegel et al., 2005). Counterstaining for cell nuclei was performed with 1:200-diluted TO-PRO-3 iodide (Molecular Probes). After incubation for 1 h at room temperature in a humidified chamber, the cell samples were washed three times in PBS, followed by incubation with FITC-conjugated goat anti-mouse IgG1 and Cy3-conjugated donkey anti-rabbit IgG at a dilution of 1:200. The samples were again washed three times in PBS and then mounted by using FluorSave reagent (Calbiochem). Apoptotic cell death was monitored by TUNEL assay according to the manufacturer’s instructions (Roche). Stained cell samples were examined by using a Leica confocal laser-scanning microscope with a ×63 NA1.4 objective (detection of viral nucleoprotein) or a ×10 objective (TUNEL assay).

Flow-cytometry analysis. iDCs and mDCs, infected, mock-infected or incubated with UV-inactivated SARS-CoV, were collected at days 1, 4 and 6 after infection, washed in PBS and incubated with one or two of the following mAbs: anti-CD1a–FITC (HI149; BD Pharmingen), anti-CD14–PE (M5E2; BD Pharmingen), anti-CD40–FITC (5C3; BD Pharmingen), anti-CD54–FITC (84H10; Immunotech), anti-CD58–PE (AICDS8; Immunotech), anti-CD80–FITC (BB1; BD Pharmingen), anti-CD83–PE (HB15; Immunotech), anti-CD86–PE (IT22; BD Pharmingen), anti-MHC class I–PE (G46-2.6; BD Pharmingen) and anti-MHC class II–FITC (Tu39; BD Pharmingen). The samples were fixed with 5% paraformaldehyde for 30 min before they were analysed on a FACSort (Becton Dickinson) using CellQuest Pro software.

RESULTS

SARS-CoV replicates in iDCs and mDCs

iDC and mDC cultures were infected at an m.o.i. of 5. At day 3 post-infection, quantitative SARS-CoV Taqman PCR (Weidmann et al., 2004) was used to investigate virus replication in SARS-CoV-infected DCs. An increase of viral RNA
molecules could be observed in SARS-CoV-infected iDCs and mDCs. No increase was documented in mock-infected controls and when UV-inactivated SARS-CoV was used (Fig. 1a).

In addition, virus replication was shown by immunofluorescence assays detecting expression of the viral N protein at days 1–6 post-infection, as outlined in Fig. 1(b). It should be noted, however, that N protein expression was reduced strongly at days 4 and 6 post-infection compared with day 1. To confirm that the infected cells were DCs, cells were additionally stained for the 96 kDa early activation/differentiation antigen, which is expressed specifically by different DC populations, including monocyte-derived DCs (Highton et al., 2000; Hock et al., 1999). Indeed, nearly all of the SARS-CoV-infected cells expressed the 96 kDa early activation/differentiation antigen, indicating that the infected cells were DCs (Fig. 1b).

Apparently, DCs are susceptible to SARS-CoV infection. To investigate whether they support production and release of progeny virus, we determined the titre of supernatants of SARS-CoV-infected DC cultures by a standard plaque assay (Spiegel et al., 2004). We obtained low but reproducible titres (around 100 p.f.u. ml⁻¹) at day 6 post-infection, indicating low-level replication of SARS-CoV in DCs (Fig. 2). To rule out the possibility that the observed titres represented residual input virus, we determined in parallel the long-term stability of SARS-CoV by inoculating growth medium with virus stock and testing for infectivity at different time points. At day 6, no viral infectivity remained, whereas supernatants of SARS-CoV-infected DCs were still infectious (Fig. 2).

As the titres obtained directly from supernatants of infected DCs were low, we additionally performed recovery experiments. To this aim, intact SARS-CoV-infected DCs, as well as supernatants and cell lysates of SARS-CoV-infected DCs, were collected at day 6 after infection. To detect infectious virus, Vero cells were co-cultivated with the collected DCs or were incubated with the DC supernatants or cell lysates. After 3 days, the supernatants of the indicator Vero cells were then tested for infectious virus by plaque assay. We could detect infectious virus in all three experimental settings, indicating that DCs are infected productively with SARS-CoV (Table 1). However, the viral titres obtained from the recovery experiments using DC supernatants were lower (≥ 10⁶ p.f.u. ml⁻¹) than the viral titres obtained with

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Fig. 1. (a) Viral load in SARS-CoV-infected DCs as measured by quantitative real-time RT-PCR. Uninfected, SARS-CoV-infected and UV-inactivated SARS-CoV-incubated iDCs and mDCs (m.o.i. of 0.5) were harvested at day 3 post-infection. The RNA of 2 x 10⁶ DCs was isolated and the number of SARS-CoV nucleocapsid RNAs was detected in a quantitative RT-PCR. The relative numbers of viral RNA of one representative experiment are given. Error bars show the intra-assay variation of the RT-PCR. (b) Replication of SARS-CoV in infected iDCs. SARS-CoV-infected iDCs were cultivated for 1, 4 and 6 days post-infection or were left untreated. Cytospin preparations of the infected cultures were used for detection of SARS-CoV N protein by immunofluorescence. Green, DC-specific 96 kDa early activation/differentiation antigen (mouse mAb CMRF-56 followed by FITC-conjugated goat anti-mouse IgG); red, SARS-CoV N protein (anti-SARS-CoV N rabbit polyclonal antibody followed by Cy3-conjugated donkey anti-rabbit IgG); blue, cell nuclei (TO-PRO-3 iodide).

Fig. 2. Virus titres of DC supernatants and long-term stability of SARS-CoV. Titres of supernatants of SARS-CoV-infected iDCs (m.o.i. of 5) were determined at days 1, 4 and 6 post-infection (bars). In parallel, residual infectivity of SARS-CoV incubated in cell-culture medium was determined at the indicated time points (curve). Data are representative of three experiments with similar results.
DC lysate or whole DCs \((\geq 10^7\) p.f.u. ml\(^{-1}\)). In summary, these data suggest that productive virus replication occurred in DCs, albeit at a low level.

**Virus replication does not induce cell death of DCs**

To investigate the consequences of SARS-CoV infection for iDCs, we monitored cell death by light microscopy and TUNEL assay. As shown in the upper panel of Fig. 3, no difference in cell numbers was observed at day 6 post-infection when mock-infected cells, infected cells and cells inoculated with UV-inactivated virus were compared. Furthermore, no signs of apoptosis were detected by TUNEL staining in the corresponding Cytospin samples (Fig. 3, lower panel), indicating that SARS-CoV replication does not induce apoptotic cell death in DCs. Interestingly, SARS-CoV-infected cultures and cultures incubated with UV-inactivated virus exhibited a higher number of adherent cells than non-infected cultures. This indicates that SARS-CoV may mediate activation and maturation of iDCs (see below).

**SARS-CoV infection induces IFN-\(\alpha\) expression**

Type I IFNs are key components of the innate immune system. They represent the first line of defence against viral infections and restrict the growth and replication of a number of viruses, including coronaviruses (Cinatl \textit{et al.}, 2003; Fuchizaki \textit{et al.}, 2003; Haagmans \textit{et al.}, 2004; Hensley \textit{et al.}, 2004; Kawamoto \textit{et al.}, 2003; Pei \textit{et al.}, 2001; Spiegel \textit{et al.}, 2004). Therefore, we investigated whether SARS-CoV infection of DCs activates the expression of IFN-\(\alpha\). iDCs were infected with SARS-CoV (m.o.i. of 5) and IFN-\(\alpha\) expression was measured 24 and 48 h post-infection by using RT-PCR. Both treatment with UV-inactivated virus and infection with replicating virus led to the production of detectable levels of IFN-\(\alpha\)-specific transcripts 24 h post-infection (Fig. 4, upper panel). Signals specific for IFN-\(\alpha\)

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**Table 1. Virus recovery from SARS-CoV-infected DCs**

iDCs were infected with SARS-CoV (m.o.i. of 5) or were left untreated. DCs, DC supernatant and DC lysate were collected at day 6 post-infection and recovery experiments were performed by using Vero cells as indicator cells. Titres of recovered virus were determined by plaque assay. –, Not detectable; ++, virus titre \(\geq 1 \times 10^6\) p.f.u. ml\(^{-1}\); ++++, virus titre \(\geq 1 \times 10^7\) p.f.u. ml\(^{-1}\).
could no longer be observed 48 h post-infection, indicating the downregulation of IFN-\(\gamma\) expression at later time points of infection. Similar experiments were performed with a clone of the IFN-competent fibroblast cell line 293, which supports efficient SARS-CoV replication (Spiegel et al., 2005). In contrast to the infection of DCs, infection of 293 cells with SARS-CoV led to a sustained activation of IFN-\(\gamma\) expression, which was even more pronounced at 48 h post-infection (Fig. 4, lower panel). Taken together, these findings demonstrate that SARS-CoV infection activates IFN-\(\gamma\) expression in both DCs and 293 cells; however, the activation is only transient in the case of SARS-CoV-infected DCs.

**Phenotype of DCs in SARS-CoV infection**

To further investigate the interaction between DCs and SARS-CoV, we analysed the expression of antigen-presenting molecules (MHC class I, MHC class II and CD1a), costimulatory molecules (CD40, CD80 and CD86), adhesion molecules (CD54 and CD58), the maturation marker CD83 and the LPS receptor CD14 by flow-cytometry analysis. For mDCs, similar expression patterns were observed for uninfected cells and for cells either incubated with UV-inactivated SARS-CoV or infected with SARS-CoV at days 1, 4 and 6 after infection (data not shown). For iDCs, SARS-CoV infection had a clear effect. When compared with uninfected iDCs, enhanced expression of CD40, CD54, CD58, CD80, CD83, CD86 (Fig. 5b) and MHC class II (Fig. 5a) was detected at day 4 after infection, whereas no differences were observed for MHC class I, CD1a (Fig. 5a) or CD14 (Fig. 5b). Similar results were obtained at days 1 and 6 after infection (data not shown). Interestingly, iDCs incubated with UV-inactivated SARS-CoV displayed the same expression pattern as SARS-CoV-infected iDCs, indicating that productive infection is not necessary for the upregulation of surface-molecule expression. The expression pattern of iDCs that came into contact with SARS-CoV particles is typical of mature DCs and the lack of CD14 expression shows clearly that the cell populations analysed did not differentiate to macrophages during cell culture. Therefore, we conclude that SARS-CoV is able to activate iDCs. However, the missing upregulation of antigen-presenting MHC class I molecules indicates that the virus is able to impair the function of DCs.

**DISCUSSION**

SARS was the first emerging infectious disease of the 21st century. It was introduced from an animal reservoir and met an immunologically naïve human population. There is evidence that, despite severe lung injury, the virus hardly activates the immune system, leading to an impairment of immunity (Ding et al., 2003, 2004).

Here, we investigated whether DCs, the key players of antigen presentation, could be involved in SARS-CoV pathogenesis and whether virus-driven immune-escape mechanisms contribute to viral pathogenesis.

The replication of SARS-CoV in DCs was shown by detection of viral RNA and the expression of viral proteins using quantitative real-time RT-PCR and immunofluorescence assays, respectively. The results were confirmed by plaque assay using supernatants of infected DCs and recovery experiments using supernatants of infected DCs, DC cell lysate and co-culture experiments. Infection could be detected in all experimental settings. Because viral titres
were much lower when supernatants of DCs were tested directly for infectious virus compared with titres obtained in recovery experiments, we concluded that only a low-level replication of SARS-CoV occurred in DCs. To confirm that it was not just input virus that we recovered from the cells, we demonstrated that all input virus infectivity was destroyed at the time point that our titration experiments were performed. In line with our results, a recent study reported low SARS-CoV titres (10<sup>4</sup> TCID<sub>50</sub> ml<sup>-1</sup>) in supernatants of infected DCs 5 days post-infection, whereas no virus at all could be detected in supernatants of infected macrophages (Tseng et al., 2005). However, we cannot formally rule out the possibility that DCs may stabilize input virus instead of supporting complete replication. Nevertheless, we think that this is less likely, as we could detect viral RNA at day 3 post-infection, as well as expression of viral nucleoprotein in SARS-CoV-infected DCs up to day 6 post-infection.

It may well be that SARS-CoV uptake into DCs is mediated by macropinocytosis. The functional SARS-CoV receptor ACE-2, described recently (Li et al., 2003), is not expressed on DCs and thus cannot be involved (Hofmann & Pohlmann, 2004; Law et al., 2005). Our results suggest that other receptor molecules are involved in virus uptake. The entry of SARS-CoV into DCs may be mediated through C-type lectins, such as CD209 (DC-SIGN), CD209L (L-SIGN) or CD206 (mannose receptor) (Jeffers et al., 2004; Marzi et al., 2004; Yang et al., 2004). The S protein of SARS-CoV contains mannose structures (Han et al., 2004) and retroviral vectors pseudotyped with SARS-CoV S protein can enter DCs via CD209 (Yang et al., 2004). Therefore, certain C-type lectins might serve as an alternative receptor for the cellular entry of SARS-CoV, which has indeed been shown for CD209L (Jeffers et al., 2004).

The human coronavirus 229E, which is related to SARS-CoV, is known to induce apoptosis in monocytes/macrophages (Collins, 2002). For SARS-CoV, however, we could not observe any cell death of infected DCs during an infection period of 6 days. Thus, the immune dysfunction observed in SARS-infected humans is probably not due to SARS-CoV-mediated cell death of DCs.

Viruses have acquired many different mechanisms to escape the immune attack of the host (Alcami & Koszinowski, 2000; Beck et al., 2003; Weber et al., 2004). Here, we investigated whether SARS-CoV has developed immune-evasion mechanisms to modulate the innate and the specific immune responses. Surprisingly, treatment of iDCs and 293 cells with UV-inactivated virus was sufficient to induce IFN-α, suggesting that SARS-CoV replication is not necessary for the activation of IFN-α expression. Replication-competent virus, however, induced a stronger IFN-α signal in both iDCs and 293 cells. Interestingly, induction of IFN-α in iDCs at 24 h post-infection was no longer present at 48 h post-infection. In contrast, in SARS-CoV-infected 293 fibroblast cells, the induced IFN-α expression persisted for at least 48 h post-infection, but the virus replicated in these cells to high titres, despite IFN-α expression. This might be explained by different kinetics of virus replication versus IFN-α expression. We have shown previously that SARS-CoV infection of 293 cells does not induce IFN-β for up to 16 h after infection (Spiegel et al., 2005) and the same applies for IFN-α (unpublished data). As the virus replication cycle of SARS-CoV is completed in approximately 6 h (Ng et al., 2003), the virus simply appears to replicate to high titres well before type I IFNs are induced. Thus, IFN-α expression, presumably induced by replicating virus, occurs too late to hamper efficient virus production. Whether this delayed activation of type I IFN expression requires the action of a virus-encoded IFN antagonist remains to be determined.

SARS-CoV infection of DCs is much less effective than infection of 293 cells, probably due to the lack of the authentic SARS-CoV receptor ACE-2. The induction of IFN-α at 24 h post-infection appears to be sufficient to restrict virus growth in DCs, which is in line with the observation of a strongly reduced expression of viral nucleoprotein at days 4 and 6 post-infection. Apparently, the restriction of virus growth leads to the downregulation of IFN-α observed at 48 h post-infection. Nevertheless, SARS-CoV was able to infect DCs productively, as demonstrated by the successful transfer of infectious virus into susceptible Vero cells. In summary, SARS-CoV has developed mechanisms to induce a delayed response of the innate immune system in both 293 fibroblast cells and DCs, which allows the production of infectious progeny virus in both cell types.

Immature DCs undergo maturation and migrate to lymphatic tissue after uptake of pathogens or antigen. As SARS-CoV replicates in these cells, iDCs may play a key role in promoting viral dissemination within the host, offering a shuttle for the virus to enter the lymphatic tissue. This might contribute to the severe damage seen in the lymphatic tissues obtained from SARS-CoV-infected subjects (Ding et al., 2003).

To study the influence of SARS-CoV infection on DC function, we analysed the expression of antigen-presenting molecules, costimulatory molecules, adhesion molecules and maturation markers. With the marked exception of MHC class I upregulation, SARS-CoV-infected iDCs were clearly activated. In contrast, two other studies (Law et al., 2005; Ziegler et al., 2005) reported the lack of enhanced CD83, CD86 and MHC class II expression in SARS-CoV-infected DCs and it was postulated that an abortive SARS-CoV infection might prevent the activation of iDCs (Ziegler et al., 2005). Productive replication, however, is not required, as we observed activation of iDCs even when UV-inactivated SARS-CoV was used and similar results were obtained for DCs treated with γ-irradiated SARS-CoV (Tseng et al., 2005).

Interestingly, neither replication-competent nor UV-inactivated SARS-CoV induced MHC class I surface expression. As virus replication is not a prerequisite for the inhibition of MHC class I upregulation, one may speculate...
that high viraemia per se may enhance the impairment of antigen-presenting cells. This may be due to a bystander effect mediated by viral antigen. This hypothesis could explain in part the immune dysfunction seen in the course of human SARS-CoV infection. Indeed, a lack of MHC class I upregulation, together with a complete lack of cytokine expression, was observed when PBMCs derived from SARS patients were analysed (Regunathan et al., 2005). The molecular mechanisms driving the inhibition of MHC class I upregulation remain to be elucidated.

Taken together, our studies demonstrated that SARS-CoV has the ability to circumvent the innate as well as the adaptive immune system. The transport of virus to the lymphatic tissue by infected DCs followed by the infection of susceptible target cells might play a crucial role in the impairment of the immune response seen in SARS patients. Identification of the underlying mechanisms may help to develop effective strategies for the treatment of SARS.

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