High-dose hydrocortisone reduces expression of the pro-inflammatory chemokines CXCL8 and CXCL10 in SARS coronavirus-infected intestinal cells

JINDRICH CINATL Jr, MARTIN MICHAELIS, BIRGIT MORGENSTERN and HANS WILHELM DOERR

Institut für Medizinische Virologie, Klinikum der J.W. Goethe-Universität, Paul Ehrlich Str. 40, D-60596 Frankfurt am Main, Germany

Received August 30, 2004; Accepted October 22, 2004

Abstract. Clinical observations and our high-density oligonucleotide microarray results demonstrated increased expression of proinflammatory chemokines after SARS-CoV infection. Here, we investigated the influence of SARS-CoV infection on CXCL8 (interleukin 8) and CXCL10 (interferon-γ-inducible protein 10) in human intestinal epithelial (Caco2) cells. RT-PCR and ELISA showed time-dependent up-regulation of both chemokines after SARS-CoV infection. Electric mobility shift assay revealed increased DNA binding activity of the cellular transcription factors activator protein 1 (AP-1) and nuclear factor (B (NF-κB) in SARS-CoV infected cells. High hydrocortisone concentrations (≥50 μg/ml) completely prevented increased DNA binding activity of AP-1 and NF-κB and inhibited up-regulation of CXCL8 and CXCL10, but did not reduce chemokine expression to basal levels. Ribavirin that does not inhibit SARS-CoV replication in Vero cells inhibited SARS-CoV replication in Caco2 cells at therapeutical concentrations. Hydrocortisone neither influenced SARS-CoV titres alone nor in combination with ribavirin. Our results show that corticosteroids may be of limited benefit in the suppression of chemokine production by SARS-CoV-infected cells.

Introduction

Severe acute respiratory syndrome (SARS) is caused by infection with the SARS coronavirus (SARS-CoV) (1,2). Approximately 25% of patients with SARS progress to severe respiratory failure with characteristics of acute respiratory distress syndrome (ARDS) (3,4). Pathologic findings demonstrated SARS to be a systemic disease that injures many organs (5,6) including the gastrointestinal tract, with diarrhoea being a common feature (7,8).

The clinical course of SARS can be divided in two phases. The first phase is characterised by viral replication (cytolytic activity) that results in tissue damage. In the second phase SARS progresses to respiratory failure in a notable proportion of patients despite lowering of virus load (9). These findings suggest an immunopathological nature of the second phase of SARS characterised by immune-mediated lung injury. Clinical investigations revealed an imbalance of inflammatory cytokines/chemokines in SARS patients (10,11).

Corticosteroids were used clinically with the aim of suppressing cytokine/chemokine storm and to stop disease progression (12). Their use often resulted in clinical improvement (9,12-17). Nevertheless, the value of corticosteroid treatment is still judged contradictory regarding efficacy, adverse immunosuppressive effects, and treatment outcome (18).

Corticosteroids were commonly co-administered with the antiviral drug ribavirin (8,9,12,13,15,19). The use of ribavirin was criticised because clinical findings did not reveal evidence for its anti-SARS efficacy and the severe adverse effects of the drug (20). First *in vitro* experiments that did not suggest anti-SARS-CoV activity of ribavirin had been performed in Vero cells (21,22), in which ribavirin is known to be of low antiviral efficacy (23,24). In other cells, ribavirin inhibited SARS-CoV replication (25).

We established a SARS-CoV infection in human intestinal epithelial cell line Caco2 allowing the investigation of SARS-CoV infection of cells from human origin (26,27). Herein, the influence of hydrocortisone on SARS-CoV replication with and without ribavirin was examined. Moreover, we examined the influence of SARS-CoV on the expression of the chemokines CXCL8 (IL-8 = interleukin 8) and CXCL10 (interferon γ inducible protein 10 = IP-10) on the mRNA and on the protein level, which were previously shown to be increased in the plasma of SARS patients. The influence of SARS-CoV infection on the DNA binding activity of the transcription factors nuclear factor κB (NF-κB) and activator protein 1 (AP-1), which are commonly involved in inflammatory processes, was investigated (28). Finally, the influence of hydrocortisone on SARS-CoV induced

Correspondence to: Professor Jindrich Cinatl, Institute for Medical Virology, University Hospital Medical School, Paul-Ehrlich Str. 40, D-60596 Frankfurt am Main, Germany
E-mail: cinatl@em.uni-frankfurt.de

Key words: Caco2, ribavirin

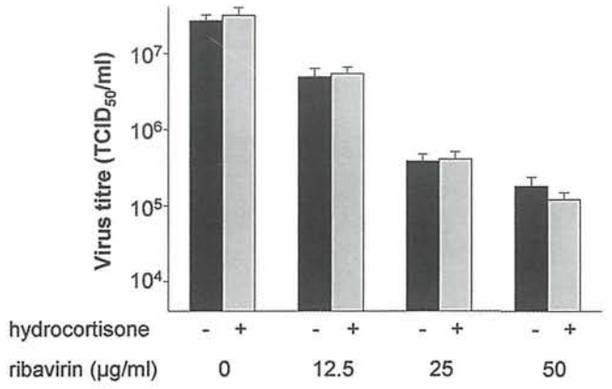

Figure 1. Influence of hydrocortisone (250 µg/ml) on SARS-CoV titres in *Caco2 cells with and without ribavirin.*

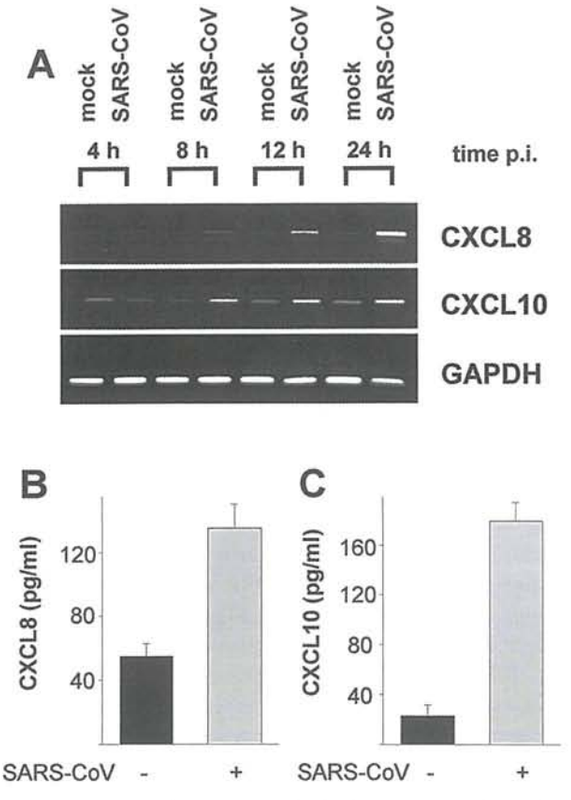

Figure 2. Influence of SARS-CoV infection on expression of pro-inflammatory chemokines CXCL8 and CXCL10. (A) Expression of CXCL8 or CXCL10 mRNA at different time points after SARS-CoV infection of Caco2 cells. (B) CXCL8 protein levels determined by ELISA in the supernatant of SARS-CoV-infected Caco2 cells 24 h after infection compared to non-infected control. (C) CXCL10 protein levels determined by ELISA in the supernatant of SARS-CoV-infected Caco2 cells 24 h after infection compared to non-infected control.

chemokine expression and proinflammatory transcription factors were examined.

Materials and methods

Cell cultures. Colon carcinoma cell line Caco2 was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). The cells were grown at 37°C in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal bovine serum (FBS), 100 IU/ml of penicillin, and 100 µg/ml of streptomycin. All culture reagents were purchased from Biochrom (Berlin, Germany).

Virus preparation. SARS-CoV strain FFM-1 was isolated from respiratory specimens of a SARS patient admitted to the Infectious Diseases Department of Frankfurt am Main University Hospital, Germany, on Vero (African green monkey kidney; ATCC CCL81) cells (21). SARS-CoV stocks used in the experiments had undergone five passages on Vero cells and were stored at -80°C. Virus titres were determined as $TCID_{50}$/ml in confluent cells in 96-well microtiter plates (21).

RT-PCR. CXCL8 and CXCL10 mRNA levels were examined by RT-PCR, according to standard protocols (29). PCR primer and amplification conditions were determined by software (Primer3, Whitehead Institute for Biomedical Research) (30).

Enzyme linked immuno sorbent assay (ELISA). ELISA was performed as described before (31). CXCL8 and CXCL10 protein levels were measured in cell culture supernatants by ELISA (R&D Systems, Wiesbaden, Germany) according to the manufacturer's instructions. Supernatants of cell cultures were stored at -80°C until measurement. Standards provided by the manufacturer were used as positive controls. Fresh culture medium was used as negative control. OD was determined using a microplate reader at 450 nm.

Electrophoretic mobility shift assay (EMSA). EMSA was performed as described before (32). Nuclear extracts (5 µg total protein) were assayed for DNA-binding activity of NF-κB or AP-1 using a gel shift assay kit (Promega, Mannheim, Germany) following the manufacturer's manual.

Results

Influence of hydrocortisone and ribavirin on SARS-CoV replication Caco2 cells. SARS patients were commonly treated with a combination of corticosteroids and antiviral drugs (9,12-17). Ribavirin was initially found to be ineffective in the inhibition of SARS-CoV replication in Vero cells (21). Newer results revealed that ribavirin inhibits SARS-CoV replication in foetal Rhesus kidney-4 cells (25). Our results show SARS-CoV replication inhibition by ribavirin in human Caco2 cells in therapeutical concentrations of 12.5-50 µg/ml in a dose-dependent fashion (Fig. 1). Hydrocortisone in concentrations up to 250 µg/ml (the maximum dose tested) neither influenced SARS-CoV titres alone nor in combination with ribavirin (Fig. 1).

Influence of SARS-CoV infection on expression of CXCL8 and CXCL10 in Caco2 cells. High-density oligonucleotide array studies had demonstrated up-regulation of different

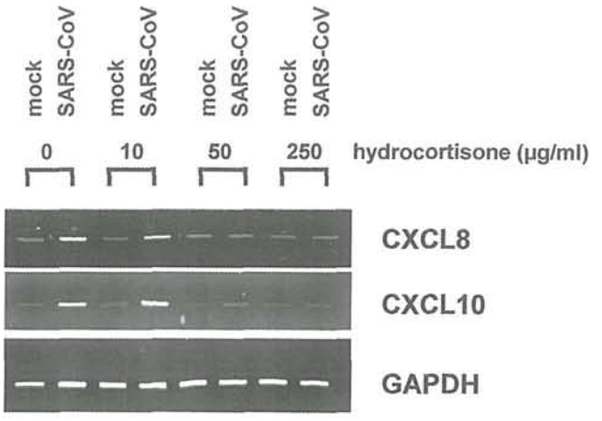

Figure 3. Concentration-dependent influence of hydrocortisone on CXCL8 or CXCL10 mRNA levels 24 h after infection.

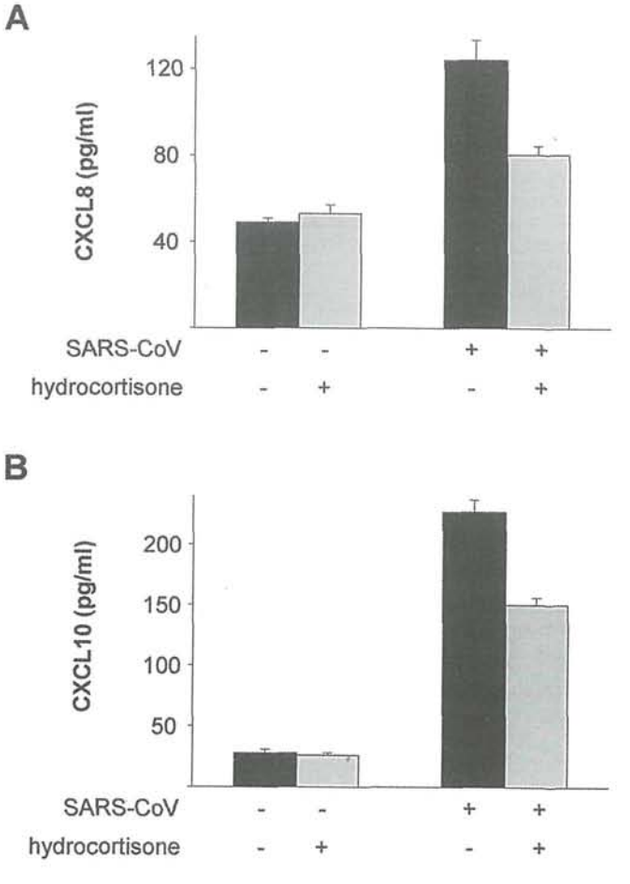

Figure 4. Influence of hydrocortisone (250 μg/ml) on SARS-CoV-induced (A) CXCL8 or (B) CXCL10 production of Caco2 cells as determined by ELISA.

pro-inflammatory cytokines and chemokines in SARS-CoV-infected Caco2 cells (27). The chemokines CXCL8 and CXCL10 had been shown to play a role in the clinical course of SARS (11,33,34). Therefore, we investigated the time-dependent influence of SARS-CoV infection on mRNA levels of CXCL8 and CXCL10 in Caco2 cells by RT-PCR

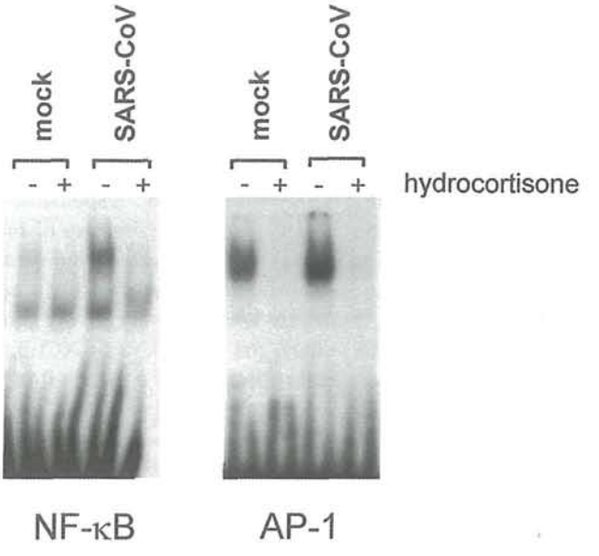

Figure 5. Influence of SARS-CoV infection on the DNA binding activity of the transcription factors AP-1 and NF-κB with and without hydrocortisone (250 μg/ml).

(Fig. 2A). An increase of CXCL8 and CXCL10 mRNA levels became visible 8 h after SARS-CoV infection compared to non-infected control. The maximum increase was detected 24 h after infection.

CXCL8 and CXCL10 protein levels were determined by ELISA. CXCL8 protein was about 2.5-fold (Fig. 2B) and CXCL10 was about 8-fold (Fig. 2C) up-regulated 24 h after SARS-CoV infection.

Influence of hydrocortisone on the SARS-CoV-induced up-regulation of CXCL8 and CXCL10. Corticosteroids are a common part of treatment strategies for SARS patients (9,12-17). Therefore, we investigated the influence of hydrocortisone on SARS-CoV-induced expression of CXCL8 and CXCL10 in Caco2 cells. As shown in Fig. 3, hydrocortisone concentrations ≥50 μg/ml inhibited increased CXCL8 and CXCL10 expression on the mRNA level 24 h after SARS-CoV infection.

Hydrocortisone 250 μg/ml also inhibited up-regulation of CXCL8 (Fig. 4A) and CXCL10 (Fig 4B) on the protein level. However, the expression of both proteins was not reduced to basal levels.

Influence of SARS-CoV infection on the DNA binding activity of the transcription factors AP-1 and NF-κB. The transcription factor AP-1 had already been found to be activated by the SARS-CoV nucleocapsid (35). AP-1 and NF-κB binding sites have been described for the CXCL8 promoter (36,37) and a NF-κB binding site has been described for the CXCL10 promoter (38). Therefore, the influence of SARS-CoV infection on the DNA binding activity of AP-1 and NF-κB was investigated. Electrophoretic mobility shift assay demonstrated increased DNA binding activity of AP-1 and NF-κB after SARS-CoV infection (Fig. 5).

Treatment of SARS-CoV infected cells with hydrocortisone 250 μg/ml prevented the increase in DNA-binding activity of both transcription factors (Fig. 5).

Discussion

Inflammatory cytokines/chemokines play both beneficial and harmful roles in infectious diseases caused by different viruses including human coronaviruses (39). Recent investigations found dynamic changes in blood cytokine levels of patients with SARS which may be responsible for injury of lung and other organs (10,11). By the use of high-density oligonucleotide arrays, we previously demonstrated that infection of cultured intestinal epithelial cell line Caco2 with SARS-CoV alters mRNA levels of several cytokines/chemokines (27). In this study, the chemokines CXCL8 and CXCL10, that play a role in the clinical course of SARS pathology (10,11), were found to be up-regulated on mRNA and protein levels after SARS-CoV infection of Caco2 cells.

The transcription factors NF-κB and AP-1 are commonly involved in inflammatory processes (28). The promoter of CXCL8 has NF-κB and AP-1 binding sites and for the CXCL10 promoter a NF-κB binding site has been described (36-38). The induction of CXCL8 by rotavirus in intestinal epithelial cells required binding of AP-1 and NF-κB transcription factors to the CXCL8 promoter (40). The porcine transmissible gastroenteritis virus (TGEV), an animal coronavirus, was shown to activate NF-κB in different cell lines (41). Recently, the SARS-CoV nucleocapsid was shown to activate AP-1 but not NF-κB pathways (35). Our results reveal that SARS-CoV infection increases the DNA binding activity of both transcription factors indicating a possible involvement in SARS-CoV induced inflammatory processes. While the present results are in concert with AP-1 activation by SARS-CoV nucleocapsid (35), they for the first time demonstrate that SARS-CoV utilises signal pathways activating NF-κB which are not induced by the SARS-CoV nucleocapsid.

Corticosteroids, that inhibit NF-κB and AP-1 activation (28,42), are a common part of SARS treatment regimens to control immunopathological processes during the course of SARS (9,12-17). Clinical findings show reduction of CXCL8 and CXCL10 in SARS patients treated with corticosteroids (11). In our model, high hydrocortisone concentrations of 250 μg/ml prevented SARS-CoV induced increase of AP-1 and NF-κB DNA binding activity and reduced SARS-CoV-induced expression of CXCL8 and CXCL10. However, CXCL8 and CXCL10 levels did not reach basal levels. This suggests that inhibition of AP-1 and NF-κB alone is not sufficient to suppress CXCL8 and CXCL10 expression. In infected patients chemokines are commonly produced by infected cells and/or immune cells. Therefore, the reduced chemokine levels that had been observed in corticosteroid-treated SARS patients (11), may be at least in part caused by inhibition of chemokine production in SARS-CoV infected cells.

Although the use of coticosteroids resulted in improved overall clinical outcome (12,13,15-17), the use of corticosteroids has to be considered with care for patients suffering from infectious diseases since immunosuppressive effects may promote the infection. This is of special importance for the first phase of SARS pathology that is characterised by viral replication and cytolytic activity (18). From our *in vitro* data, we can conclude that hydrocortisone does not affect SARS-CoV replication when given alone. Ribavirin which was commonly used for SARS patients in combination with corticosteroids inhibited SARS-CoV replication in Caco2 cells in a dose-dependent manner. This result is in accordance with other findings showing ribavirin to inhibit SARS-CoV infection in other cells than Vero (25). Hydrocortisone did not affect the anti-SARS-CoV activity of ribavirin.

In spite of general improved outcome of SARS patients treated with corticosteroids, severe side-effects have been reported. The 30-day mortality was reported to be increased and a disseminated fungal disease was described (43). Moreover, avascular necrosis of bone was detected in >30% of SARS cases (44). Other clinical observations reported corticosteroid therapy to be of low efficacy or ineffective (19,45). The different treatment outcomes may be due to different dosing, timing, and/or duration of corticosteroid treatment. Therefore, exact criteria for the use of corticosteroids for SARS patients have been defined (15-18). Our results, showing that corticosteroids may be of limited benefit in the suppression of chemokine production by SARS-CoV-infected cells, strongly support that corticosteroids should be carefully used for SARS patients.

References

1. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RA, Berger A, Burguiere AM, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra JC, Muller S, Rickerts V, Stürmer M, Vieth S, Klenk HD, Osterhaus AD, Schmitz H and Doerr HW: Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 348: 1967-1976, 2003.
2. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, Tong S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell SF, Ling AE, Humphrey CD, Shieh WJ, Guarner J, Paddock CD, Rota P, Fields B, DeRisi J, Yang JY, Cox N, Hughes JM, LeDuc JW, Bellini WJ, Anderson LJ and SARS Working Group: A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 348: 1953-1966, 2003.
3. Lew TW, Kwek TK, Tai D, Earnest A, Loo S, Singh K, Kwan K, Chan Y, Yim CF, Bek SL, Kor AC, Yap WS, Chelliah YR, Lai YC and Goh SK: Acute respiratory distress syndrome in critically ill patients with severe acute respiratory syndrome. JAMA 290: 374-380, 2003.
4. Fowler RA, Lapinsky SE, Hallett D, Detsky AS, Sibbald WJ, Slutsky AS, Stewart TE and Toronto SARS Critical Care Group: Critically ill patients with severe acute respiratory syndrome. JAMA 290: 367-373, 2003.
5. Ding Y, Wang H, Shen H, Li Z, Geng J, Han H, Cai J, Li X, Kang W, Weng D, Lu Y, Wu D, He L and Yao K: The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. J Pathol 200: 282-289, 2003.
6. Nicholls JM, Poon LL, Lee KC, Ng WF, Lai ST, Leung CY, Chu CM, Hui PK, Mak KL, Lim W, Yan KW, Chan KH, Tsang NC, Guan Y, Yuen KY and Peiris JS: Lung pathology of fatal severe acute respiratory syndrome. Lancet 361: 1773-1778, 2003.
7. Leung WK, To KF, Chan PK, Chan HL, Wu AK, Lee N, Yuen KY and Sung JJ: Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. Gastroenterology 125: 1011-1017, 2003.
8. Booth CM, Matukas LM, Tomlinson GA, Rachlis AR, Rose DB, Dwosh HA, Walmsley SL, Mazzulli T, Avendano M, Derkach P, Ephtimios IE, Kitai I, Mederski BD, Shadowitz SB, Gold WL, Hawryluck LA, Rea E, Chenkin JS, Cescon DW, Poutanen SM and Detsky AS: Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. JAMA 289: 2801-2809, 2003.
9. Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, Law KI, Tang BS, Hon TY, Chan CS, Chan KH, Ng JS, Zheng BJ, Ng WL, Lai RW, Guan Y, Yuen KY and HKU/UCH SARS Study Group: Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet 361: 1767-1772, 2003.

10. 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 116: 1283-1287, 2003.
11. Wong CK, Lam CW, Wu AK, Ip WK, Lee NL, Chan IH, Lit LC, Hui DS, Chan MH, Chung SS and Sung JJ: Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol 136: 95-103, 2004.
12. Ho JC, Ooi GC, Mok TY, Chan JW, Hung I, Lam B, Wong PC, Li PC, Ho PL, Lam WK, Ng CK, Ip MS, Lai KN, Cahn-Yeung M and Tsang KW: High-dose pulse versus nonpulse corticosteroid regimens in severe acute respiratory syndrome. Am J Respir Crit Care Med 168: 1449-1456, 2003.
13. Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, Ahuja A, Yung MY, Leung CB, To KF, Lui SF, Szeto CC, Chung S and Sung JJ: A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med 348: 1986-1994, 2004.
14. Tsang OT, Chau TN, Choi KW, Tso EY, Lim W, Chiu MC, Tong WL, Lee PO, Lam BH, Ng TK, Lai JY, Yu WC and Lai ST: Coronavirus-positive nasopharyngeal aspirate as predictor for severe acute respiratory syndrome mortality. Emerg Infect Dis 9: 1381-1387, 2003.
15. So LK, Lau AC, Yam LY, Cheung TM, Poon E, Yung RW and Yuen KY: Development of a standard treatment protocol for severe acute respiratory syndrome. Lancet 361: 1615-1617, 2003.
16. Lau AC and So LK: Severe acute respiratory syndrome treatment: present status and future strategy. Curr Opin Investig Drugs 4: 918-920, 2003.
17. Lau AC, So LK, Miu FP, Yung RW, Poon E, Cheung TM and Yam LY: Outcome of coronavirus-associated severe acute respiratory syndrome using a standard treatment protocol. Respirology 9: 173-183, 2004.
18. Kamps BS and Hoffmann C (eds). SARS reference. Flying Publisher, www.SARSreference.com, 2003.
19. Tsui PT, Kwok ML, Yuen H and Lai ST: Severe acute respiratory syndrome: clinical outcome and prognostic correlates. Emerg Infect Dis 9: 1064-1069, 2003.
20. Cyranoski D: Critics slam treatment for SARS as ineffective and perhaps dangerous. Nature 423: 4, 2003.
21. Cinatl J Jr, Morgenstern B, Bauer G, Chandra P, Rabenau H and Doerr HW: Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet 361: 2045-2046, 2003.
22. Ströher U, DiCaro A, Li Y, Strong JE, Aoki F, Plummer F, Jones SM and Feldmann H: Severe acute respiratory syndrome-related coronavirus is inhibited by interferon-α. J Infect Dis 189: 1164-1167, 2004.
23. Huffman JH, Sidwell RW, Khare GP, Witkowski JT, Allen LB and Robins RK: *In vitro* effect of 1-ß-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (virazole, ICN 1229) on deoxyribonucleic acid and ribonucleic acid viruses. Antimicrob Agents Chemother 3: 235-241, 1973.
24. Smee DF, Sidwell RW, Clark SM, Barnett BB and Spendlove RS: Inhibition of bluetongue and Colorado tick fever orbiviruses by selected antiviral substances. Antimicrob Agents Chemother 20: 533-538, 1981.
25. Chu CM, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, Kao RY, Poon LL, Wong CL, Guan Y, Peiris JS, Yuen KY and HKU/UCH SARS Study Group: Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax 59: 252-256, 2004.
26. Cinatl J Jr, Morgenstern B, Bauer G, Chandra P, Rabenau H and Doerr HW: Treatment of SARS with human interferons. Lancet 362: 293-294, 2003.
27. Cinatl J Jr, Hoever G, Morgenstern B, Preiser W, Vogel J-U, Hofmann W-K, Bauer G, Michaelis M, Rabenau HF and Doerr HW: Infection of cultured intestinal epithelial cells with severe acute respiratory syndrome coronavirus. Cell Mol Life Sci 61: 2100-2112, 2004.
28. Cato AC and Wade E: Molecular mechanisms of anti-inflammatory action of glucocorticoids. Bioessays 18: 371-378, 1996.
29. Cinatl J Jr, Cinatl J, Kotchetkov R, Vogel J-U, Woodcock BG, Matousek J, Pouckova P and Kornhuber B: Bovine seminal ribonuclease selectively kills human multidrug-resistant neuroblastoma cells via induction of apoptosis. Int J Oncol 15: 1001-1009, 1999.
30. Rozen S and Skaletsky H: Primer 3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132: 365-386, 2000.
31. Cinatl J Jr, Blaheta R, Bittoova M, Scholz M, Margraf S, Vogel JU, Cinatl J and Doerr HW: Decreased neutrophil adhesion to human cytomegalovirus-infected retinal pigment epithelial cells is mediated by virus-induced up-regulation of Fas ligand independent of neutrophil apoptosis. J Immunol 165: 4405-4413, 2000.
32. Cinatl J Jr, Margraf S, Vogel JU, Scholz M, Cinatl J and Doerr HW: Human cytomegalovirus circumvents NF-κB dependence in retinal pigment epithelial cells. J Immunol 167: 1900-1908, 2001.
33. Hsueh PR, Chen PJ, Hsiao CH, Yeh SH, Cheng WC, Wang JL, Chiang BL, Chang SC, Chang FY, Wong WW, Kao CL, Yang PC and SARS Research Group of National Taiwan University College of Medicine and National Taiwan University Hospital: Patient data, early SARS epidemic, Taiwan. Emerg Infect Dis 10: 489-493, 2004.
34. Lee CH, Chen RF, Liu JW, Yeh WT, Chang JC, Liu PM, Eng HL, Lin MC and Yang KD: Altered p38 mitogen-activated protein kinase expression in different leukocytes with increment of immunosuppressive mediators in patients with severe acute respiratory syndrome. J Immunol 172: 7841-7847, 2004.
35. He R, Leeson A, Andonov A, Li Y, Bastien N, Cao J, Osiowy C, Dobie F, Cutts T, Ballantine M and Li X: Activation of AP-1 signal transduction pathway by SARS coronavirus nucleocapsid protein. Biochem Biophys Res Commun 311: 870-876, 2003.
36. Mukaida N, Okamoto S, Ishikawa Y and Matsushima K: Molecular mechanism of interleukin-8 gene expression. J Leukoc Biol 56: 554-558, 1994.
37. Mukaida N, Morita M, Ishikawa Y, Rice N, Okamoto S, Kasahara T and Matsushima K: Novel mechanism of glucocorticoid-mediated gene repression. Nuclear factor-κB is target for glucocorticoid-mediated interleukin 8 gene repression. J Biol Chem 269: 13289-13295, 1994.
38. Ohmori Y and Hamilton TA: Cooperative interaction between interferon (IFN) stimulus response element and κB sequence motifs controls IFN γ- and lipopolysaccharide-stimulated transcription from the murine IP-10 promoter. J Biol Chem 268: 6677-6688, 1993.
39. Glass WH, Rosenberg HF and Murphy PM: Chemokine regulation of inflammation during acute viral infection. Curr Opin Allergy Clin Immunol 3: 467-473, 2003.
40. Casola A, Garofalo RP, Crawford SE, Estes MK, Mercurio F, Crowe SE and Brasier AR: Interleukin-8 regulation in intestinal epithelial cells infected with rotavirus: role of viral-induced IκB kinase activation. Virology 298: 8-19, 2002.
41. Eleouet JF, Chilmonczyk S, Besnardeau L and Laude H: Transmissable gastroenteritis coronavirus induces programmed cell death in infected cell through a caspase-dependent pathway. J Virol 72: 4918-4924, 1998.
42. Pelaia G, Vatrella A, Cuda G, Maselli R and Marsico SA: Molecular mechanisms of corticosteroid actions in chronic inflammatory airway diseases. Life Sci 72: 1549-1561, 2003.
43. Wang H, Ding Y, Li X, Yang L, Zhang W and Kang W: Fatal aspergillosis in a patient with SARS who was treated with corticosteroids. N Engl J Med 349: 507-508, 2003.
44. Hong N and Du XK: Avascular necrosis of bone in severe acute respiratory syndrome. Clin Radiol 59: 602-608, 2004.
45. Hsu LY, Lee CC, Green JA, Ang B, Paton NI, Lee L, Villacian JS, Lim PL, Earnest A and Leo YS: Severe acute respiratory syndrome (SARS) in Singapore: clinical features of index patient and initial contacts. Emerg Infect Dis 9: 713-717, 2003.