Elevated Levels of Interleukin-1β (IL-1β) and IL-6 in Serum and Increased Production of IL-1β mRNA in Lymph Nodes of Patients With Polyneuropathy, Organomegaly, Endocrinopathy, M Protein, and Skin Changes (POEMS) Syndrome


To evaluate a possible implication of cytokines in the pathogenesis of polyneuropathy, organomegaly, M protein, skin changes (POEMS) syndrome, we studied five consecutive patients with this condition, of which four had sclerotic bone lesions and four had multicentric Castleman’s disease. Interleukin-1β (IL-1β) and IL-6 serum levels were determined in these patients (13 serum samples) and in patients with multiple myeloma (5) and Waldenström’s macroglobulinemia (5). In situ hybridization of the relevant mRNAs was performed on lymph node specimens of two patients with POEMS syndrome who had Castleman’s disease. Elevated serum levels of IL-1β (13/13 samples), and IL-6 (7/13 samples) were found in patients with POEMS syndrome.

The POEMS SYNDROME is a multisystem disorder characterized by the combination of polyneuropathy, organomegaly, M protein, skin changes, and various other clinical and pathologic signs such as fever, cachexia, edema, thrombocytosis, and multicentric Castleman’s disease.1 The POEMS syndrome is typically associated with osteosclerotic myeloma or solitary plasma cytomma, but in a minority of cases Castleman’s disease without concurrent plasma cell tumor may be found.1,4 The nosologic questions raised by the striking overlap between POEMS syndrome and some types of multicentric Castleman’s disease with IgA dysproteinemia, polyneuropathy, papilledema, and thrombocytosis are still debated.6

Attempts to identify a specific auto-antibody activity of the monoclonal protein have failed or have not been substantiated in POEMS syndrome, raising the possibility of a pathogenetic role of nonimmunoglobulinic mediators. High serum IL-6 levels have been previously reported in two patients with POEMS syndrome, suggesting a role for IL-6 in the pathogenesis of the disease.7,8 Interleukin-6 (IL-6) is likely implicated at the origin of some systemic manifestations associated with Castleman’s disease6,10 or POEMS syndrome, such as polyclonal gammapathy, fever, hepatomegaly, and thrombocytopenia. In fact, it is currently believed that IL-6 has little direct toxicity and rather behaves as an important cofactor in the expression of diseases,11 factors other than IL-6 have been considered necessary for the full expression of POEMS syndrome.8 Such factors might include other cytokines, such as IL-1, which is an early mediator of inflammation produced by monocytes/macrophages.12 IL-1 participates in the so-called monokine network in which IL-1 stimulates IL-6.13 IL-1 acts on nearly all body tissues, including those of the immune, nervous, and endocrine systems, and can induce a wide spectrum of systemic effects when released in large amounts in the circulation.12 Whereas IL-1α remains primarily cell associated, IL-1β is secreted mainly in the extracellular fluid.11

In the present study, we evaluated serum levels of IL-6 and IL-1β in five patients with POEMS syndrome and looked for relevant mRNAs in lymph nodes of 2 of them who had Castleman’s disease. We also looked for Epstein-Barr virus (EBV) protein and genome in lymph nodes, as EBV may play a role in Castleman’s disease,14 and EBV-transformed B lymphocytes may produce IL-1β.15

PATIENTS AND METHODS

Patients

Serum cytokines levels were evaluated in five consecutive patients with POEMS syndrome (four men, one woman, age range: 38 to 72 years), followed at Henri Mondor hospital from 1977 to 1991. The main clinical and biologic findings are listed in Table 1. Some findings in patients 1, 3, 4, and 5 have been previously published.5,6 Polyneuropathy (5/5) met the criteria for chronic demyelinating neuropathy (median nerve conduction velocities less than 30 m/s, proteinorachia greater than 100 mg/mL). Organomegaly included hepatomegaly (5/5), splenomegaly (5/5), and lymphadenopathy (5/5). Endocrinopathy (5/5) included hypothyroidism (3/5), hypogonadism (4/5), hyperprolactinemia (2/5), and glucose intolerance (1/5). The monoclonal protein consisted of a free λ light chain (4/5), IgGκ (2/5), IgGλ (2/5), and serum levels ranged from undetectable (the free λ light chain) to 4 g/ L. Polyclonal IgA gammapathy was observed in 2 of 5 patients. Typical skin changes (hyperpigmentation, thickening, hypertrophic infiltration) were observed in 4 of 5 patients. Other manifestations previously described in POEMS syndrome were found, including edema (5/5), anasarca (4/5), cachexia (4/5), psychiatric disturbances (4/5), chronic diarrhea (3/5).
Immunocytochemistry and in situ hybridization for cytokines and EBV were performed on lymph node frozen tissue of patients 2 and 3. Tonsilar lymphoid tissues displaying a benign follicular hyperplasia without evidence of Castleman’s disease were used as controls for in situ hybridization.

Serum Cytokine Levels

**Serum sampling.** Peripheral blood (5 mL) was taken in dry tubes at 8 am; the storage of the whole blood was performed at room temperature. The serum was separated within 3 hours, and the sera were kept frozen at −70°C until analyzed. Blood samples were obtained in the absence of fever, shock, and overt infection.

**IL-1β and IL-6** were measured using a sandwich type enzyme immunoassay (Immunotech, Marseille, France) according to the kit procedure. Using these kits, the upper limit of IL-1β serum levels in healthy subjects is 5 pg/mL, that of IL-6 is 10 pg/mL.

Immunocytochemistry and In Situ Hybridization

**Tissues.** Lymph nodes and tonsilar lymphoid tissues used as controls were frozen in liquid nitrogen immediately after surgical biopsy, and were kept at −70°C.

**Immunocytochemistry.** Immunocytochemistry was performed on frozen material using an indirect immunoperoxidase assay (APAAP) with antibodies directed against α, μ, γ heavy and κ and λ light Ig chains, against B-cell antigens CD19 (B4), CD20 (B1), and CD22 (panB), against T-cell antigens CD3 (Leu-4), CD4 (Leu-3), and CD8 (Leu-2), against dendritic cell antigens dendritic reticulum cell (DRC) (no CD assigned) and CD35 (C3B receptor), against activation antigen CD25 (Tac), and against EBV-encoded latent membrane protein (LMP-1). A direct immunofluorescence study was performed on the paraffin-embedded material using antibodies directed against κ and λ chains.

**In situ hybridization.** Several nucleic acid probes were used in the study. The IL-1β and IL-6 probes have been previously described, and correspond to antisense 3SS-labeled riboprobes recognizing the coding sequences of the relevant mRNA. In situ hybridization was performed as previously described. Sense probes were used as controls. Enumeration of cytokine gene expressing cells was expressed as the mean number of positive cells per 40 power field, observed in five consecutive areas. Detection of EBV genomes was performed as previously described by some of us, using a biotinylated EBV DNA probe corresponding to the 3.1-kb BamHIW internal repeat fragment of the EBV genome (ENZO Diagnostics, New York), and the fluorescein-conjugated oligonucleotides complementary to nuclear RNAs portions of the EBER 1 and 2 genes of EBV (Dakopatts, Glostrup, Denmark), which are actively transcribed in latently infected cells.

### Table 1. Main Clinical and Biologic Findings in Patients With POEMS Syndrome

<table>
<thead>
<tr>
<th>Age of onset/sex</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
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<tbody>
<tr>
<td>Polyneuropathy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Organomegaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hepatomegaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Endocrinopathy</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hyperprolactinemia</td>
<td>+</td>
<td>+</td>
<td>Not tested</td>
<td>Not tested</td>
<td>−</td>
</tr>
<tr>
<td>Hypogonadism</td>
<td>+</td>
<td>+</td>
<td>Not tested</td>
<td>Not tested</td>
<td>+</td>
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<tr>
<td>Glucose intolerance</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Monoclonal protein</td>
<td>λ</td>
<td>IgG, IgA, IgM</td>
<td>IgA, IgG</td>
<td>IgG, IgA</td>
<td>+</td>
</tr>
<tr>
<td>Skin changes</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Edema</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Cachexia</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Polyclonal hypergammopathy</td>
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<td>−</td>
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<tr>
<td>Castleman’s disease</td>
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<td>+</td>
<td>Not tested</td>
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</tr>
<tr>
<td>Bone lesions</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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</tbody>
</table>

### Table 2. Serum Cytokine Levels in Patients With the POEMS Syndrome, Multiple Myeloma, and Waldenström’s Macroglobulinemia

<table>
<thead>
<tr>
<th>Cases</th>
<th>No. of Samples</th>
<th>IL-1β (N ≤ 5 pg/mL)</th>
<th>IL-6 (N ≤ 10 pg/mL)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Median Value</td>
<td>Range</td>
</tr>
<tr>
<td>POEMS syndrome</td>
<td>1</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>365</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>5</td>
<td>9.5</td>
<td>1 - 12</td>
</tr>
<tr>
<td>Waldenström’s macroglobulinemia</td>
<td>5</td>
<td>1</td>
<td>0 - 5</td>
</tr>
</tbody>
</table>
RESULTS

Serum Cytokines Measurements

Results of serum cytokines measurements are given in Table 2. Moderate to marked (up to 73-fold) elevation of IL-1β was found in all 13 serum samples of the 5 patients with POEMS syndrome (Fig 1). Seven of 10 patients with other plasma cell dyscrasias had normal serum IL-1β levels. The remaining 3 had multiple myeloma and mild increase of serum IL-1β. Elevated serum levels of IL-6 were found in 3 patients with POEMS syndrome (7 of 13 samples, up to 5-fold elevation), and one patient with Waldenström's macroglobulinemia (Fig 2).

Serial evaluations in patient 1 (Fig 3) showed that the high levels of IL-1β were unaffected by 10 monthly courses of cytotoxic chemotherapy including vinblastin, melphalan, cyclophosphamide, and prednisone, whereas the IL-6 levels transiently returned within normal limits. Afterwards, at a time of worsening of the clinical condition assessed by repeated episodes of anasarca, both IL-1β and IL-6 increased until death.

Immunocytochemistry and In Situ Hybridization for Cytokines and EBV

Axillary lymph node biopsies of patient 2 and 3 with POEMS showed angiofollicular hyperplasia. As in patients 1 and 4, the germinal centers were hyperplastic or, more often, atrophic and were surrounded by small lymphocytes in an "onion skin pattern" (Fig 4A). Interfollicular regions showed small vessel proliferation with mild sinus histiocytosis and contained small lymphocytes and a low number of plasma cells. Immunocytochemistry showed numerous B-cell follicles with dendritic reticulum cell pattern (DRC, CD35) and T cells that predominated in interfollicular areas. Neither the APAAP technique on frozen sections nor direct immunofluorescence on paraffin-embedded material could disclose a monotypic B cell population.

In situ hybridization with IL-6 probes showed rare positive cells in the interfollicular spaces in lymph nodes of both patients 2 and 3 in control tissue (data not shown). In contrast, in situ hybridization with IL-1β probe showed abundant IL-1β-mRNA producing cells. Sense probes used as controls gave no positive signals (Fig 4B). The IL-1β gene-
DISCUSSION

In the present study, increased levels of IL-1β were found in all serum samples of 5 patients with POEMS syndrome, whereas increased levels of IL-6 were detected in 3 of 5 patients. In contrast, patients with other plasma cell dyscrasias had no or very slight increase of circulating IL-1β, as previously reported. Marked elevation of serum IL-6 was detected in one patient with Waldenström’s macroglobulinemia, a finding consistent with the role ascribed to IL-6 in this disease. The marked intersubject variability observed for IL-1β levels was consistent with the previous finding of high and low “IL-1β producers.” Release of cytokines is usually pulsatile, which may account for intrasubject variables. However, in a number of individuals, IL-1 production is strikingly stable, and this was the case in two patients. In one of them (patient 1), longitudinal evaluations showed that levels of IL-1β were unaffected by cytotoxic chemotherapy, unlike IL-6 levels, which transiently returned within normal limits. Worsening of the clinical condition assessed by anasarca episodes were associated with elevation of both IL-1β and IL-6.

In situ hybridization on lymph nodes with Castleman’s disease lesions showed abundant cells producing IL-1β mRNA in interfollicular areas without evidence of conspicuous local production of IL-6 in the two evaluated patients. Lymph nodes showed a normal mixture of κ and λ light chain immunoreactivity, and distribution and localization of IL-1β mRNA-producing cells were those of macrophages, the most important source of IL-1β. However, plasma cell lines secreting IL-1β have been described, and it could not be excluded that IL-1β was produced by sparse monoclonal B cells present in a too-small proportion to be recognized by usual histochemical techniques, as previously reported in multicentric Castleman’s disease. EBV protein and genome were not found in lymph nodes.

Abnormal cytokine production in B-cell disorders may be clinically relevant in two ways: it may be responsible for some clinical manifestations and may participate in genesis and/or maintenance of the plasma cell clone.

Gammopathy, thrombocytosis, and hepatomegaly in our patients could be related to overproduction of IL-6, as IL-6 acts as a B-cell differentiation factor with induction of antibody production as a major function, stimulates thrombopoiesis, and is a strong hepatocyte-stimulating factor. IL-6 might also have acted as a cofactor for the toxic effects of IL-1β. Overproduction of IL-1β had presumably participated to the expression of some clinical manifestations in our patients. For example, skin hyperpigmentation was associated with abnormal production of α-melanocyte stimulating hormone (MSH) by pituitary cells in patient 4 of the present study. IL-1β activates the gene for proopiomelanocortin, a product of which is α-MSH, a pigmenting agent that exerts a negative feed back on IL-1-induced fever. Fibrogenic properties of IL-1β and angiogenic properties of IL-6 may have accounted for dermal fibrosis and hemangiomata, that are other skin changes of POEMS syndrome. IL-1β acts on the central nervous system where it can induce anorexia and sympathetic activation of brown adipose tissue, leading to cachexia, sickness behavior, and slow wave sleep, and increased production of corticotropin releasing hormone. IL-1 suppresses thyroid, testicle, and pancreatic β-cell functions. In addition, monokines are likely implicated in the genesis of demyelinating polyneuropathies, and the patients were severely disabled by their neuropathy at time of serum evaluations. Endothelium is an important target of cytokines, and this probably accounted for edema and other manifestations of our patients, such as episodes of hypotension, liability to thrombosis, severe arteriopathy, and microangiopathic glomerulopathy.
High serum IL-6 levels in patients with POEMS syndrome have been previously reported. This finding is in keeping with the evidence that human myeloma cells constitutively secrete IL-6 and proliferate in response to this molecule. However, IL-6 stimulates B-cell and vascular proliferations that are both observed in Castleman’s disease, and there are some arguments suggesting that IL-6 overproduction in patients with POEMS syndrome could be related to Castleman’s disease more than myeloma: (1) increased production of IL-6 mRNA was detected in germinal centers of lymph nodes of some patients with Castleman’s disease; (2) increased production of IL-6 has been documented in several conditions that may be associated with Castleman’s disease-like lesions in lymph nodes, such as acquired immunodeficiency syndrome, Kaposi’s sarcoma, and rheumatoid arthritis; and (3) circulating levels of IL-6 were not detected in a series of 9 patients with POEMS syndrome without Castleman’s disease.

In our patients, lymph nodes appeared as one site of increased production of IL-1β. This finding likely reflected systemic activation of monocyte/macrophages. The lack of evidence for strong local production of IL-6 in lymph nodes, and the decrease of IL-6 levels under cytotoxic chemotherapy in patient 1, suggested extranodal, possibly tumoral, secretion of this cytokine. Whether the plasma cell clone, or marrow accessory cells, of patients with POEMS syndrome abundantly produce IL-6 and molecules able to stimulate IL-1 production remains to be determined, but the hypothesis of a crucial role of myeloma products at the origin of POEMS syndrome is supported by the complete recovery that occurs in patients with a solitary plasmocytoma after surgery and local irradiation. New insights into the pathogenesis of POEMS syndrome will probably come from studies of the plasma cell tumor of affected patients and evaluation of a possible interference of other cytokines and cytokine inhibitors, in the overall biologic and clinical setting.

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REFERENCES

33. Dubuis JM, Dayer JM, Siegriest-Kaiser CA, Burger AG: Human recombinant interleukin-1β decreases plasma thyroid hormone and thyroid stimulating hormone levels in rats. Endocrinology 123:2175, 1988
34. Hales DB: Interleukin-1 inhibits Leydig cell steroidogenesis primarily by decreasing 17 alpha hydroxylase/C17-20 lyase cytochrome P450 expression. Endocrinology 131:2165, 1992
38. Klein B, Zhang XG, Jourdan M, Content J, Houssiau F, Delfraissy JF, Dormont J, Galanaud P Production of interleukin-1 by an...
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