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Original article

Th2 predominance and CD8+ memory T cell depletion in patients with severe acute respiratory syndrome

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Abstract

The immune spectrum of severe acute respiratory syndrome (SARS) is poorly understood.

To define the dynamics of the immune spectrum in SARS, serum levels of cytokines, chemokines, immunoglobulins, complement and specific antibodies against SARS-associated coronavirus (SARS-CoV) were assayed by enzyme-linked immunosorbent assay (ELISA), and phenotypes of peripheral lymphocytes were analyzed by flow cytometry in 95 SARS-infected patients. Results showed that interleukin (IL)-10 and transforming growth factor β (TGF-β) were continuously up-regulated during the entirety of SARS. Regulated on activation normally T cell-expressed and secreted (RANTES) levels were decreased, while monocyte chemoattractant protein-1 (MCP-1) was elevated in acute patients. Immunoglobulins and complement were elevated during the first month of SARS. Both serum-positive rates and titers of specific IgM and IgG antibodies responding to SARS-CoV peaked at days 41–60 from the onset of SARS. CD4+ and CD8+ T lymphocytes decreased significantly in acute-phase. CD3+CD8+CD45RO+ T lymphocytes were decreased by 36.78% in the convalescent patients. Conclusion: SARS-CoV seemed to elicit effective humoral immunity but inhibited cellular immunity, especially CD8+ memory T lymphocytes over time. Prolonged overproduction of IL-10 and TGF-β may play an important role in the disease.

Keywords: Severe acute respiratory syndrome; Immune monitoring; Immune system

Abbreviations: ALT, aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; C, complement; CoV, coronavirus; ctr, control; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; IFN-γ, interferon γ; Ig, immunoglobulin; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor κB; PBMC, peripheral blood mononuclear cell; RANTES, regulated on activation normally T cell-expressed and secreted; SARS, severe acute respiratory syndrome; SARS-CoV, SARS-associated coronavirus; S.D., standard deviation; TGF, transforming growth factor; TNF, tumor necrosis factor.

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1. Introduction

Severe acute respiratory syndrome (SARS) is caused by SARS-associated coronavirus (SARS-CoV) [1,2]. It is known that human coronaviruses usually infect the upper respiratory tract and cause the common cold [3], whereas SARS-CoV infects the lower respiratory tract, leading to pulmonary destruction [4]. Although antibody induction and lymphopenic responses to SARS-CoV have been briefly described elsewhere [5], the precise immune and inflammatory responses following SARS-CoV infection remain unclear. Moreover, the rapidly reported results by other authors deal merely with one or two aspects of anti-viral immunity, i.e. either antibody induction, changes in T lymphocytes or alteration of cytokines. The initial studies showed that not only lung but also immune cells were targets of SARS-CoV [4]. What then, is the overall immune spectrum of SARS: the profile of humoral and cellular immunity and their importance in SARS; whether cytokines and chemokines play a role in pathogenesis of SARS; the status of immune memory function of lymphocytes in SARS? It is particularly important to explore a full, informative description of the immune response and pathogenesis in SARS. This will greatly help us in understanding the pathogenic mechanisms, as well as improving patient management and developing a vaccine to completely control SARS epidemics.

The panel of cytokines (Th1 cytokines interferon γ (IFN-γ), tumor necrosis factor (TNF)-a, interleukin (IL)-2 and IL-12; Th2 cytokines IL-4, IL-6 and IL-10) reflects the overall balance within the immune system; chemokines function briefly in inflammatory processes, acting as regulatory bridge molecules between innate and acquired immunity. The complement system is an important component of innate immunity and major anti-viral effectors. Besides the soluble mediators mentioned above, lymphocytes, especially T and B lymphocytes, play a central role in specific anti-viral immunity for clearing the virus. We thus decided to define the immune response profile, focusing mainly on cytokine/chemokine balance and lymphocyte subtypes to give an overview of the immune spectrum against SARS-CoV.

Therefore, we characterized systemically the spectrum of immune and inflammatory responses in 95 SARS-infected healthcare workers. Our results indicate that SARS-CoV seem to elicit effective humoral immunity but inhibit cellular immunity. An imbalance of Th2 over Th1 immunity, i.e. prolonged overproduction of IL-10 and transforming growth factor β (TGF-β), may play an important role in the disease. These observations are hypothesized to create an imbalance in immune function that could be associated with SARS pathogenesis, through direct destruction of lymphocytes by SARS or indirectly through impairment of cellular immunity by SARS-induced humoral mediators.

2. Materials and methods

2.1. Patients and clinical features

From February 1 to March 9, 2003, we identified 95 hospital-contact-exposed healthcare workers (nurses, physicians, radiologists, clerical staff, trainees and paramedics) who participated in caring for other SARS patients in our region. whose disease met the case definition of SARS (revised by the Chinese Ministry of Health on April 14, 2003) at the Second Affiliated Hospital of Sun Yat-sen University, in Guangzhou. The 95 patients were enrolled in the study. All had a definite close-contact history with a person who was a suspect, and alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum creatinine levels were elevated significantly within 10–14 days from onset of SARS, as described previously [6]. Approximately 10% of the patients suffered from hypoxemia. There was a 3–7 days (3.6 ± 1.5) latency period prior to the onset of symptoms. All 95 patients developed fever (temperature > 38 for more than 24 h), and 25 of them had rigor (Table 1). About 30% of patients presented with dyspnea, pleurisy and myalgia. Over 80% of the patients reported a productive cough and malaise. Mild leukopenia and thrombocytopenia were observed at initial presentation in about 30% of the patients. Within 1 week of onset of SARS, significant leukopenia (decreased to 3.4×10^9 per l) and thrombocytopenia (decreased to 159.0×10^9 per l) were observed. ALT was present in most cases, which is similar to previous reports [7,8]. Three patients were treated with mechanical ventilation. After empirical treatment with antiviral regimens, gamma globulin and/or corticosteroids, all of these patients survived to recover from all symptoms. Most

Table 1
Characteristics of the patients on presentation*

Characteristic	No. (%) of individuals
Sex	
Male	19 (20)
Female	76 (80)
Age	28.7 ± 9.5
Major symptoms	
Fever (temperature > 38 C for 24 h)	95 (100)
Rigor	25 (26.3)
Cough	95 (100)
Sputum production	84.21 (80)
Dyspnea	30 (31.6)
Pleurisy	28 (29.5)
Malaise	95 (100)
Myalgia	32 (33.7)
Physical signs	
Crackles	34 (35.8)
Percussion dullness	88 (92.7)
Red blood cell count ($\times 10^{12}$ per l)	4.38 ± 0.5
Hemoglobin (g/dl)	129.3 ± 16.8
White blood cell count ($\times 10^9$ per l)	5.6 ± 2.1
Lymphocyte (%)	13.9 ± 13.5
Platelet count ($\times 10^9$ per l)	176.6 ± 72.6
ALT (U/l)	14.6 ± 6.0
AST (U/l)	22.7 ± 5.8
Creatinine (μmol/l)	79.2 ± 13.2
Oxygen saturation on room air (%)	97 ± 2.3

* Plus-minus values are mean ± S.D. Normal ranges are as follows: for red blood cell count, 3.5×10^{12} to 5.5×10^{12} per l; for hemoglobin, 110–160 g/dl; for white blood cell count, 4×10^9 to 10×10^9 per l; for lymphocyte percentage, 20–40%; for platelet, 100×10^9 to 300×10^9 per l; for ALT, 5–40 U/l; for AST, 5–40 U/l; for creatinine, 44–133 μmol/l.