Cell-Mediated Immune Response during Primary Chlamydia pneumoniae Infection

S. HALME,† J. LATVALA, R. KARTTUNEN, I. PALATSI, P. SAIKKU, AND H.-M. SURCEL

National Public Health Institute, Pohja Military Hospital, and Department of Microbiology, University of Oulu, Oulu, and Lapland Air Command, Rovaniemi, Finland

Received 26 May 2000/Returned for modification 12 July 2000/Accepted 13 September 2000

The development of Chlamydia pneumoniae-specific cell-mediated immunity was studied during a primary C. pneumoniae infection. The immune response was detected as positive lymphocyte proliferation and secretion of interferon gamma. C. pneumoniae-induced activation of both CD4⁺ and CD8⁺ T cells was detected in the early phase of infection, but activation of only CD4⁺ T cells was detected in the later stage.

Chlamydia pneumoniae is an obligate intracellular bacterium that can infect a number of cell types, including monocytes and macrophages (1, 7, 10, 13). Activation of cell-mediated immune (CMI) responses (3, 17) is supposed to be important for protective immunity. Contributory factors participating in the outcome or eradication of the primary C. pneumoniae infection have not been investigated in humans.

In this work we followed the development of humoral immunity and CMI in cases of primary C. pneumoniae infection over a period of 4 months. We measured C. pneumoniae-specific antibodies and the development of CMI responses, including the activation of CD4 and CD8 T-cell subsets and the secretion of selected proinflammatory (tumor necrosis factor alpha [TNF-α] and interferon gamma [IFN-γ]) and anti-inflammatory ( interleukin-10 [IL-10]) cytokines, which according to experimental infections play a role in the final fate of the host defence against Chlamydia (14, 15, 21).

The series included 291 male patients (age range, 18 to 20 years) who had consulted a doctor because of respiratory tract symptoms (range of duration, 2 to 14 days) and acute fever. The diagnosis of a primary C. pneumoniae infection was based on the detection of specific Immunoglobulin M (IgM) antibodies either at admission or 2 weeks later. The positive diagnosis was used as an inclusion criterion for enrollment in the tests for CMI responses. For that purpose, citrated blood samples were drawn soon after the diagnosis with follow-up specimens taken at weeks 8 and 16 after the symptoms appeared.

The presence of IgM antibodies in 16 of the 291 patients (5.5%), and 9 of these 16 patients were within reach for the follow-up study of CMI responses. The IgM response to C. pneumoniae continued to increase after admission (geometric mean titer [GMT] of 31.8) and reached its maximum value (GMT of 160.0) 2 to 3 weeks after diagnosis. The C. pneumoniae-specific IgG antibodies were positive (IgG ≥ 32) 8 weeks after the diagnosis in eight of the nine patients (GMT of 50.8) and at 16 weeks in all 9 patients (GMT of 36.8). A positive C. pneumoniae-specific IgA response (IgA ≥ 16) was found in only three patients.

### TABLE 1. Follow-up analysis of C. pneumoniae-specific antibodies and simultaneously analyzed lymphocyte proliferative responses (SI) in nine patients with a primary C. pneumoniae infection

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Time (wk)</th>
<th>Antibody titer</th>
<th>LP test (SI)</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>IgA</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>1,280</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1,280</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>640</td>
<td>64</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>160</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>160</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>640</td>
<td>64</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>160</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>1,280</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>160</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>160</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>160</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>
of the nine patients (33%) at some point in the follow-up (Table 1), and C. trachomatis-specific IgG antibodies were detected in none of them.

The PBL proliferative response to C. pneumoniae was positive (stimulation index [SI] $\geq 3$) in all nine patients at 3 weeks after the disease symptoms appeared (Table 1; median SI, 86.1; range, 3.1 to 162.0). CMI responses were also studied in five control patients who did not have detectable antibodies to C. pneumoniae, and the median PBL response in these cases was 2.3 (range, 1.0 to 7.1). The PBL responses to C. pneumoniae antigen started to decline after the active phase of the infection but remained clearly positive up to 16 weeks (24.3; range, 3.5 to 97.3) in seven patients. As shown in Table 1, the PBL responses were low (SI $< 3$) at week 16 in two patients, one of whom no longer had detectable antibodies to C. pneumoniae.

The PBL responses to C. trachomatis did not change and were significantly lower than those to C. pneumoniae at every time point (median responses of 1.6, 7.9, and 2.7 at weeks 3, 8, and 16, respectively; $P < 0.05$).

The PBL activation induced by the infectious C. pneumoniae (0.1 and 0.01 EB/PBL) was detected in vitro as an increased number of CD8$^+$ DR$^+$ lymphocytes in comparison to non-stimulated lymphocytes in the specimens taken at 3 weeks ($P < 0.01$) but less clearly in the later specimens (Fig. 1). The expression of HLA-DR antigen on the nonstimulated CD4$^+$ lymphocytes tended to be high at week 3 relative to the other time points and did not differ statistically significantly from the stimulated level at any time point.

C. pneumoniae-induced cytokine secretion (IFN-γ, IL-10, and TNF-α) was analyzed in the 5-day culture supernatants. The strong PBL proliferative response at week 3 was also reflected in increased production of IFN-γ in response to C. pneumoniae EB (0.1 IFU/PBL) compared to the nonstimulated secretion (Fig. 2). Thereafter, the C. pneumoniae-induced IFN-γ secretion tended to decline, but the difference between the nonstimulated and stimulated PBL remained significant over the whole period. Secretion of TNF-α and IL-10 was clearly detectable even in the culture supernatants of nonstimulated PBL at all the time points, but stimulation with C. pneumoniae enhanced the secretion significantly (Fig. 2).

According to our results, C. pneumoniae-specific CMI responses during acute C. pneumoniae infection appeared early after the disease symptoms and simultaneously with the humoral response. The strength of the proliferation did not correlate with the antibody responses, however, which is in accordance with immune responses during C. trachomatis infection (4, 12). Although the PBL responses differed widely in the background secretion of cytokines was measured in cultures in the absence of antigen.
patients, in spite of antimicrobial therapy, these responses appeared to be strong during the active stage of the infection compared to corresponding results for C. pneumoniae seronegative subjects included in this and an earlier series of ours (17) and with subjects having IgA antibodies to C. pneumoniae (9). Strong PBL reactivity to C. trachomatis antigens has been associated with spontaneous clearance of Chlamydia infection (2), suggesting a protective role for CMI in the development of trachoma.

The fact that the C. pneumoniae-induced PBL reactivity was significantly stronger than that to C. trachomatis antigen shows that CMI reactivity to C. pneumoniae is species specific during a primary infection (17), as was the case in healthy C. pneumoniae-immune responders (9). On the other hand, C. pneumoniae-specific CMI responses in patients with coronary heart disease have been shown to be predominantly induced by antigenic structures that are common among chlamydial species (8). It is difficult to know whether immune responses to chlamydial antigens are linked to the immunopathological mechanism that operates in C. pneumoniae-associated chronic diseases (19); in any case, the PBL cross-reactivity to chlamydial species that was typically found in male patients with coronary heart disease but not in female ones (8) is not a general phenomenon related to the male sex because all of the patients with species-specific PBL reactivity in this series were men.

According to our results, C. pneumoniae-induced T-cell activation seemed to be linked with CD8+ cells during the active stage of infection, since the difference between nonstimulated and C. pneumoniae-stimulated cells expressing the HLA-DR molecule was larger in CD8 than in CD4 cells. Alternatively, the nonsignificant difference in the activated CD4+ T cells between nonstimulated and stimulated cells early in the acute infection suggests that the CD4+ cells were activated in vivo and are thereby important for recovery from the infection.

IFN-γ has proved to be crucial in terms of the eradication of infection and providing immunity to experimental Chlamydia (14, 15). On the other hand, susceptibility and impaired host defence against Chlamydia has been shown to be a consequence of a cytokine production switch to prominence of IL-10 (20), an anti-inflammatory cytokine that attenuates CMI reactions that are needed for host defence against intracellular pathogens (5, 6). According to our results, IFN-γ production was clearly detectable after PBL stimulation with infectious C. pneumoniae EBs in all of the patients studied. IL-10 secretion was simultaneously detected as well, but never in the absence of IFN-γ secretion by the same cells, which argues against the immune response being immunosuppressive in these subjects. Although it is not possible to draw conclusions about the physiological significance of the cytokine concentrations, the presence of IL-10 in the Chlamydia-stimulated cell cultures may reflect a possibility that overproduction of IL-10 at some stages may inhibit the immune response and IFN-γ secretion upon C. pneumoniae stimulation.

In conclusion, we have shown that a primary C. pneumoniae infection induces the development of a positive PBL proliferative response to C. pneumoniae but not to C. trachomatis and also the secretion of IFN-γ in all subjects. C. pneumoniae-induced lymphocyte activation involves CD8+ T cells in the early phase of infection but CD4+ T cells in the later stage. However, in vivo activation of CD4+ cells during the acute disease may relate to their role in recovery from infection. No significant differences were observed in the immune responses among the subjects, although a larger population would be needed to determine whether any immunopathological markers of susceptibility to chronic C. pneumoniae infection (11, 16, 19) can be seen at the acute stage of infection or only with time or after repeated infections.

This work was supported by grants from the Academy of Finland and the Jalmari and Rauha Ahokas Foundation.

REFERENCES