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Cytokine Activation During Attacks of the Hyperimmunoglobulinemia D and Periodic Fever Syndrome

By Joost P.H. Drenth, Marcel van Deuren, Johanna van der Ven-Jongekrijg, Casper G. Schalkwijk, and Jos W.M. van der Meer

The hyperimmunoglobulinemia D and periodic fever syndrome (hyper-lgD) is a rare entity characterized by periodic occurrence of febrile attacks. All patients have a clearly elevated (poly)clonal serum IgD (>100 U/mL). So far, we have diagnosed 59 patients (31 male and 28 female) from nine, mainly European, countries. The episodes last 3 to 7 days, occur every 4 to 6 weeks, start early, and persist throughout life, and, between attacks, patients are nonsymptomatic. Symptoms are not triggered by known infectious agents and include abdominal distress (vomiting, diarrhea, and pain), joint involvement (arthralgia/arthritis), skin lesions, headache, and lymphadenopathy. The main feature of the disease is the abrupt onset of fever that parallels with an acute-phase reaction as evidenced by leukocytosis, neutrophilia, and a raised erythrocyte sedimentation rate.

The pathogenesis of the hyper-lgD syndrome is unclear, but, because fever and the acute-phase reaction are considered to be mediated by cytokines such as interleukin-1α (IL-1α), IL-1β, IL-6, and tumor necrosis factor α (TNFa) and the inhibitory compounds IL-1 receptor antagonist (IL-1ra), IL-10, and the soluble TNF receptors p55 (sTNFr p55) and p75 (sTNFr p75) in 22 patients with the hyper-lgD syndrome during attacks and remission. Serum CRP and PLA2 concentrations were elevated during attacks (mean, 213 mg/L and 1,452 ng/mL, respectively) and decreased between attacks. Plasma concentrations of IL-1α, IL-1β, or IL-10 were not increased during attacks. TNFr concentrations were slightly, but significantly, higher with attacks (104 ν 117 pg/mL). Circulating IL-6 values increased with attacks (19.7 ν 147.9 pg/mL) and correlated with CRP and PLA2 values during the febrile attacks. The values of the antiinflammatory compounds IL-1ra, sTNFr p55, and sTNFr p75 were significantly higher with attacks than between attacks, and there was a significant positive correlation between each. The ex-vivo production of TNFa, IL-1α, and IL-1ra was significantly higher with attacks, suggesting that the monocytes/macrophages were already primed in vivo to produce increased amounts of these cytokines. These findings point to an activation of the cytokine network, and this suggests that these inflammatory mediators may contribute to the symptoms of the hyper-lgD syndrome.

The study group consisted of 22 Dutch patients, 14 male and 8 female patients, with the hyper-lgD syndrome. The mean age at the time of the study was 28.3 years (SD ± 14.4 years). The diagnosis was made following standard clinical criteria, and a recent review with a pertinent description of the clinical and biologic features of the hyper-lgD syndrome has been published elsewhere. Briefly, all patients were suffering from recurrent febrile attacks accompanied by a combination of the following features: lymphadenopathy, splenomegaly, arthralgias/arthritis, abdominal pain/vomiting/diarrhea, and skin lesions. The attacks are self-limiting and last 3 to 7 days. The main clinical features of these patients are listed in Table 1.

Blood samples. Sampling of plasma was performed during attacks as well as during remission, using special endotoxin-free tubes (Vacutainer Systems; Becton Dickinson, Rutherford, NJ). The plasma was immediately processed to avoid ex vivo cytokine excretion. We defined attacks if the patient was febrile (>=38°C not caused by infection) and showed signs of active disease with symptoms as mentioned before. Remission was defined as the absence of symptoms for at least 1 month. No medication, including antipyretics, were allowed during the study period. All patients underwent physical examination, and results are listed in Table 1. Cytokine production was measured using a whole blood culture system as described elsewhere. Briefly, two 4-mL tubes containing 48 μL EDTA-K, and 250 μL aprotinin (10,000 kallikrein-inactivating units per mL; Bayer, Leverkusen, Germany) were drawn.

One tube was incubated immediately; the other tube was incubated after addition of 50 μL lipopolysaccharide (LPS, Escherichia coli serotype 055:B5), final concentration 10 μg/mL; Sigma, St Louis, from a disturbed cytokine synthesis which may be responsible for some of its clinical manifestations and could also contribute to the polyclonal serum IgD elevation. Therefore, we studied the pattern of circulating and ex vivo production of inflammatory mediators IL-1α, IL-1β, IL-6, TNFa, C-reactive protein (CRP), soluble type-II phospholipase A2 (PLA2), and inhibitory compounds such as IL-1 receptor antagonist (IL-1ra), IL-10, and soluble TNF receptors p55 (sTNFr p55) and p75 (sTNFr p75).

MATERIALS AND METHODS

Patients. The study group consisted of 22 Dutch patients, 14 male and 8 female patients, with the hyper-lgD syndrome. The mean age at the time of the study was 28.3 years (SD ± 14.4 years). The diagnosis was made following standard clinical criteria, and a recent review with a pertinent description of the clinical and biologic features of the hyper-lgD syndrome has been published elsewhere. Briefly, all patients were suffering from recurrent febrile attacks accompanied by a combination of the following features: lymphadenopathy, splenomegaly, arthralgias/arthritis, abdominal pain/vomiting/diarrhea, and skin lesions. The attacks are self-limiting and last 3 to 7 days. The main clinical features of these patients are listed in Table 1.

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One tube was incubated immediately; the other tube was incubated after addition of 50 μL lipopolysaccharide (LPS, Escherichia coli serotype 055:B5), final concentration 10 μg/mL; Sigma, St Louis,
MO). After 24 hours of incubation at 37°C both tubes are centrifuged at 2,250g for 10 minutes and, second, at 15,000g for 5 minutes to obtain platelet-poor plasma. Aliquots were stored at −70°C until assay. Cytokine production with LPS stimulation was measured and compared with production without LPS stimulation. Control values were obtained from 10 healthy volunteers (20 to 45 years of age) and were 6,847 ± 2,175 pg/mL (IL-1β); 3,344 ± 969 pg/mL (TNFα), and 9,827 ± 2,597 pg/mL (IL-1ra). TNFα was determined by radioimmunoassay as described by Van der Meer et al10 (lower detection level, 20 pg/mL; upper detection limit, 10,000 pg/mL). IL-1α and IL-1β were measured by radioimmunoassay (without choloroform extraction) according to Lisi et al11 (IL-1α lower detection limit, 5 pg/mL; IL-1β lower detection limit, 40 pg/mL). IL-1ra was determined by radioimmunoassay according to Poutsila et al12 (lower detection limit, 60 pg/mL). sTNFα were measured by an enzyme-linked immunosorbent assay (ELISA [detection level, 80 pg/mL for TNFα p55 and 300 pg/mL for TNFα p75]; a kind gift of H. Gallati, Hoffmann-La Roche, Basel, Switzerland). This assay measures both free and total receptor-bound concentrations. Because sTNFα is not produced ex vivo, its LPS-stimulated production was not assessed in this study. IL-6 was measured by an ELISA as described earlier (detection level, 14 pg/mL).13 IL-10 was measured by sandwich ELISA with the 4G6 and 5D1 I mouse antihuman IL-10 monoclonal antibodies for capture and the 5A10 antihuman IL-10 monoclonal antibodies for detection (kindly provided by Michael, Amersfoort, The Netherlands). The detection limit was 11 pg/mL.14

CRP measurements were performed with an immunoturbidimetric assay using an automatic analyzer HITACHI 747 (Hitachi Co, Tokyo, Japan). The measuring range was from 3 to 440 mg/L.

Secreted nonpancreatic type-II PLA2 antigen concentrations in plasma were determined with an ELISA modified from Smith et al.15 Two different monoclonal antibodies against human PLA2 (a kind gift from Dr F.B. Taylor, Jr, Oklahoma Medical Research Foundation, Oklahoma City, OK) were used as coating and catching antibodies. Because previous experiments showed a very good correlation between results from ELISA measurements and those obtained with cultured medium from Hepatoma G2 cells stimulated with human IL-6, only the former method was used (Schalkwijk CG, unpublished observations). The lower limit of detection was 1 ng/mL. To minimize analytical errors, all samples from the same patient were analyzed in the same run in duplicate.

Statistical analysis. The paired nonparametric Wilcoxon signed rank test was used for statistical comparison of values obtained during active disease versus remissions. The unpaired nonparametric Mann-Whitney U test was used where appropriate. In patients with multiple samples taken during separate episodes of active disease, the mean value was used. Probability (P) values were calculated on the basis of two-tailed tests. A correlation coefficient was calculated with the Pearson’s correlation test. A P value of less than .05 was considered to be the lowest level of significance. Data are given in mean ± standard deviation (SD).

RESULTS

Results of circulating cytokines and other inflammatory mediators are given in Figs 1 and 2. The CRP concentrations, a reflection of the acute-phase response, clearly modulated with the attacks in the patients with the hyper-IgD syndrome. The CRP concentrations were uniformly high during attacks (213.3 ± 98.2 mg/L) but decreased to low values (22.6 ± 26.8 mg/L) in between attacks (P < .0001; see Fig 1A). Concentrations of PLA2 increased to a mean of 1,452 ± 1,665 ng/mL during the febrile attacks, as compared with values during nonsymptomatic periods (113 ± 240 ng/mL; P < .0001; see Fig 1B). One patient even had a 253-fold elevation (maximum, 6,066 ng/mL) of PLA2 during attack as compared with convalescence. The concentrations of PLA2 and CRP obtained during febrile attacks showed a very good correlation during attacks (r = 0.74; P < .0001).

We could not detect measurable quantities of circulating IL-1α between and during attacks in patients with the hyper-IgD syndrome (Table 2). The mean plasma concentrations of IL-1β during active disease did not differ from the values

### Table 1. Main Clinical Features of 22 Patients With the Hyper-IgD and Periodic Fever Syndrome

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Lymphadenopathy</th>
<th>Splenomegaly</th>
<th>Abdominal Distress</th>
<th>Skin Lesions</th>
<th>Arthritis</th>
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<tr>
<td>1</td>
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<td>32</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>2</td>
<td>M</td>
<td>30</td>
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<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
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</tr>
<tr>
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</tr>
<tr>
<td>6</td>
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<tr>
<td>7</td>
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<td>M</td>
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<td>M</td>
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<td>–</td>
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<td>M</td>
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<td>+</td>
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<td>M</td>
<td>27</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>20</td>
<td>M</td>
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<td>+</td>
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<td>–</td>
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<td>–</td>
</tr>
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<td>21</td>
<td>F</td>
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<td>22</td>
<td>F</td>
<td>27</td>
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</tbody>
</table>

Abbreviations: +, present; –, absent; M, male; F, female.
obtained during remissions. The TNFα concentrations increased from 104 ± 20 pg/mL to 117 ± 15 pg/mL, a small but significant increase (P = .0075). Nevertheless, the TNFα concentrations during attack remained within normal values of our laboratory (106 ± 25 pg/mL). IL-6 was also significantly elevated during active disease (147.9 ± 258.3 pg/mL; P < .0001; see Fig 2A). Three patients had values above 150 pg/mL (471, 720, and 1,074 pg/mL, respectively). Exclusion of data from these 3 patients did result in a lower mean IL-6 plasma concentration during attacks (54.9 ± 27.2 pg/mL). However, the difference between values obtained in attacks and between attacks remained significant despite exclusion (P < .0001). During febrile episodes, there was a moderate, albeit significant, correlation between IL-6 and the acute-phase proteins CRP (r = 0.47; P = .014) and PLA2 (r = 0.45; P = .018).

No correlation could be detected between IL-6 and either IL-1β or TNFα.

IL-10, measured in plasma of 8 patients, remained unchanged regardless of the phase of the disease (Table 2).

The anti-inflammatory compounds IL-1ra, sTNFr p55, and sTNFr p75 were significantly increased during attacks compared with remissions (Fig 2B through D). The mean values of IL-1ra were 996 ± 329 pg/mL during attacks, whereas they were 300 ± 167 pg/mL during remission (P < .0001; see Fig 2B). The mean sTNFr p55 and sTNFr p75 concentrations during active disease increased to 5,043 ± 1,703 pg/mL and 7,103 ± 2,378 pg/mL, respectively (both P < .0001; see Fig 2C and D). The sTNFr p75/sTNFr p55 ratio did not change with attacks (data not shown). We could not establish a correlation between either the individual concentrations of IL-1ra, sTNFr p55, or sTNFr p75 and the temperature at the time of blood drawing or the frequency of the attacks (data not shown). During attacks, there was a significant correlation between circulating plasma concentrations of IL-1ra and sTNFr p55 (r = 0.48; P = .0231), IL-1ra and sTNFr p75 (r = 0.67; P = .0006), and sTNFr p55 and sTNFr p75 (r = 0.58; P = .0041); however, no correlation could be detected between individual CRP levels and IL-1ra, sTNFr p55, or sTNFr p75 levels. In 3 patients, the course of the circulating IL-1ra, sTNFr p55, and sTNFr p75 concentrations during the first 5 days after onset of the attack was followed as shown in Fig 2B. The course of values for the various cytokines is as follows. sTNFr p55 and sTNFr p75 only decrease 3 days after onset of the febrile attack. IL-1ra reaches the highest values on the second day of the attack and normalizes within 5 days. IL-6 was rapidly cleared from the circulation, similar to the acute-phase proteins CRP and PLA2.

Ex vivo cytokine production. Figure 4, upper panel, shows the nonstimulated production of the various cytokines tested. The unstimulated production of TNFα between attacks was significantly higher as compared with that for healthy controls (564 ± 304 vs 225 ± 11 pg/mL). Only the values for IL-1ra were significantly higher during attacks than during remissions, 3,642 ± 1708 pg/mL as compared with 2,048 ± 2,398 pg/mL (P = .015). The production of IL-1α in whole blood in the presence or absence of LPS was negligible and did not differ between attacks and remission. The LPS-stimulated production of IL-1β and TNFα in patients with the hyper-IgD was significantly higher during attacks as compared with that in remissions and for healthy controls (Fig 4, lower panel; P < .0001). During remissions, both the nonstimulated and LPS-stimulated production of TNFα, but not of IL-1β, was significantly higher compared with that of controls (P < .05). Fourteen patients had TNFα concentrations beyond the upper detection limit of 10,000 pg/mL during attacks, compared with only 6 patients during remission. LPS-stimulated production of IL-6 during attacks was similar to that measured during nonsymptomatic periods. The LPS-stimulated production of IL-1α was also significantly higher during attacks than in between attacks and than that in healthy volunteers (P < .0001).
During attacks, there were modest correlations between IL-1ra and IL-1β production ($r = 0.42; P = .027$) and IL-1ra and IL-6 production ($r = 0.3; P = .027$). The production of IL-1β correlated well with that of IL-6 during febrile episodes ($r = 0.53; P = .0057$).

**DISCUSSION**

We investigated the pattern of circulating cytokines and the ex vivo production of cytokines in 22 patients with the hyper-IgD syndrome. We found evidence for a vigorous acute-phase response in the hyper-IgD syndrome, as reflected by the significant increase of CRP and PLA$_2$ concentrations that subsided during remission. The significant elevation of the proinflammatory cytokines IL-6 and, albeit very modest, TNFα together with the elevation of the natural cytokine inhibitors IL-1ra, sTNFr p55, and sTNFr p75 point to activation of the cytokine network during the attacks of the hyper-IgD syndrome. Although it is not clear to what extent these cytokines contribute to the clinical features of the hyper-IgD syndrome, many of its symptoms are compatible with the effects of proinflammatory cytokines. However, it is unlikely that only one factor can cause the clinical picture; instead, activation of the cytokine network and involvement of various cytokines probably contribute to the pathogenesis of the attacks.

The elevation of these inflammatory mediators occurs re-
Table 2. Circulating Cytokines in 22 Patients With Hyper-lgD Syndrome

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Circulating Concentration (pg/mL) ± SD</th>
</tr>
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<tbody>
<tr>
<td>IL-1α</td>
<td>83.1 ± 20.3</td>
</tr>
<tr>
<td>IL-1β</td>
<td>80.4 ± 21.6</td>
</tr>
<tr>
<td>IL-10*</td>
<td>20.3 ± 21.1</td>
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</tbody>
</table>

* Tested in 8 patients.

The absence of any elevation of IL-10 concentration, a potent inhibitor of synthesis of cytokines, argues against an important role for this cytokine in the pathogenesis of the hyper-lgD syndrome.66 Lastly, there is increased ex vivo production of IL-1β, TNFα, and IL-1ra during attacks compared with nonsymptomatic periods, as measured in a whole blood culture system.

The acute-phase response in humans is considered to be mediated by the proinflammatory cytokines, and we detected a modest but significant elevation of IL-6 together with a significant correlation between IL-6 and the acute-phase proteins CRP and PLA2 in the blood during the febrile attacks.

In view of these findings, it can be speculated that the increased plasma IL-6 concentration results from overproduction rather than underutilization, because renal function was normal in all patients. In addition, the elevated IL-6 values are associated with increased CRP and PLA2 values. Underutilization would probably have resulted in low concentrations of these acute-phase proteins. IL-6, as the major mediator for the regulation of acute-phase protein synthesis in humans, also influences the glycosylation of these proteins.7,17,18 In recent studies in the hyper-lgD syndrome, we have shown elevated α1-fucosylation of the acute-phase protein α1-acid glycoprotein (AGP), and this finding is compatible with a role of IL-6 during the recurrent febrile attacks.19 In addition, IL-6 is a stimulus for PLA2 synthesis in the liver, and the strongly elevated (13-fold) PLA2 concentrations found during the febrile attacks, together with the correlation between IL-6 and PLA2, corroborate these findings.20 By cleavage of arachidonic acid from cell membrane, PLA2 in its turn is able to elicit an inflammatory response. Enhanced PLA2 activity in serum propagates tissue damage through membrane digestion and liberation of cytotoxic fatty acids.21 For example, when administered intra-articulary in rats, it causes synovitis,22 and serum PLA2 concentrations correlate well with activity of juvenile rheumatoid arthritis (JRA) in humans.23 Furthermore, intradermal injection of PLA2 causes local dermal inflammatory infiltrates.24 These biologic effects of PLA2 may be responsible for some of the clinical features of the hyper-lgD syndrome, because arthritis and cutaneous vasculitis are main symptoms of the disease.2,3

Despite our efforts, we have not been able to establish which endogenous pyrogen is responsible for the fever in the hyper-lgD syndrome.

A possible reason for our failure to trace appreciable elevations of pyrogenic proinflammatory cytokines such as IL-1β and TNFα may be the delay between onset of attack and sampling. Although we sampled all our patients on their first day with fever, this may already have been too late. The slight elevation of TNFα during attacks perhaps reflects the descending slope of an earlier peak. The increased CRP levels at time of drawing of blood indicate an already ongoing acute-phase response thought to be the consequence of the action of cytokines, and these may have already disappeared from the circulation. This hypothesis is in line with the finding of increased cytokine inhibitors such as IL-1ra, sTNFr p55, and sTNFr p75, of which it is known, from experimental endotoxemia, that they can still be detected in the circulation hours after the disappearance of IL-1β and TNFα.25,27 The same holds for PLA2, which peaks 18 to 24 hours later than TNFα after endotoxin infusion in human volunteers.28

In our study, elevation of IL-1ra, sTNFr p55, and sTNFr p75 was present at the first day of an attack, and appreciable concentrations were found on the second and third day of the attack. Endogenously produced IL-1ra, a major natural inhibitor of IL-1, may ameliorate the severity of attacks in the hyper-lgD syndrome.29,31 Because we did not find clear elevations of IL-1 concentrations, it is impossible to judge whether the amount of IL-1ra is adequate to alleviate the acute inflammation. Our finding of predominant concentrations of IL-1 antagonist recalls the results obtained in systemic JRA, another periodic febrile disorder. In JRA, inhibition of IL-1 bioactivity by serum and urine during febrile attacks was found.32 Also, for TNFα, we merely find inhibition, as judged by the elevated concentrations of soluble receptors for TNF.33 sTNFrs bind TNF and form a biologic, inactive complex, blunting the deleterious effects of TNFα.26

How do our results of circulating cytokines compare with measurements in another periodic fever syndrome such as familial Mediterranean fever (FMF)? In FMF patients, TNF and IL-1 activity has been investigated.

In a study with Sephardic Jews with FMF there was no evidence of TNFα bioactivity in plasma obtained during attack and remission.34 Nevertheless, the investigators suggested a role for TNFα in FMF and impute the lack of elevated circulating plasma TNFα to the short half life (6 minutes) and the possible binding to soluble receptors. In a similar study of Turkish FMF patients, using ELISA, TNFα was detected, albeit in modest concentrations (33.1 pg/mL in between attacks compared with 62.2 pg/mL during attacks).35 Here, again, one may wonder whether the values measured during an attack represent a tail of initially elevated TNF concentrations. The higher concentrations we found during remissions (104 ± 20 pg/mL) and attacks (117 ± 15 pg/mL) in the hyper-lgD syndrome are most likely due to detection of both free TNF and TNF bound to sTNFrs.

The role of IL-1 was tested in a study of 18 FMF patients, 9 symptomatic and 9 asymptomatic, and mean IL-1 activity in patients (either during attack or remission) was comparable with the values obtained in controls.36

We studied the effect of bacterial LPS on the cytokine production as generated in whole blood. In contrast to T-cell mitogens such as concavalin A, in our experience, LPS is a reliable stimulator of cytokine production. The effect of
LPS is dose-dependent, and dosages at 10 μg/mL, as used in our study, have yielded reproducible results.\textsuperscript{37} It is of interest that we detected an augmented LPS-stimulated ex vivo production of TNFα, IL-1β, and IL-1ra in our whole blood culture system during attacks.

This enhanced production capacity in the hyper-IgD syndrome during attacks is in contrast with findings obtained in various infectious diseases such as meningococcal sepsis and typhoid fever, where the ex vivo production of TNF, IL-1, and IL-6 is depressed during the acute stage and is gradually restored during recovery.\textsuperscript{38,39}

Also, TNFα and IL-1 production, as stimulated by LPS, has been reported to be markedly decreased during attacks in FMF.\textsuperscript{34,36,40} We could confirm these results in three FMF patients and detected decreased ex vivo production of TNFα, IL-1β, and IL-1ra during attacks (Drenth JPH, unpublished observations). These findings clearly differ from the increased production during the febrile attacks of the hyper-IgD syndrome. It is possible that the monocytes/macrophages in the hyper-IgD syndrome are already primed in vivo to produce increased amounts of these cytokines. This could be the result of increased concentrations of AGP and CRP, because these acute-phase proteins have been shown to potentiate the in vitro production of cytokines and of
TNFα in particular. In addition, CRP and, to a lesser extent, AGP are able to induce the synthesis of IL-1ra in vitro.

We have shown earlier that serum AGP concentrations are markedly elevated during attacks of the hyper-IgD syndrome and are somewhat elevated during remission. In contrast, lower concentrations of the acute-phase protein AGP are found in FMF, and this perhaps contributes to the diminished LPS-stimulated cytokine production. These data underscore differences in pathogenesis of the disorders. Elsewhere, we have already alluded to the genetic differences and histologic findings in the hyperimmunoglobulinemia D syndrome.}

**ACKNOWLEDGMENT**

We are indebted to Gerard Pesman and Mariëlle Spruytenburg (Laboratory of Endocrinology and Reproduction, University Hospital St Radboud, Nijmegen, The Netherlands) for the analysis of IL-1α, IL-1β, TNFα, and IL-1ra. We are grateful to Marie-Cécile Dekker for her help with collecting the sera.

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