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Severe Acute Respiratory Syndrome Associated Coronavirus Is Detected in Intestinal Tissues of Fatal Cases

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OBJECTIVES: A significant percentage of confirmed severe acute respiratory syndrome (SARS) patients experienced gastrointestinal symptoms, and the viral sequence was detectable in the stool of most patients. At present, the knowledge of the pathology of the digestive system in SARS patients is limited. Because a resurgence of the SARS epidemic is constantly possible, there is an urgent need to understand the involvement of the digestive system in this new disease.

METHODS: We performed seven SARS autopsies in which samples of alimentary tract and digestive glands were examined with routine pathology, electron microscopy (EM), *in situ* hybridization (ISH), immunohistochemistry, and real-time polymerase chain reaction (PCR).

RESULTS: The main histopathological finding was atrophy of the mucosal lymphoid tissue. A few mucosal epithelial cells and lymphocytes in the intestine were positively stained for coronavirus with ISH. SARS-coronavirus (CoV)-like particles were found in the mucosal epithelial cells under EM and mild focal inflammation was detected in the alimentary tract. One patient who experienced severe diarrhea had pseudomembranous enteritis of the ileum. Fatty degeneration and central lobular necrosis were observed in the liver. No evidence of direct viral infection was found in the esophagus, the stomach, the salivary gland, the liver, or the pancreas.

CONCLUSIONS: In addition to the lungs, the gastrointestinal tract is another target of SARS-CoV infection, as the intestinal epithelial cells and mucosal lymphoid tissue are infected. The findings provide possible explanations for the gastrointestinal symptoms and the presence of virus in the stool of SARS patients.

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INTRODUCTION

In November 2002, a life-threatening respiratory disease first appeared in Guangdong Province, China, and rapidly spread around the world. This disease was designated "severe acute respiratory syndrome (SARS)" on March 2003 by the World Health Organization (WHO) (1). Clinically, the disease was characterized by fever, nonproductive cough, dyspnea, lymphopenia, and rapidly progressing changes on chest radiography. Soon afterwards, many investigators found that gastrointestinal symptoms were not uncommon in SARS patients. These included diarrhea (7–73%), nausea and vomiting (14–22.2%), and abdominal pain (3.5–12.6%) (2–6). Further studies reported that the SARS-associated coronavirus (SARS-CoV) was detectable in the stool of most of the SARS patients (5, 7). Biochemical tests showed the elevation of aminotransferases indicating liver injury in SARS patients (4, 8, 9). All this research suggests that the digestive system might be injured during the course of SARS. Until now, only two reports specifically describing the pathology of the digestive system have appeared in the literature (10, 11). Both, however, did not investigate the submucosal lymphoid tissue or digestive glands.

Our research team at the Peking University Health Science Center, Beijing, China, performed 14 cases of autopsies on clinically confirmed and suspected SARS patients. To date, this was the largest series of SARS autopsies performed anywhere in the world. Among them, seven were confirmed to have contracted SARS by identifying the pathogen in these patients. The digestive system of these seven patients was thoroughly examined with a number of techniques. We attempted to answer two questions: First, what were the

pathological damages to the digestive system in SARS patients? Second, was the digestive system a direct target of SARS viral infection? Here we report our original observations and analysis below.

MATERIALS AND METHODS

Patients

Seven patients who met the WHO case definition of SARS underwent postmortem examinations at a Biosafety Level 3 laboratory built specifically for autopsy examination of SARS victims at Ditan Hospital, Beijing, China. All patients died between May 9 and July 27, 2003. The case definition included fever (temperature over 38°C), cough, or shortness of breath, new pulmonary infiltrates on chest radiograph, history of exposure to a patient with SARS, and a positive serological test for the SARS-CoV antibody or a positive RT-PCR test in sera, urine, pharyngeal aspirates, or gargle. The diagnoses were further confirmed by real-time RT-PCR as detecting the SARS-CoV sequence in postmortem tissue samples of the lung. Clinical and laboratory information for each patient before death is presented in Table 1.

Investigations

Full autopsies were performed on all the seven cases, and samples from different parts of the alimentary tract and digestive glands were obtained, including the tongue, the pharynx, the esophagus, the stomach, the small intestine, the colon, the appendix, the salivary glands, the pancreas, and the liver. Identical tissue samples from age and clinically matched non-SARS patients were also examined as controls.

For histopathologic examination, small pieces (1–2 cm^3) of autopsy materials were fixed in 10% formalin for 24 h. Paraffin-embedded blocks of the tissue were sectioned at 5 μm, dewaxed, and stained with hematoxylin and eosin (HE).

For transmission electron microscopy, the small pieces ($\sim$1 mm^3) of the liver and the mucosa of the ileum were fixed in 2.5% glutaraldehyde and postfixed in osmium tetroxide, and then embedded in Epon 812. Ultrathin sections were cut and stained with uranyl acetate and lead citrate.

For immunohistochemistry (IHC), all the immunohistochemistry reagents, except those that are pointed out, were supplied by DakoCytomation Company (Carpinteria, CA). Monoclonal antibodies to CD20 (B cell marker, diluted 1:50), CD68 (macrophage marker, diluted 1:50), CD4 (T helper cell marker, by Novocastra Laboratories Ltd., Newcastle, UK), CD8 (cytotoxic T cell marker, dilution 1:50), and polyclonal antibody to CD3 (T cell marker, diluted 1:100) were applied on tissue sections of the tongue, the pharynx, the esophagus, the stomach, the small intestine, the colon, and the appendix. Monoclonal antibody to HBsAg (diluted 1:50) and polyclonal antibody to HBcAg (diluted 1:200) were applied on liver sections. The staining was carried out with the DAKO automated immunohistochemical stainer using the DAKO Envision system. The controls included omission of the primary antibody and the use of an unrelated antibody as the first layer in place of the specific antibodies.

RNA probes and the protocol of *in situ* hybridization (ISH) were prepared as previously described by Nagoshi *et al*. (12) with specific modifications. Briefly, a pair of primers was designed based on the SARS coronavirus genome sequence (GenBank, Accession No. AY274119). The sequences of the primers were: **A**: 5′-GCGCAAGTATTAAGTGAGATG-3′ (15348–15368 nt), **B**: 5′-GAAGTGCATTTAC ATTGGCT-3′ (15473–15492 nt). Total RNA was extracted from the peripheral blood of a SARS patient with TRIZOL reagent (Roche, USA). After the reaction, the RT-PCR products were purified and confirmed by sequencing. Then, the RNA probe was prepared using *in vitro* transcription with the label of digoxigenin. The reaction of ISH took place at 55°C for 16 h. The washes for posthybridization were carried out in 50% formamid/2×SSC at 55°C for 30 min, three times in 2×SSC at 37°C and 0.1×SSC for 20 min at room temperature. After blocking the background staining with horse sera (1:100) for 60 min, we added AP-labeled anti-Dig antibody (1:500) to the sections for 60 min at room temperature. The color development was carried out with NBT/BCIP and the counter staining with methyl-green.

The real-time RT-PCR was performed with a pair of primers encasing a highly conserved region of the SARS viral genome (15301–15480) from the GenBank (G130027616). A 145 bp sequence of the PCR product was further confirmed by conventional PCR assay. The experiment was carried out according to a protocol published previously by Wu *et al.* (13).

RESULTS

Macroscopic Findings

Macroscopically, despite the autolytic changes, the entire alimentary tracts of cases 2, 4, and 6 appeared to be largely

Table 1. Summary of Major Relative Clinical Presentations

Case No.	Age (yr)	Gender	Course of Disease (d)	Digestive System Symptom	Lymphopenia	Elevation of ALT or AST
1	51	Male	45	Upper gastrointestinal hemorrhage	+	+
2	50	Male	33	No	+	−
3	31	Male	35	Hematochezia	+	−
4	49	Female	32	No	+	+
5	24	Male	21	Diarrhea	+	−
6	20	Male	62	No	+	+
7	58	Male	92	Upper gastrointestinal hemorrhage	+	+

normal. In case 5, who had diarrhea before death, there were plaques of pseudomembrane with a few shallow ulcers in the terminal ileum. Scattered hemorrhagic spots were present in the gastric mucosa of this case. In cases 1, 3, and 7, coffee ground-like liquid was observed in the lumens of the stomach and/or intestine, which suggested that these patients might have had acute stress ulcers before death.

There was no enlargement of the liver in all cases, although the weight of the liver was heavier in case 1 (1725 g) and case 3 (1658 g) (the reference liver weight was less than 1500 g). The gross surface and cut-surface of the livers showed no positive findings except in case 4, whose cut-surface showed mild fatty degeneration. Another exception appeared in case 1, whose liver showed mild congestion, a commonly noted observation in autopsies of patients with fulminant diseases. We observed no obvious changes in the pancreas or salivary glands of all cases.

Histopathologic and Immuohistochemical Findings

There were slight histological changes in the mucosa of the alimentary tract in the six patients without diarrhea as compared to the controls. The height of villous in the small intestine and depth of crypts in the large intestine were normal in all cases. Only a mild degree of focal lymphoid and plasma cell infiltration was observed in the lamina propria of the gastrointestinal mucosa in cases 1, 3, and 6. Occasional apoptotic epithelial cells were present, although no obvious increase of mitosis was observed. Immunohistochemical stain showed that the lymphoid cells were mainly CD3 positive T cells or CD68 positive macrophages (Fig. 1A–C). Further studies showed that the T cells mainly consisted of CD8 positive cytotoxic cells and fewer CD4 positive helper cells. Hyaline thrombi found in small blood vessels of the small and large intestines in two cases were believed to be a manifestation of disseminated intravascular coagulation (DIC). Nonspecific changes that are usually seen in autopsy specimens, such as vasodilation and/or edema of the submucosa, were observed in different parts of the gastrointestinal tract in all cases.

The patient in case 5 suffered from pseudomembrane enteritis of the terminal ileum. During examination we came to know the pseudomembrane, which was present in the luminal surface, was composed of mucin, fibrin, nuclear debris, bacteria, and a large number of neutrophils. The underlying mucosa was partially necrotic. Inflammatory cells infiltrating into the lamina propia included neutrophils, mononuclear cells, and plasma cells (Fig. 1D, E).

In addition to the changes in the mucosa, a prominent depletion of the mucosal lymphoid tissues was present in all seven cases in comparison to non-SARS controls. These changes were most pronounced in the pharynx, the small intestine, and the appendix, where in normal subjects, the lymphoid tissue was particularly abundant. In these regions, a decrease of lymphocytes, depletion of follicles, and a burned-out appearance of germinal centers were evident. We encountered enlarged lymphocytes within the remaining lymphoid tissue of cases 1, 2, and 5; this observation was also noted in the lamina propria of case 5. The changes were more severe in patients who had a longer course of the disease. The reductions of both CD20 positive B cells and CD3 positive T cells were detected in the atrophic mucosal lymphoid tissue by immunohistochemistry (Fig. 1F–H); however, it was not clear which type of cell decreased more severely.

In all cases, no obvious inflammatory cells infiltrated into the portal areas of the liver. Fatty degeneration of hepatocytes was mainly seen in the centrilobular area in cases 2, 5, and 6. Various degrees of hepatic central lobular necrosis occurred in cases 1, 4, and 6 (Fig. 2A, B). Immunostainings of HBsAg and HBcAg were negative, except in case 7, which showed hepatocytic swelling and a mild degree of fatty degeneration. Focal and a milder degree of fatty degeneration was observed in non-SARS control cases, but there was no evidence of hepatic central lobular necrosis.

Aside from mild autolysis, occasional lymphocyte infiltration into the mesenchyma of the pancreas and salivary glands was observed with no obvious differences from the controls.

Etiological Findings

Positive signals of SARS-CoV were detected in the intestines of five cases (cases 1–5) with ISH. The positive reactions were observed in cytoplasm of scattered mucosal epithelial cells and that of the mucosal and submucosal lymphocytes (Fig. 3A, B).

No positive ISH signal was observed in the tongue, the esophagus, the stomach, the liver, the pancreas, or the salivary glands. Coronavirus-like particles were found in the dilated endoplasmic reticulum of mucosal epithelial cells in the ileum under transmission electron microscopy in case 5 (Fig. 3C, D). Those particles were not detectable in the other six cases. No viral particles were observed in the liver. Major findings of the digestive system are summarized in Table 2.

DISCUSSION

Previous studies of the clinical symptoms and the pathology of SARS patients showed that the lungs were the main targets of SARS-CoV infection (2–4, 14, 15). Our simultaneous study of the respiratory tract on the SARS autopsies confirmed extensive respiratory tract damages in these patients (16). Despite predominant respiratory symptoms, a few groups reported that diarrhea was common in SARS patients (2–6, 17). The positive rates of SARS-CoV in the stool were high (57.4–97%) when compared with those in the gargle, urine, or nasopharyngeal aspirates (5, 18, 19). These data strongly suggested that the alimentary tract was involved in the SARS-CoV infection.

This report described the pathological changes in the alimentary tracts and digestive glands of SARS patients. We detected SARS-CoV sequences and viral particles in the mucosal epithelial cells and lymphocytes of the intestinal mucosa with ISH and electron microscopy. The reliability of the techniques employed was verified with

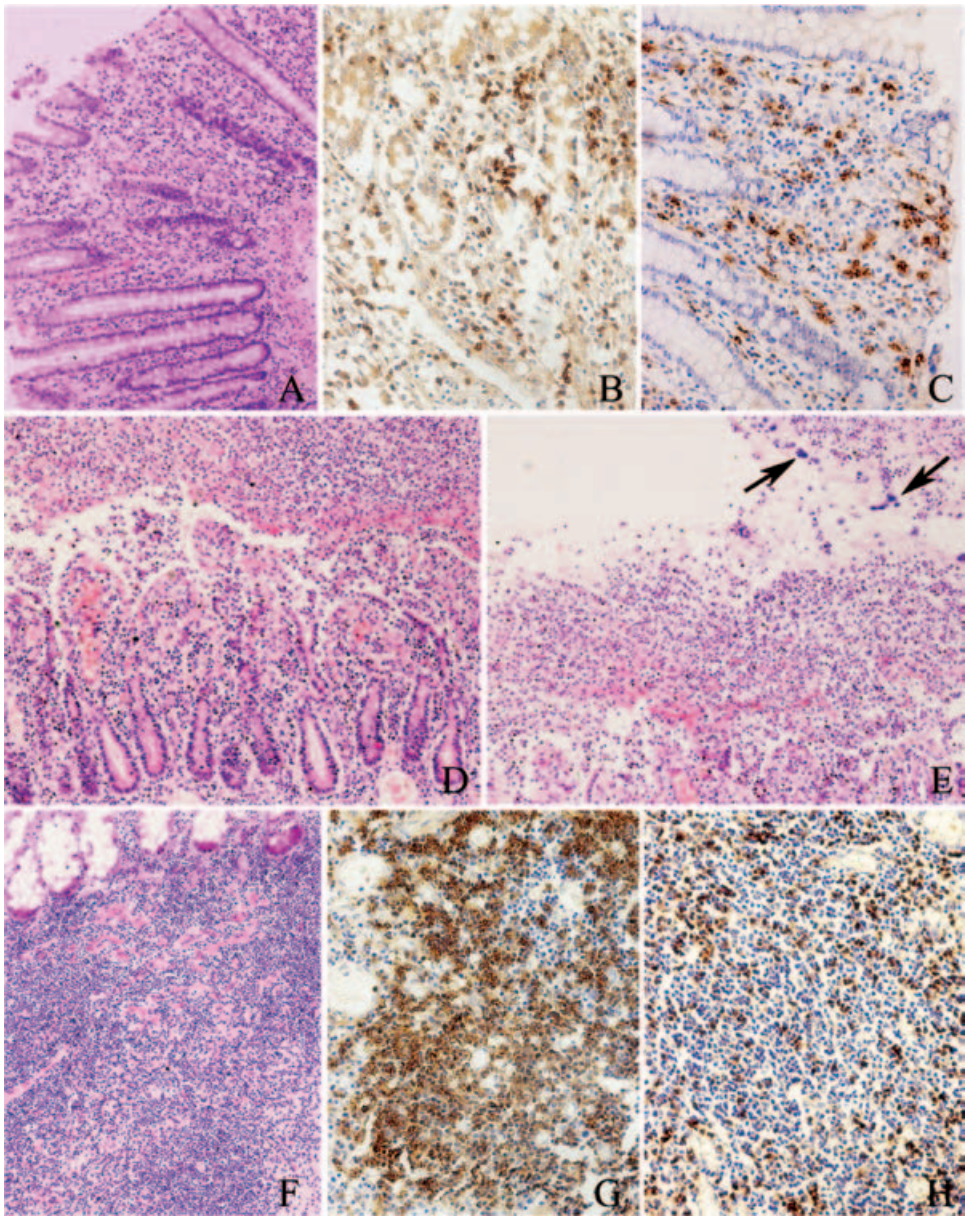

Figure 1. (*A*) Focal mild lymphoid and plasma cell infiltration in the mucosa of the colon from a patient without diarrhea (case 6) (H&E, ×100). (*B*) In the same case, the inflammatory cells in the lamina propria were CD3 positive T cells (IHC, (H&E, ×200)), or (*C*) CD68 positive macrophages (IHC, (H&E, ×200)). (*D*) Pseudomembrane enteritis in the ileum of case 5, and (*E*) plaque of bacteria (arrows) can be seen in the pseudomembrane (H&E, ×100). (*F*) Depletion of mucosal lymphoid tissue, showing decreased amount of lymphocytes and depletion of the germinal center in the ileum of case 1 (H&E, ×100). (*G*) Most of the remaining lymphocytes of mucosal lymphoid tissue were CD20 positive B cells (IHC, ×200), and (*H*) scattered CD3 positive T cells (IHC, ×200).

extensive negative and positive controls. Therefore, it appears that the intestine is another organ that is vulnerable to SARS viral infection. This may also provide an explanation for the presence of SARS virus in the stool of SARS patients.

Recently, Leung *et al.* (10) and To *et al* (11) reported their studies on enteric involvement of SARS infection. Our findings, in part, agree with their report; however, additional information was obtained. In this study, ISH unveiled that scattered epithelial cells in the small and large intestines were infected by the SARS-CoV. We found that the viral particles were present in the dilated endoplasmic reticulum of the mucosal epithelial cells but not on the surface of the microvilli of superficial enterocytes, as reported by Leung *et al.* (10). This might be attributed to the better preservation of samples from biopsy than from autopsy. Aside from the etiological findings, we found that mucosal damage was fairly mild in the alimentary tracts of patients without diarrhea and that only focal mild inflammation was observed. The various cell types and ratios among the inflammatory foci had no obvious deviations from the normal range. Since the six patients, other than patient 5, did not experience diarrhea,

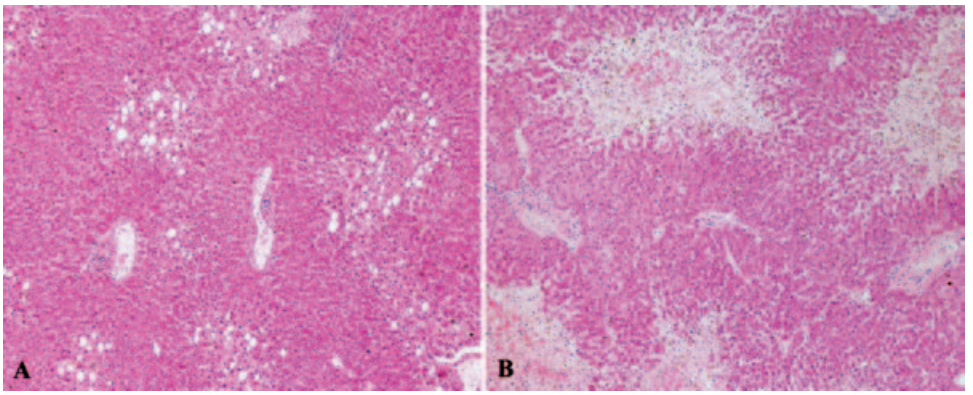

Figure 2. The damages of the livers include (*A*) fatty degeneration of hepatocytes with cytoplasmic vesicles (H&E, ×100) and (*B*) extensive hepatic central lobular necrosis (H&E, ×100).

and the inflammatory foci were observed in both the stomach and the intestines, while the virus could only be detected in the intestines, we believe that this inflammation should be attributed to the long duration of disease or to the medications, rather than a response to SARS-CoV infection. In previous veterinary studies on transmissible gastroenteritis (TGE), a fatal disease in piglets caused by transmissible gastroenteritis coronavirus (TGEV), typical findings within the intestine included villous blunting with abnormal cuboidal epithelium, crypt hyperplasia, and an increase of inflammatory cells in the lamina propia (20–22). These morphological changes, combined with the abnormalities of ion transport in the intestine, were thought to be the major determinants of diarrhea. Another coronavirus, porcine respiratory coronavirus (PRCV), is one that has only limited differences in molecular structure to TGEV (23), but does not cause villous atrophy, or any clinical symptoms; however, the replication of virus was detected in the villous enterocytes (24). This information suggests that SARS-CoV may replicate in the gut with no obvious tissue response in patients without diarrhea.

Lymphopenia is one of the earliest and the most important symptoms in all SARS patients (3, 26). Lymphoid depletion

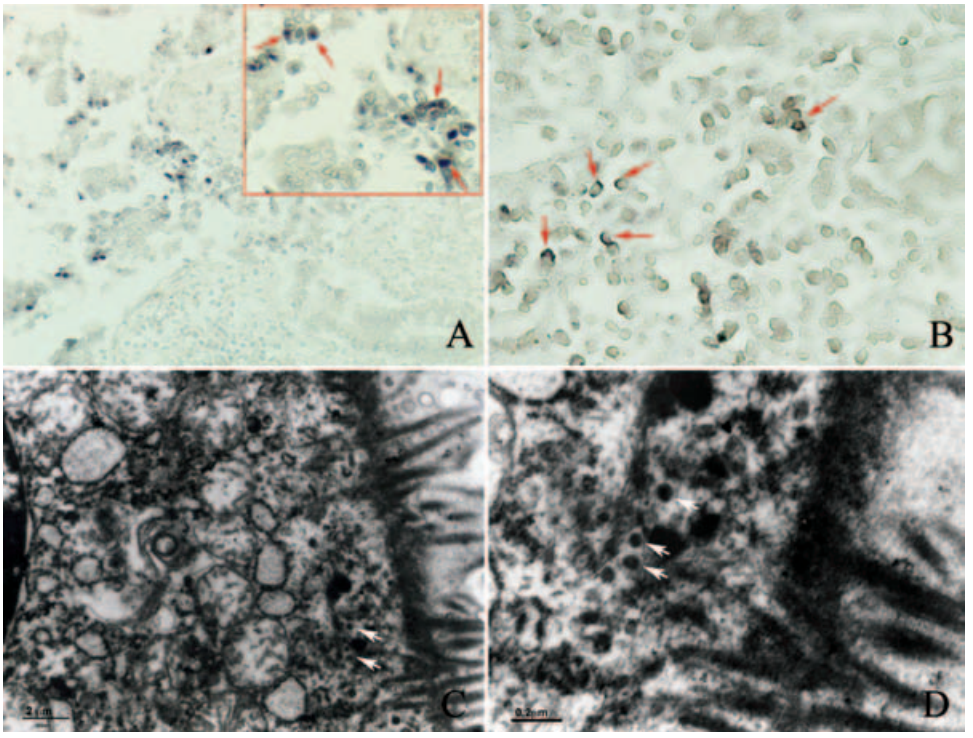

Figure 3. (*A*) ISH showing SARS CoV positive signals (arrows) in the cytoplasm of a few mucosal epithelial cells (×200, upper right corner ×400) and (*B*) lymphocytes of the mucosa in case 5 (×400). (*C*) Coronavirus-like particles (arrows) were observed in the dilated endoplasmic reticulum of the mucosal epithelial cells in the ileum. Microvilli can be seen on the right (EM, bar = 2 μm). (*D*) High-power view of Figure 5A (bar = 0.2 μm).

Table 2. Summary of Major Findings

Case No.	Inflammations in Digestive Tract	Depletion of Lymph Tissue	SARS-CoV Like Particles in Epithelium under EM	ISH Results of Intestine	Liver Pathology	ISH Results of Liver
1	Focal, mild inflammation	+	−	+	Extensive hepatic central lobular necrosis	−
2	No	+	−	+	Fatty degeneration	−
3	Focal, mild inflammation	+	−	+	Approximately normal	−
4	No	+	−	+	Extensive hepatic central lobular necrosis	−
5	Pseudomembranous enteritis in ileum	+	+	+	Fatty degeneration	−
6	Focal, mild inflammation	+	−	−	Fatty degeneration, small foci of hepatic central lobular necrosis	−
7	No	+	−	−	Fatty degeneration	−

of splenic follicles was observed in fatal SARS patients (26) and in macaques infected with SARS-CoV (27). In addition to these findings, extensive infection and severe destruction of lymphocytes in the lymphoid organs was evident in our series of autopsies. We hypothesized that acute immune damage is one of the key mechanisms in SARS pathology (28). Marked depletion of the mucosal lymphoid tissues in the gut was not reported previously. The damage to the mucosal lymphoid tissues and infection of other lymphocytes in the gut by the SARS virus was likely a part of the general deficiency of the immune system. Another possible explanation is that steroids could have caused lymphoid tissue atrophy because they can induce T cell apoptosis (29). But to refute this possibility, lymphopenia occurred before the use of steroids in case 1 and, moreover, in case 3, no steroid was administered at all. Although atrophy of lymphoid tissues was observed in treatment-matched control patients, it was not as severe as that in the SARS patients and most of the germinal centers were still preserved. Therefore, the steroids could not be the only cause for the depletion of lymphoid tissues. In the remaining lymphoid tissues of the alimentary tract, both B and T cells were decreased from the normal range and scatter SARS-CoV infected lymphocytes were found. Although we are not certain about the phenotype of SARS-CoV infected lymphocytes, studies of lymphocytes in the peripheral blood showed that the reduction of T cells was more severe than that of B cells (30, 31), and thus it was suggested that the lymphocytes, particularly T cells, might be the target of SARS-CoV. In this study, SARS-CoV sequence or coronavirus-like particles were detected in both the epithelial cells and the lymphocytes of the intestines. This suggests that the SARS-CoV sequence found in the feces of SARS patients (5, 10) might have two sources—the shedding of SARS-CoV infected mucosal epithelial cells and the shedding of infected lymphocytes. In addition, SARS infected mucosal epithelial cells were seen in the walls of the intestine but not in the tongue, the esophagus, or the stomach. We speculate that there are a number of possibilities for this discrepancy. Clinically, the digestive tract symptoms appeared at an average of 3.5–7.5 days after the onset of the initial symptoms (5, 10), indicating that the infection of the gut occurred after that of the lungs. Moreover, the microenvironments of the stomach were not suitable for the survival of SARS-CoV. Therefore, the alimentary tract was unlikely to be the initial route of viral transmission, and the source of the virus in the intestine was probably blood-borne, by way of the infected circulating lymphocytes. In this study, the SARS-CoV sequence was detectable in the intestines in five of the seven cases. The two negative cases have much longer courses of infection than the rest (>60 days compared to <45 days) and because of this, it is possible that the infection of the intestines represents a transient phase of the disease. Furthermore, considerable autolysis was observed in the two cases might be the reason for their negativity. In addition, because only a limited number of enterocytes were found to be infected by the virus, the possibility that not all SARS patients have digestive tract involvement could not be ruled out.

In the only case (case 5) that had severe watery diarrhea in our study, the mucosal morphology was the same as that of the other six cases, except for the presence of pseudomembrane and plaque of bacteria that were observed in exudates in the ileum. This suggested that the patient developed a secondary bacterial infection. Given the history of broad-spectrum antibiotic treatment of this patient, an antibiotic-associated pseudomembranous enteritis was most probable (32). The incidence of antibiotic-associated diarrhea was just 5–25% (33, 34) while in Peiris (5) and Leung's (10) reports, 38.4–73% patients developed watery diarrhea. Furthermore, no common diarrhea pathogens, including the *Clostridium difficile* cytotoxin was detected in the stools from any of those patients (5, 10). Therefore, the high incidence of diarrhea in SARS patients could not be completely attributed to antibiotic-associated enteritis. Even today, the mechanism of diarrhea in SARS patients is still unknown. However, based on the ISH and morphological findings, we speculate that the replication of SARS-CoV within the intestinal cells and depletion of mucosal associated lymphoid tissue might reduce the mucosa's ability of immune defense, and therefore, cause an opportunistic infection, leading to diarrhea in at least some of the SARS patients. The differences in clinical symptoms and alimentary damage among different patients could be attributed to the variability of the viral load and the strength of their respective immune defense systems.

Elevations of AST and ALT were common features in SARS patients (4, 8, 9). Hepatic lipidosis and emaciation

were also reported in SARS-infected ferrets (35). In this study, two patients (cases 1 and 4) had obvious elevations of AST and ALT. The same patients also had extensive hepatic central lobular necrosis. Pathological changes were mild in other five cases whose AST and ALT levels were normal or only slightly increased. Although there was fatty degeneration and necrosis in the liver, no evidence of SARS-CoV infection was found in the liver by ISH and EM. It is possible that the liver injury and the elevations of AST and ALT in SARS were not caused by direct viral infection. It has been reported that plasma cytokines, such as IL-8, IFN-α, IFN-γ, and TNF-α, were significantly increased in SARS patients (31, 36). The lesions in the liver might be the consequence of hypoxemia or immune system overreaction caused by cytokine dysfunction. Moreover, steroids could increase lipolysis in fat depots (37), thus the fatty degeneration in the liver of some cases might be caused or enhanced by a high dose of steroids.

In conclusion, our study of the digestive system demonstrates that SARS-CoV has the ability to infect mucosal epithelial cells and lymphocytes in the intestinal mucosa in SARS patients. This infection was likely caused by the SARS-CoV carried by infected lymphocytes. The absence of the SARS virus in the esophagus and stomach suggests that the alimentary tract might not be the initial route of transmission. The lesions in the liver may not be caused by a direct SARS-CoV infection but may be a secondary effect. As the viral sequence was detectable in the gut and the stool, oral-fecal transmission could be a real possibility through the respiratory tract. Data obtained from this study increase our understanding of the pathogenetic mechanism of SARS and its relationship to the digestive system. This should improve the treatment and prevention of this deadly disease, should it re-appear in the future.

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REFERENCES

1. WHO. World Health Organization issues emergency travel advisory. Available at http://www.who.int/csr/sarsarchive/2003˙03˙15/en/. Accessed on May 1, 2003.
2. Donnelly CA, Ghani AC, Leung GM, et al. Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. Lancet 2003;361:1761–6.
3. Booth CM, Matukas LM, Tomlinson GA, et al. Clinical features and short-term outcomes of 144 patients with SARS in the Greater Toronto Area. JAMA 2003;289:2801–9.
4. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med 2003;348:1986–94.
5. Peiris JSM, Chu CM, Cheng VCC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: A prospective study. Lancet 2003;361:1767–72.
6. Rainer TH, Cameron PA, Smit DV, et al. Evaluation of WHO criteria for identifying patients with severe acute respiratory syndrome out of hospital: Prospective observational study. Br Med J 2003;326:1354–8.
7. WHO. First data on stability and resistance of SARS coronavirus compiled by member of WHO laboratory network. Available at http://www.who.int/csr/sars/survival˙2003˙05˙04/en/. Accessed on May 7, 2003.
8. Poutanen SM, Low DE, Henry B, et al. Identification of severe acute respiratory syndrome in Canada. N Engl J Med 2003;348:1995–2005.
9. Tsang KW, Ho PL, Ooi GC, et al. A cluster of cases of severe acute respiratory syndrome in Hong Kong. N Engl J Med 2003;348:1977–85.
10. Leung WK, To KF, Chan PKS, et al. Enteric involvement of severe acute respiratoy syndrome-associated coronavirus infection. Gastroenterology 2003;125:1011–7.
11. To KF, Tong JHM, Chan PKS, et al. Tissue and cellular tropism of the coronavirus associated with severe acute respiratory syndrome: An in situ hybridization study of fatal cases. J Pathol 2004;202:157–63.
12. Nagoshi J, Nomura S, Uchida N, et al. Expression of genes encoding connective tissue proteins in androgen-dependent SC115 tumors after androgen removal. Lab Invest 1994;70:210–6.
13. Wu BQ, Zhong HH, Gao JP, et al. Gene detection of severe acute respiratory syndrome-related coronavirus. Zhonghua Bingli Zazhi 2003;32:212–4.
14. Nicholls JM, Poon LLM, lee KC, et al. Lung pathology of fatal severe acute repiratory syndrome. Lancet 2003;361:1773–8.
15. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003;348:1953–66.
16. Xie ZG, Lu M, Gao ZF, et al. Pathological investigation of 5 autopsy of severe acute respiratory syndrome. AIMM 2003;11:292.
17. Zhao Z, Zhang F, Xu M, et al. Description and clinical treatment of an early outbreak of severe acute respiratory syndrome in Guangzhou, PR China. J Med Microb 2003;52:715–20.
18. Ren Y, Ding HG, Wu QF, et al. Survey on pathogenic diagnosis of 28 SARS patients. AIMM 2004;12:93.
19. He ZP, Dong QM, Song SJ, et al. The excretion of SARS coronavirus in stool of SARS patients. AIMM 2003;11:298.
20. Shepherd RW, Butler DG, Hamilton JR. Determinants of diarrhea in viral enteritis. Gastroenterology 1979;76:20–4.

21. Kerzner B, Kelly MH, Gall DG, et al. Transmissible gastroenteritis: Sodium transport and the intestinal epithelium during the course of viral enteritis. Gastroenterology 1977;72:457–61.
22. Sirinarumitr T, Paul PS, Kluge JP, et al. In situ hybridization technique for the detection of swine enteric and respiratory coronaviruses, transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV), in formalin-fixed paraffin-embedded tissues. J Virol Methods 1996;56:149–60.
23. Ballesteros ML, Sanchez CM, Enjuanes L. Two amino acid changes at the N-terminus of transmissible gastroenteritis coronavirus spike protein result in the loss of enteric tropism. Virology 1997;227:378–88.
24. Otoole D, Brown I, Bridges A, et al. Pathogenicity of experimental infection with pneumotropic porcine coronavirus. Res Vet Sci 1989;47:23–9.
25. Wong RSM, Wu A, To KF, et al. Haematological manifestations in patients with severe acute respiratory syndrome: Retrospective analysis. Br Med J 2003;326:1358–62.
26. Ding Y, Wang H, Shen H, et al. The clinical pathology of severe respiratory syndrome (SARS): A report from China. J Pathol 2003;200:282–9.
27. Kuiken T, Fouchier RAM, Schutten M, et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 2003;362:263–70.
28. Gu J, Zheng J, Gong EC, et al. The pathogenetic mechanisms of SARS. AIMM 2003;11: 286–7.
29. Tuosto L, Cundari E, Montani MS, et al. Analysis of susceptibility of mature human T lymphocytes to dexamethasone-induced apoptosis. Eur J Immunol 1994;24:1061–5.
30. Cui W, Fan Y, Wu W, et al. Expression of lymphocytes and lymphocyte subsets in patients with severe acute respiratory syndrome. Clin Infect Dis 2003;37:857–9.
31. Wang FS, Li YG, Fan R. Association of disease progression with dynamic alterations of clinical immunological and virological parameters in patients with severe acute respiratory syndrome. AIMM 2003;11:305
32. Jabbar A, Wright RA. Gastroenteritis and antibiotic-associated diarrhea. Prim Care 2003;30:63–80.
33. Bergogne-Berezin E. Treatment and prevention of antibiotic associated diarrhea. Int J Antimicrob Ag 2000;16:521–6
34. Beaugerie L, Flahault A, Barbut F, et al. Antibiotic-associated diarrhea and clostridium difficile in the community. Aliment Pharmacol Ther 2003;17:905–12.
35. Martina BEE, Haagmans BL, Kuiken T, et al. SARS virus infection of cats and ferrets. Nature 2003;425:915.
36. Xie J, Han Y, Li TS, et al. Dynamic changes of plasma cytokine levels in patients with severe acute respiratory syndrome. Zhonghua Nei Ke Za Zhi 2003;42:643–5.
37. Miner JN. Designer glucocoraticoids. Biochem Pharmacol 2002;64:355–61.