Interferon Gamma-induced Formation of Neopterin and Degradation of Tryptophan in Cerebrospinal Fluid of Children with Meningitis but Not with Febrile Convulsions

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Summary

Endogenously formed cytokines may play an important role in the development of meningitis, and it is necessary to understand in more detail inflammatory cascades involved in the disease process. We were interested to investigate biochemical changes which are inducible by interferon gamma. Therefore, we examined concentrations of neopterin, tryptophan, kynurenine and interferon gamma in cerebrospinal fluid (CSF) of 11 children with bacterial or viral meningitis and of 20 children with febrile convulsions, who suffered from viral or bacterial disease not involving the central nervous system. Concentrations of neopterin (mean $\pm$ S.D. : 43.9 ± 34.6 nmol/l) kynurenine (0.41 ± 0.56 μ mol/l) and interferon gamma (0.64 ± 0.81 U/ml) levels were higher in CSF of children with meningitis than in those with febrile convulsions (all $p < 0.01$). In addition, positive correlations resulted between increase of neopterin and changes of tryptophan metabolism in the CSF. Interestingly no difference was found between viral and bacterial meningitis. Our findings indicate that intrathecal immune activation takes place as part of the pathogenesis of meningitis.

Key words : Cerebrospinal fluid, Interferon gamma, Kynurenine, Meningitis, Neopterin

Introduction

Meningitis in paediatric patients remains a serious disease harbouring high incidence of morbidity and mortality (1, 2). Most commonly meningitis is caused by bacterial and/or viral pathogens. Clinical signs and symptoms in children with meningitis do not allow to distinguish between different etiologic agents. Therefore, bacterial or viral meningitis has to be ruled out in all cases of febrile convulsions by examining cerebrospinal fluid (CSF). The early detection of the infectious process plays a crucial role for successful therapy, while a late diagnosis, even if treated correctly, may be associated with poor outcome (3~6). A role of immune activation in the pathogenesis of meningitis was suggested by results obtained from experimental studies in animal models, and it proved to play an important role in aggravating the disease

(7-11); immune-mediated injury was suggested by enhanced formation of proinflammatory cytokines such as tumour necrosis factor alpha, interleukin-1 and interferon gamma in the CSF of patients with meningitis. Similarly, T-cell involvement in the pathogenesis of the disease was demonstrated during the acute phase of echovirus meningitis (12). In this study, we examined concentrations of interferon gamma and of the interferon gamma-inducible factor neopterin (13) in children with infectious meningitis and with febrile convulsions. In addition, we measured concentrations of tryptophan and kynurenine, since catabolism of tryptophan is induced by interferon gamma (14) namely by activation of indoleamine-(2, 3)-dioxygenase which cleaves tryptophan to form N-formyl-kynurenine.

Material and Methods

Figure 1. Concentrations of interferon gamma, neopterin, kynurenine and tryptophan in children with meningitis (Bact=bacterial, Vir=viral) and with febrile convulsions (Co). Black dots indicate specimens containing concentrations below the detection limit of the test used (Interferon gamma <0.04 U/ml, neopterin <3 nmol/l, kynurenine <0.2 μ mol/l, tryptophan <0.3 μ mol/l).

Eleven children with infectious meningitis and 20 children with febrile convulsions caused by infections not involving the central nervous system were studied (median ages: febrile convulsions 2.1 years, bacterial meningitis 7.8 years, viral meningitis 6.1 years). Diagnosis was made by clinical assessment, blood and cerebrospinal fluid cultures, standard laboratory measurements such as white blood cell counts, erythrocyte sedimentation rate and leukocyte counts and differential blood picture as well as protein and glucose levels in the CSF. Of the 11 children with meningitis, 5 had severe bacterial meningitis caused by *Neisseria meningitidis* (3 cases) and *Haemophilus influenzae* (2 cases), and 6 children had viral meningitis or encephalitis (3 *Mumps virus*, 2 *Varicella zoster virus*, 1 unclarified serous (non-bacterial) meningitis). CSF samples were available from all children whereas serum samples were only available from 7 patients (two with bacterial meningitis and 5 with febrile convulsions). By radioimmunoassays we examined CSF and serum levels of interferon gamma (Centocor, Malvern, PA, USA) and of neopterin (Henning-Berlin, Berlin, GFR). For measurements of interferon gamma an optimized protocol was used (15) to increase sensitivity of the test, CSF specimen were diluted 1:1 by adding dilution buffer provided

by the manufacturer for specimen with low protein content. No such pretreatment of samples is necessary for neopterin measurements (16). Tryptophan and kynurenine was measured by HPLC with on-line deproteinization of samples (17). Tryptophan was monitored by UV-absorption (365 nm emission wavelength), kynurenine was monitored by UV-absorption (365 nm wavelength). Statistical comparison of diagnostic categories was done by Wilcoxon Mann Whitney U-test. Correlation analysis was performed using Spearman rank regression coefficients. Probability values less than 0.05 were regarded to indicate statistical significance.

Results

On average children with febrile convulsions had CSF neopterin levels (mean $\pm$ S.D.: 6.0 ± 3.77 nmol/l, Table 1) only slightly higher than levels obtained from healthy adults in an earlier study (median: 4.2, range 3.2~5.5 nmol/l, Ref. 16). Concentrations of neopterin, kynurenine and interferon gamma in CSF were significantly higher in patients suffering from meningitis compared to children with febrile convulsions (Table 1). There was no apparent difference of

Table 1. Concentrations of interferon gamma, neopterin, kynurenine and tryptophan (mean $\pm$ S.D.) in children with meningitis (n=11) and with febrile convulsions (n=20).

	Meningitis	Febrile Convulsions	Wilcoxon-test
Interferon gamma (U/l)	0.64 $\pm$ 0.81	0.11 $\pm$ 0.07	U=2.64 p<0.01
Neopterin (nmol/l)	43.9 $\pm$ 34.6	6.0 $\pm$ 3.8	U=3.60 p<0.001
Kynurenine (μ mol/l)	0.41 $\pm$ 0.56	n.d.*	U=2.64 p<0.01
Tryptophan (μ mol/l)	2.1 $\pm$ 1.9	1.49 $\pm$ 0.65	n.s.*

* n.d. not detectable (<0.2 μ mol/l)

* n.s. not significant

neopterin and interferon gamma levels in CSF between bacterial and viral meningitis (Fig. 1). In all samples available from children with febrile convulsions and in meningitis with systemic involvement certified by blood culture (n=4), we found elevated concentrations of neopterin, kynurenine and interferon gamma in serum. Serum neopterin, kynurenine and interferon gamma levels were not increased in 2/2 patients who had meningitis but did not suffer from a systemic disease. There existed a weak correlation between neopterin and kynurenine concentrations in CSF in children with meningitis (n=10, $r_s=0.598$, $p=0.07$). A correlation was observed between CSF neopterin concentrations and the kynurenine per tryptophan ratio (n=31; $r_s=0.565$, $p=0.002$). Six of 8 children with CSF neopterin concentrations greater than 25 nmol/l had detectable kynurenine concentrations in CSF but none of 23 children with neopterin levels below 25 ($\chi^2=21.39$, $p<0.0001$; χ^2 -test). Two children with very high interferon gamma and neopterin concentrations in CSF (two of them had also high kynurenine concentrations in CSF namely 0.8 μ mol/l) had tryptophan concentrations below 0.3 μ mol/l which is less than 20% of the average tryptophan concentrations found in children with febrile convulsions.

Discussion

Elevated neopterin levels have been documented earlier in disease entities which are associated with cellular immune activation such as infectious diseases, autoimmune disorders as well as neoplastic diseases (13). Elevated neopterin levels in serum are usually accompanied by elevated interferon gamma (15, 18). In CSF, increased neopterin levels have been described in patients with measles encephalitis

(19), Lyme neuroborreliosis (20), subarachnoid haemorrhage (21) and in human immunodeficiency virus (HIV) infection (16, 22-25). Increased formation of tryptophan degradation products such as kynurenine or quinolinic acid was observed in patients with infections like HIV infection (26, 27) and Lyme disease (28, 29) but also in patients treated with immunostimulatory cytokines (30). This was demonstrated in serum and in CSF of patients as well. In our study, we found higher concentrations of neopterin and kynurenine in the CSF of children with meningitis than in the control group of children with febrile convulsions. In healthy children neopterin levels decrease slightly with age (13), thus taking into account the difference in age of patients with meningitis versus febrile convulsions in our study, the data further extend the significance of neopterin elevations in children with meningitis. The observation of increased neopterin concentrations in children with meningitis agrees with earlier data of W. Koelfen *et al.* (31), and all the data at hand indicate intrathecal immune response. This conclusion is supported on the one hand by data obtained from 2/2 patients with bacterial meningitis who had highly elevated neopterin in CSF (49 and 28 nmol/l, respectively) but low serum neopterin concentrations (10.9 and 12.2 nmol/l, respectively), on the other hand 5/5 children with febrile convulsions had low CSF neopterin but high serum levels. This data agree with earlier observations that neopterin does not easily penetrate the blood-brain-barrier (22, 23). In agreement, high neopterin concentrations in the CSF together with lower serum levels have been described in adults with meningitis (16) and with Lyme neuroborreliosis (20). We assume that the situation is similar in children with meningitis, although our experience is still limited due to the low number of available serum specimens. We also found high interferon gamma levels in CSF of patients with meningitis indicating local production of the cytokine. The presence of high interferon levels in CSF of patients with certain neurological disorders and meningitis was documented earlier (10, 32, 33). Increased neopterin levels and enhanced degradation of tryptophan indicate that endogenously formed interferon gamma is biologically active in children with meningitis. Similarly, increased interferon gamma levels were found concomitantly with increased neopterin levels in CSF of children suffering from post measles encephalitis (19). The data suggest that monocytic cells are activated by interferon gamma, most probably monocytes from the peripheral blood invading the CSF or monocytic cells of brain tissue like astrocytes or microglia cells are the source of neopterin. Whereas interferon-induced degradation of trypto-

phan is not specific for a certain cell type, e.g., it can be induced in a variety of tumour cell lines and in human peripheral blood monocytes/macrophages in vitro (34), neopterin formation is rather specific for peripheral blood monocytes/macrophages (13), but also human microglia cells have been found to produce detectable amounts of neopterin upon stimulation with interferon gamma (34). Recently, increased concentrations of tumour necrosis factor alpha have been described in CSF of children with meningitis preferentially in cases with bacterial origin (9, 11, 35). Endogenously formed tumour necrosis factor alpha may synergistically enhance interferon gamma-induced formation of neopterin and kynurenine as it was found in vitro (36). It is interesting that neopterin levels in CSF were reported to be higher in children with bacterial meningitis when compared to viral meningitis (31). In our patients such a difference was relatively small, however, meningitis caused by different bacteria may also differ immunologically. Enhanced catabolism of tryptophan may be involved in the pathogenesis of symptoms in children with meningitis. On the one hand, low availability of tryptophan may disturb neurotransmitter biosynthesis (37), on the other hand tryptophan degradation products are potent neurotoxins (27, 29). Longitudinal studies are required to clarify this point.

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