Cytokine and chemokine responses to Japanese encephalitis live attenuated vaccine in a human population

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SUMMARY

Objectives: The SA14-14-2 Japanese encephalitis (JE) live attenuated vaccine is licensed for use only in China, and has provided excellent efficacy in reducing the incidence of JE. The humoral immune response related to the JE vaccination has been well characterized, however cellular immune responses are less well known.

Methods: Thirty-four healthy males who had recently received inoculation with the SA14-14-2 live attenuated vaccine were recruited. Serum samples from these subjects were analyzed for cytokine and chemokine levels using the FlowCytomix method.

Results: Eighteen of 34 subjects were positive for JE virus-specific IgG antibodies. Levels of interleukin (IL)-8, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, and MIP-1β were significantly higher in the vaccinees than in a control group (p < 0.0001, p < 0.0001, p = 0.021, and p < 0.0001, respectively). IL-6 was detectable in 64.7% of vaccinees, but was not detectable in any of the controls. IL-1β, IL-2, IL-4, IL-5, IL-9, IL-10, IL-12p70, IL-13, IL-17A, IL-22, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ were detected in very few subjects or were undetectable in both groups.

Conclusions: IL-6, IL-8, MCP-1, MIP-1α, and MIP-1β may play important roles in the immune response to JE live attenuated vaccine.

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1. Introduction

Japanese encephalitis (JE) is prevalent in eastern and southern Asia. An estimated three billion persons live in areas where JE is endemic, and the annual incidence of the disease in these areas is 30 000–50 000 cases.1 Approximately 25–30% of cases are fatal, and 50% result in irreversible neuropsychiatric sequelae. In China, JE is the most important viral encephalitis and is one of four currently circulating arbovirus diseases.2 After a nationwide vaccination program was initiated in the late 1970s, the incidence of JE dramatically decreased.3 Both inactivated and live attenuated JE vaccines have been developed in China.4 The inactivated vaccine was introduced in the mid-1970s and played a major role in reducing the incidence of JE in the 1980s and 1990s. The SA14-14-2 JE live attenuated vaccine, developed and licensed for use only in China in 1988, provides excellent efficacy and has replaced the inactivated vaccine in many provinces.5–7

The humoral immune response related to JE vaccination has been well characterized in humans and in animal models,8–10 however cellular immune responses during JE virus (JEV) infection are less well known. Studies on the immune response related to cytokine action during JEV infection have shown that various proinflammatory mediators like interferon (IFN)-α, tumor necrosis factor (TNF), interleukin (IL)-6, IL-8, and RANTES (regulated upon activation, normal T-cell expressed and secreted) are associated with the severity of JE disease.11–13 There is less information with respect to serum patterns of cytokines and chemokines in JE vaccination or unapparent JEV infection in both humans and experimental animals. The present study investigated cytokine and chemokine responses in human recipients of SA14-14-2 live attenuated vaccine.

2. Materials and methods

2.1. Subjects and sample collection

Thirty-four healthy males (mean ± standard deviation (SD) age, 31.9 ± 6.7 years) without any acute or chronic infectious diseases were recruited at a medical examination and formed the study population. They had received a single-dose inoculation of JE live attenuated vaccine (The Lanzhou Institute of Biological Products, Lanzhou, China) 20 days prior to presentation, but no other
vaccination. Sixteen healthy males (mean ± SD age, 21.8 ± 2.2 years) were recruited as controls; they had no history of vaccination, or of acute or chronic infections, within the last 6 months. The mean age of vaccinated subjects was significantly higher than that of the controls. Serum samples from all participants were collected and stored at −80 °C until use.

2.2. JEV-specific antibody detection

JEV-specific immunoglobulin (Ig) M and IgG antibodies from serum were measured by IgM antibody-capture ELISA (MAC-ELISA) and indirect ELISA (Shanghai B & C Enterprise Development Co. Ltd, Shanghai, China), respectively.

2.3. Measurement of cytokines and chemokines

Serum cytokines IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17A, IL-22, IFN-γ, and TNF-α, and chemokines IL-8, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, monokine induced by interferon-γ (MIG), and granulocyte colony stimulating factor (G-CSF), were analyzed using a FlowCytomix™ kit (Bender MedSystems, Vienna, Austria), in accordance with the manufacturer’s instructions. Briefly, beads that had distinct fluorescence intensities and that had been coated with specific capturing antibodies were incubated with the serum sample. The cytokine/chemokine-captured beads were then mixed and inoculated with phycoerythrin-conjugated detection antibodies. Fluorescence intensities were detected on a FACSCalibur flow cytometer using CellQuest Pro software (BD Biosciences). Based on a standard curve, analysis was performed using FlowCytomix Pro (version 2.3) software (Bender MedSystems). The thresholds of detection were as follows: 1.2 pg/ml for IL-6; 1.5 pg/ml for IL-9 and IL-12p70; 1.6 pg/ml for IFN-γ and IL-5; 1.9 pg/ml for IL-10; 2.5 pg/ml for IL-17A; 3.2 pg/ml for TNF-α; 4.2 pg/ml for IL-1β; 4.5 pg/ml for IL-13; 16.4 pg/ml for IL-2; 20.8 pg/ml for IL-4; 43.3 pg/ml for IL-22; 0.9 pg/ml for MIG; 1.0 pg/ml for MIP-1α and MIP-1β; 2.2 pg/ml for IL-8 and MCP-1; and 3.4 pg/ml for G-CSF.

2.4. Statistical analysis

Group differences in frequency were tested with the Chi-square test. The Mann–Whitney U-test was used for comparing the levels of cytokines and chemokines between vaccinees and controls because of the non-parametric distribution of data and the relatively small number of observations. All analyses were performed using SPSS v.11.5 software. p-Values of <0.05 were considered to indicate statistical significance. Two-sided p-values are given.

3. Results

Of all 13 cytokines measured, only IL-6 showed a higher detectable rate (64.7%) in the vaccinated subjects; IL-6 was not detectable in any of the controls. TNF-α was positively detected in 26.5% of vaccinees and in 18.8% of controls. IL-1β, IL-2, IL-4, IL-5, IL-9, IL-10, IL-12p70, IL-13, IL-17A, and IFN-γ were detected in very few subjects or were undetectable in both groups. IL-22 was positive in four of 16 controls, but was undetectable in all vaccinees (Table 1).

In contrast with the cytokines, chemokines IL-8, MCP-1, MIP-1α, MIP-1β, and MIG had high percentages of detectable levels in the vaccinated subjects (97–100%) and controls (44–75%), while one of 34 vaccinees and three of 16 controls had detectable G-CSF (Table 2). The detectable rate of IL-8 in the vaccinated group was significantly higher than that in the controls (p < 0.0001). There were no significant differences in the detectable rate between the two groups for the other chemokines.

IL-6 and chemokine levels in vaccinated subjects and controls are shown in Table 3. Levels of IL-8, MCP-1, MIP-1α, and MIP-1β were significantly higher in vaccinated subjects than those in controls. The median MIG level was higher in vaccinees than that in controls, but the difference did not reach statistical significance (p = 0.116). No significant correlation of IL-6 and the chemokines with age was found.

Only one subject was IgM-positive. Eighteen (52.9%) of 34 subjects were positive for IgG antibodies. There were no significant differences in any of the cytokines or chemokines detected between IgG-positive and IgG-negative vaccinees.

4. Discussion

Our results demonstrate that serum cytokine IL-6 and chemokines IL-8, MCP-1, MIP-1α, and MIP-1β were elevated in vaccination.
the vaccine recipients compared to controls; however, most of the cytokines studied, including IL-1β, IL-2, IL-4, IL-5, IL-9, IL-10, IL-12p70, IL-13, IL-17A, IL-22, TNF-α, and IFN-γ, were detected in very few subjects or were undetectable in the two groups.

Chemokines are divided into four subfamilies (CC, CXC, CX3 C, and C) on the basis of the arrangement and number of positionally conserved cysteine motifs. MCP-1 (CCL2), MIP-1α (CCL3), and MIP-1β (CCL4) belong to the CC subfamily. They are the major chemoattractants for monocytes during the inflammatory response, whereas CXC chemokine IL-8 (CXCL8) commonly attracts and activates neutrophil leukocytes.14,15 MCP-1, MIP-1α, and MIP-1β were found to be expressed in some central nervous system (CNS) infections and to play a protective or immunopathological role in the host immune response.16–18 Following JEV infection, MCP-1 or MCP-1 mRNA was mainly detected in microglia and astrocytes and related to neuronal death.19 Few studies have indicated the role of these chemokines in the immune response to JEV in the periphery, especially in the human population. Levels of serum MCP-1 were reported to be increased in patients with live attenuated yellow fever vaccine-associated viscerotonic disease.20,21 These findings have also been reported in rat serum with non-lethal wild-type JEV infection. A significant correlation between viral replication and the MCP-1 or MIP-1α level has been suggested in several virus infections in vitro and in vivo.17,22,23 Our observations of high levels of MCP-1, MIP-1α, and MIP-1β in the mice may result from a SA14-14-2 strain replication in serum. IL-6 and IL-8 are believed to contribute to the antiviral response indirectly by modulating various aspects of the immune response.24 In a study on JE patients, IL-6 and IL-8 were significantly higher in the cerebrospinal fluid of non-survivors compared to survivors.12 Similarly, there were elevated levels of IL-6 and IL-8 in the cerebrospinal fluid in viral encephalitis compared to controls.25 A significantly higher serum IL-8 concentration was detected in acute JE patients than in convalescent patients or healthy controls, and a good correlation was shown between IL-8 and neutrophil count, which has been suggested to relate to JEV degradation26,27. There were increased levels of IL-6 in the serum of rats during non-lethal JEV infection.21

Our results indicate that the cytokine profile induced by SA14-14-2 live attenuated vaccine in the human differs from that caused by wild-type JEV. In the present study, serum IL-4, IL-10, IL-17A, IL-22, and IFN-γ were not detected in any of the vaccines, and IL-1β, IL-2, IL-5, IL-9, IL-12p70, IL-13, and TNF-α were detected in very few. Conversely, higher percentages of JE patients with detectable IFN-γ and IL-4 in plasma have been observed.12 These discrepancies may be due to differences in sampling time. In this study, collection of the serum sample was conducted at 20 days post-vaccination, whereas the patients tended to present earlier to the hospital, generally within 7 days after the onset of illness. The association of cytokine secretion with sampling time has been indicated in animal models. During unapparent JEV infection by non-neuroinvasive JEV in mice, TNF-α, IL-4, and IL-10 were found to be significantly increased within 5 days post-inoculation (dpi), but decreased to the levels before infection at 7 dpi.28 Similarly, in JEV-infected rats, the serum levels of cytokines TNF-α, IFN-γ, IL-4, and IL-10 increased gradually only within 10 dpi, but significantly increased IL-6 and MCP-1 levels continued to 20 dpi.22 Other possibilities are differences in species (murine vs. human) or viral dose and strain (wild-type strain vs. vaccine strain). In addition, a limitation of this study is that only a single time-point was used for serum sampling. Other cytokines or chemokines may have been produced and may play an important role in immunity, but were missed.

In conclusion, proinflammatory cytokine IL-6 and chemokines IL-8, MCP-1, MIP-1α, and MIP-1β may play important roles in the immune response to SA14-14-2 live attenuated vaccine in the human. Further investigations based on multiple samplings are necessary.

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Ethics statement: The study was performed after consultation with the subjects and after receipt of written consent. The study-related information was used anonymously. The Institutional Review Board of Beijing Institute of Microbiology and Epidemiology approved the research involving human materials.

Conflict of interest: No competing interest declared.

References