The Role of RANTES in Meningococcal Disease

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The chemokine RANTES (regulated on activation, normal T cell expressed and secreted) is a potent regulator of leukocyte trafficking. RANTES preferentially attracts mature CD4 cells as well as macrophages and eosinophils, but not neutrophils. In total, 128 children with meningococcal disease were prospectively studied, and the role of RANTES in the pathophysiology of meningococcal disease was assessed. Plasma RANTES, interleukin (IL)-8, IL-6, IL-1 receptor agonist, and tumor necrosis factor–α were measured at admission. Severity of disease was stratified by the Glasgow meningococcal septicemia prognostic score (GMSPS). RANTES levels correlated significantly with IL-8 levels, admission lactate levels, platelets, prothrombin time, and activated partial thromboplastin time. RANTES levels were lower in children with severe disease (GMSPS ≥ 8; P = .001), in those with septic shock (P < .0005), and in nonsurvivors (P = .048; Mann-Whitney test). RANTES is a potential mediator in the pathophysiology of meningococcal disease.

Cytokines are important in the pathophysiology of meningococcal sepsis [1–4]. Interleukin (IL)-8, a chemotactic chemokine, also participates in the complex cytokine network during the initial phase of invasive meningococcal infection [5]. IL-8, like the chemokine RANTES (regulated on activation, normal T cell expressed and secreted) belongs to a family of structurally related proteins involved in leukocyte trafficking. Individual chemokines selectively attract different leukocyte populations [6].

RANTES protein is an 8-kDa nonglycosylated polypeptide produced by T cells, endothelial cells, and platelets. It belongs to a family of ≥10 distinct 8- to 10-kDa cytokines that include IL-8, macrophage inflammatory proteins–1α and –1β (MIP-1α, MIP-1β), monocyte chemoattractant protein–1, and interferon-inducible protein–10. This chemokine superfamily is divided into 2 branches, depending on whether the first 2 cysteine molecules are adjacent (the C-C branch, β chemokines) or separated by an amino acid (the C-X-C branch, α chemokines). The C-X-C chemokines, which include IL-8 and MIP-2α, are potent chemoattractants for neutrophils [6], whereas the C-C branch, which includes RANTES and MIP-1β, are potent chemoattractants for monocytes and eosinophils.

RANTES is produced by mature CD4+/CD8+ lymphocytes, and its expression is regulated by cell activation, hence the name. It attracts monocytes, macrophages, and eosinophils and also preferentially attracts T cells of the CD4+/CD45RO+ phenotype. These CD4+/CD45RO+ cells are thought to be primed helper T cells involved in memory T cell function [7]. These properties suggest that it may play a key role in immune regulatory functions and in inflammatory processes. The role of RANTES has not been studied in meningococcal disease.

Recent studies show that in RANTES-stimulated T cells there is induction of expression and up-regulation of certain adhesion and activation molecules in a lymphocyte function–associated antigen–1/intercellular adhesion molecule (ICAM)–3–dependent process, with a possible contribution from ICAM–1 [8]. This suggests that these cells undergo cell activation, which may significantly alter their phenotype and enhance their capacity to respond to bacterial or viral pathogens. ICAMs play a central role in tissue damage that occurs in meningococcal disease secondary to the inflammatory response [9].

Patients and Methods

We prospectively studied 128 children with a clinical diagnosis of meningococcal disease. A clinical diagnosis of meningococcal disease was made in an ill child with fever and a petechial or purpuric rash or with signs of meningitis or with both. Children studied ranged in age from birth to 16 years. They were included in the study if they presented with a petechial or purpuric rash with or without signs of meningitis or proven meningitis and if no
Figure 1. RANTES concentrations (pg/mL) in different groups of children with meningococcal disease. Upper bars, 75th percentile; lower bars, 25th percentile; middle circle, median. GMSPS, Glasgow meningococcal septicemia prognostic score; MM, meningococcal meningitis alone; MM/MS, meningococcal meningitis/septicemia; MS, septicemia alone.

other pathogen was isolated despite thorough microbiologic investigation. Symptoms, clinical findings, and laboratory data were recorded on a specially designed form. Symptoms at presentation included fever, vomiting, impaired consciousness, headache, rash, myalgia, irritability, upper respiratory symptoms, seizures, reduced feeding, and diarrhea. Severity of disease was assessed by the Glasgow meningococcal septicemia prognostic score (GMSPS) [10, 11]. Severe disease was defined as a GMSPS ≥8. Children were classified into 3 groups: meningitis (MM)—meningitis without shock or impaired peripheral perfusion; septicemia (MS)—no evidence of meningism (nuchal rigidity or irritability and lethargy in an infant) but features of shock with impaired peripheral perfusion, tachycardia, acidosis, or oliguria; and mixed meningitis/septicemia (MM/MS)—both septicemia and signs of meningism or irritability and lethargy in an infant.

Children were classified as having meningitis alone if they had either a positive cerebrospinal fluid (CSF) microscopy, culture, antigen, or polymerase chain reaction (PCR) or abnormal CSF parameters with positive serology for meningococcal infection in acute- and convalescent-phase specimens. Classification by acute- and convalescent-phase serology was used for 2 children with symptoms and signs of meningitis and abnormal CSF parameters but no other microbiologic confirmation of meningococcal disease. The serology assays were done at the Public Health Laboratory Service (PHLS; London) Meningococcal Reference Unit (which provides a national service for England and Wales), with a screening assay based on the outer membrane protein, followed by an assay to measure serogroup B and C polysaccharide antibody.

Septic shock was defined as systolic blood pressure (mm Hg) by age as follows: <75, <1 year old; ≥80, 1–5 years old; ≥85, 6–12 years old; and ≥100, >12 years old. Blood lactate, platelet count, total white cell count, neutrophil and lymphocyte count, prothrombin time, and activated partial thromboplastin time (APTT) were measured on admission.

RANTES, IL-8, IL-6, IL-1 receptor antagonist (Ra), and tumor necrosis factor (TNF)-α concentrations were determined in plasma samples collected on admission and stored at −70°C until assayed by commercial solid-phase enzyme amplified sensitivity immunoassay (EASIA) on a microtiter plate (Medgenix Biosource, Fleurus, Belgium). If the cytokine concentrations were above the upper limit of the standard curve of the immunoassay, additional dilutions were not performed. In the statistical analysis, nonparametric tests for ordinal or ranked data were used to allow for cytokine concentrations above the upper limit of normal, to avoid inaccuracies in data analysis. Antibody detection tests were done by a latex agglutination method (Wellcogen; Wellcome, Dartford, UK), and the PCR assays were performed at the PHLS Meningococcal Reference Unit with an automated PCR system (Taqman; ABI, Warrington, UK). Statistical analyses were done by computer (version 8.0 for Windows; SPSS, Chicago). We determined differences in medians by Mann-Whitney test and correlations by Spearman’s correlation coefficient.

Results

We studied 52 girls (41%) and 76 boys (59%). Ages were 0.1–15.9 years (median, 2.9; interquartile range [IQR], 1.0–7.5). RANTES concentrations were 260 to 4500 pg/mL (the upper limit of detection of the assay; median, 1395; IQR, 835–4117). Of the children, 61 (49%) had severe disease, 24 (19%) had septic shock, and 7 (6%) died. Seven children had MM (5%), 71 had mixed MM/MS (56%), and 50 had septicemia (39%). Diagnoses were confirmed microbiologically in 88 children.
Table 1. Cytokine and chemokine concentrations (pg/mL) by Glasgow meningococcal septicemia prognostic score (GMSPS).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>GMSPS &gt;8</th>
<th>GMSPS &lt;8</th>
<th>Significancea</th>
</tr>
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<tbody>
<tr>
<td>TNF-α</td>
<td>159 (56–658)</td>
<td>72 (35–124)</td>
<td>.001b</td>
</tr>
<tr>
<td>IL-8</td>
<td>100 (13–750)</td>
<td>19 (10–80)</td>
<td>&lt;.0005c</td>
</tr>
<tr>
<td>IL-6</td>
<td>1480 (1710–1700)</td>
<td>1395 (304–700)</td>
<td>.162</td>
</tr>
<tr>
<td>IL-1Ra</td>
<td>&gt;1750 (&gt;750)</td>
<td>&gt;1750 (&gt;1750)</td>
<td>.005b</td>
</tr>
</tbody>
</table>

NOTE. TNF, tumor necrosis factor; IL, interleukin; Ra, receptor antagonist.
a By Mann-Whitney test.
b P < .05.

differences in RANTES concentrations found on the basis of either of the 2 comparisons: duration of symptoms <12 versus ≥12 h or duration of symptoms <24 versus ≥24 h. TNF-α, IL-8, and IL-1Ra concentrations were significantly higher in children with severe disease than in those without. IL-6 concentrations were also higher but did not reach statistical significance (table 1).

Discussion

The pathophysiology of meningococcal disease involves a complex interplay of cytokines, complement, and clotting factors. High circulating levels of TNF-α, IL-6, IL-1, and IL-8 have been detected in children with meningococcal septicemia [1–4]. The chemokine RANTES plays an important role in directing the migration of monocytes, memory T cells, eosinophils, basophils, and NK cells and in activation of T cells. RANTES suppresses human immunodeficiency virus (HIV) type 1 because it is a natural ligand for the chemokine receptor CCR5, a coreceptor for HIV-1 [13]. Its role has not been previously determined in meningococcal disease. RANTES does play a specific role in T cell intercellular adhesion and adhesion molecule expression [8]. Adhesion molecule expression is increased in meningococcal disease [9], and this process may be modulated through RANTES.

We found that RANTES correlated negatively with the proinflammatory cytokines, TNF-α and IL-8, and other laboratory variables known to be associated with more-severe disease. RANTES responses do not appear to mature through childhood nor do they appear to be involved in determining type of disease presentation. Our results are consistent with those of other studies in which concentrations of TNF-α, IL-8, and IL-1Ra were significantly higher in more-severe disease. The Mann-Whitney test and Spearman’s correlation coefficient were calculated both with and without the 30% of study patients with no microbiologic confirmation, and the results were the same in both cases. Thus, it is reasonable to assume that these children also had meningococcal disease.

Polymorphisms in the TNF-α promoter gene are associated with more-severe disease and a worse outcome [14]. Further work is needed to determine if polymorphisms in the RANTES chemokine promoter might be associated with less-severe disease, as this polymorphism delays the progression of HIV-1 disease in HIV-infected persons [15]. Experimental studies remain to be performed to determine the exact role of RANTES in the pathophysiology of meningococcal disease and to establish any potential therapeutic value.

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References