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Intrathecal production of neopterin in aseptic meningo-encephalitis and multiple sclerosis

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SUMMARY

Neopterin levels in cerebrospinal fluid (CSF) and serum were measured with a sensitive radioimmunoassay in patients with multiple sclerosis (MS), aseptic meningo-encephalitis (AM), other mostly non-inflammatory neurological diseases, and controls with tension headache or psychoneurosis. Elevated levels of neopterin were found in CSF in most patients during acute phase of AM, and normalization after clinical recovery. The elevation in CSF was not reflected in serum. Only four of 24 MS patients—three of them examined during exacerbation—had slight elevation of neopterin in CSF and all had normal serum levels. Neopterin levels in CSF correlated with mononuclear cell count. The elevation of neopterin observed in CSF in inflammatory CNS diseases despite low cell numbers in CSF compared to blood might reflect a high activation level of cells locally in the CNS. Neopterin in CSF is a valuable marker of acute cellular immune response.

Keywords neopterin multiple sclerosis aseptic meningitis cerebrospinal fluid

INTRODUCTION

Neopterin is a pyrazino (2,3d) pyrimidine derivative formed from guanosintriphosphate (GTP) in the synthetic pathway of biopterin, which acts as cofactor in neurotransmitter synthesis, i.e. in hydroxylation of phenylalanine, tyrosine and tryptophan (Huber *et al.*, 1984b; Kern, Rokos & Dietrich, 1984). It is still unclear whether neopterin is a by-product of biopterin or if it has physiological functions of its own.

Neopterin is released from monocytes and macrophages after stimulation *in vitro* by γ-interferon (γ-IFN) from activated T cells (Huber *et al.*, 1984a). Release from peripheral blood mononuclear cells *in vitro* was earlier shown to be induced through activation of T cells by stimulation with alloantigens or virally modified autologous cells (Huber *et al.*, 1983). Increased neopterin excretion *in vivo* has been described in serum and urine from patients with viral infections (Wachter, Hausen & Grassmayr, 1979; Reibnegger *et al.*, 1984; Kern *et al.*, 1984), rheumatoid arthritis, systemic lupus erythematosus, coeliac disease (Hausen *et al.*, 1983; Fuchs *et al.*, 1983), malaria (Kern *et al.*, 1985), graft vs host disease (Margreiter *et al.*, 1983) and certain tumour states (Wachter *et al.*, 1979). Neopterin has also been used as a biochemical marker of cellular immune response and T cell activation (Margreiter *et al.*, 1983; Huber *et al.*, 1984b) and has been used to follow the clinical course during different therapeutical trials, e.g. with IL-2 in patients with acquired immunodeficiency syndrome (AIDS) (Kern & Dietrich, 1985).

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Since it has been shown that cellular immune mechanisms are involved in inflammatory diseases of the central nervous system (CNS) such as acute aseptic meningo-encephalitis (AM) (Link *et al.*, 1983) and multiple sclerosis (MS) (for review see Waksman & Reynolds, 1984), cerebrospinal fluid (CSF) and serum from patients with these disorders and from controls were examined for neopterin levels, one goal being to identify a variable reflecting functional and activation states of CSF cells.

MATERIALS AND METHODS

Twenty-four patients (18 females) had clinically definite MS according to the criteria of Schumacher *et al.* (1965). Their age varied between 22 and 55 years (mean 37 years). Fourteen of the patients had no or slight disability, i.e. they were in all respects able to manage on their own, whereas the remaining 10 patients had moderate or severe disability, implying dependence or assistance at various degrees in so far as the patients professional and social lifes were considered. The duration of MS varied between 1 and 20 years (mean 7 years). Of the 24 patients with MS, seven showed a chronic progressive form of disease, 11 patients were in exacerbation (i.e. there had been a sudden appearance of new symptoms and signs or a sudden reapperance or worsening of previous findings within 1 month before examination) and six were in remission at the time of sampling. Mononuclear pleocytosis ($> 5/\mu l$) was present in CSF in 15 of the patients, ranging between 5·1 and 36·6 (median 9·5). All except one patient had oligoclonal IgG in CSF demonstrated by agarose isoelectric focusing (Link & Kostulas, 1983) and all except two patients had intrathecal IgG production as reflected by elevated ($> 0{\cdot}7$) CSF IgG index (Link & Tibbling, 1977). None of the patients had been treated with immunomodulatory drugs.

Thirteen patients (seven females) had AM of unknown aetiology except in two (varicellae-zoster). Their age varied between 20 and 58 years (mean 36 years). Benign clinical course was registered in all patients. The mononuclear cell count in CSF varied between 42 and 715 (median $147/\mu l$). One patient had elevated CSF IgG index and two had oligoclonal IgG bands in CSF.

Ten patients (five females) with other neurological diseases were also examined. Their age varied between 19 and 77 years (mean 43 years). The following diagnoses were encountered: Transitory ischemic attacks (two patients), cerebral infarction (one), pituitary tumour (one), epilepsy (one), Guillain-Barré syndrome (one), myasthenia gravis (one), torticollis (one), neuralgia (one) and post-traumatic cerebral syndrome (one).

Control subjects consisted of 19 patients (14 females) with tension headache or psychoneurotic disorders. Their age varied between 24 and 55 years (mean 39 years). Inquiry among them revealed no signs or symptoms of other diseases, and they displayed normal findings at physical and neurological examinations. Normal values were also registered for concentrations of albumin, C reactive protein (CRP), haptoglobin and IgG in serum, as well as for CSF studies including cell count, CSF/serum albumin ratio as indicator of blood-brain barrier status (Tibbling, Link & Öhman, 1977), CSF IgG index and agarose isoelectric focusing.

Blood and CSF samples were usually taken between 0800 and 1100 h. After dividing the specimens to obtain material for routine analysis, the remaining CSF was centrifuged, and CSF and corresponding serum were stored at $-70^{\circ}C$ in the dark.

Neopterin was measured with a standard radioimmunoassay (Neopterin RIAcid, Henning-Berlin, Berlin, FRG) (Rokos, Rokos & Zeigler, 1983). There was no cross-reactivity to biopterin in this assay.

Attempts to oxidize the CSF specimens did not significantly change the neopterin levels as measured by RIA (Rokos *et al.*, 1985). Within-assay coefficient of variation was less than 6% and between-assay coefficient of variation was below 11%.

Wicoxon's rank sum test and Pearson's coefficient of correlation were used for statistical calculations.

RESULTS

Neopterin concentrations in CSF and serum from each of the patients in the four groups are shown in Fig. 1 and ranges, mean and median values summarized in Table 1.

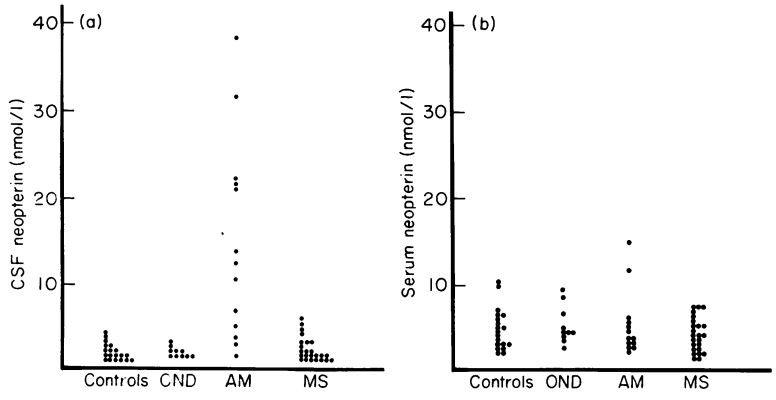

Fig. 1. Concentrations of neopterin in CSF (a) and serum (b) in controls consisting of patients with tension headache or psychoneurosis, and in patients with other neurological diseases (OND), aseptic meningo-encephalitis (AM) and multiple sclerosis (MS).

Table 1. Ranges, mean and median values of neopterin concentrations in CSF and serum given in nmol/l in controls consisting of patients with tension headache or psychoneurosis, and in groups of patients with aseptic meningitis (AM), multiple sclerosis (MS) and other neurological diseases (OND), respectively

		CSF (nmol/l)	Serum (nmol/l)
Controls ($n=19$)	Range	1·25–3·3	2·3–9·8
	Mean ± s.d.	1·6±0·6	4·7±2·1
	Median	1·25	4·3
AM ($n=13$)	Range	1·25–37	2·1–14·3
	Mean ± s.d.	13·9±10·8	4·95±3·5
	Median	11·4	3·4
MS ($n=4$)	Range	1·25–5·1	1·5–6·9
	Mean ± s.d.	2·1±1·2	4·0±1·7
	Median	1·25	3·25
OND ($n=10$)	Range	1·25–2·6	2·6–8·3
	Mean ± s.d.	1·5±0·4	4·96±1·8
	Median	1·4	4·2

Controls and other neurological diseases. The upper reference limits of neopterin (mean + 3 s.d.) based on the observations from the 19 control subjects were 3·4 nmol/l for CSF and 11 nmol/l for serum. None of these control subjects, nor any of those 10 patients with other mostly non-inflammatory neurological disease had neopterin concentrations in CSF or in serum above these reference limits.

Acute aseptic meningo-encephalitis. Ten of the 13 patients with AM had elevated neopterin concentrations in CSF. Only two of them displayed slightly increased neopterin levels in serum. These two patients had signs of mild viral infections outside the CNS, with symptoms from respiratory and gastrointestinal tracts.

The patients with AM had significantly higher neopterin values ($P<0·001$) in CSF in comparison with each of the other three patient groups.

The median time between onset of symptoms and sampling in the patients with elevated neopterin levels in CSF and those with normal values, was 5 days and 19 days, respectively.

The normalization of neopterin in CSF during the course of AM was further investigated by analysing samples obtained after clinical recovery. CSF was available from nine of the 13 patients after clinical improvement and all showed normal values (Fig. 2).

A strong correlation was found between neopterin concentration in CSF and number of mononuclear cells in CSF ($r=0{\cdot}74$). Only a weak correlation ($r=0{\cdot}45$) was demonstrable between neopterin level in CSF and CSF IgG index. With regard to blood-brain barrier status, damage as reflected by an increased CSF/serum albumin ratio was registered in 10 of the 13 patients. However, no correlation between neopterin concentrations in CSF and serum was demonstrable ($r=0{\cdot}11$), which indicates that the neopterin level in CSF is determined by mechanisms in addition to transudation from serum.

Multiple sclerosis. Of the 24 patients with MS, four had slightly elevated neopterin levels in CSF. Three of these four patients were examined during exacerbation of the disease. The mean value for the MS patients did not differ from that of the patients with other neurological diseases or that of the controls. There was a strong correlation between neopterin levels in CSF and CSF mononuclear cell count ($r=0{\cdot}66$) but no correlation to CSF IgG index ($r=0{\cdot}05$). All 24 patients with MS had normal neopterin concentrations in serum.

DISCUSSION

Elevated neopterin levels have previously been described in serum from patients with diseases involving a cellular immune response. In MS as well as AM, systemic as well as intrathecal cellular immune mechanisms are generally considered to be involved in the pathogenesis of these diseases (for reviews see Reder & Arnason, 1985; Link *et al.*, 1983).

The present study revealed elevated neopterin concentrations in CSF from patients with AM. In some of them, these levels reached those previously reported in serum in certain diseases, e.g. AIDS or hepatitis (Rokos *et al.*, 1983). This should be considered in conjunction with the fact that the number of mononuclear cells in CSF is normally 1,000 times less than in peripheral blood. Even though elevated cell counts are found in CSF in AM, their actual numbers are many times lower than in peripheral blood. Therefore, the degree of elevation of neopterin levels encountered in CSF from some of our patients with AM may reflect a high degree of functional activation of the cells present within the CSF–CNS compartment. This elevation was not observed in serum despite the frequent occurrence of blood–brain barrier damage, favouring the hypothesis that the elevated neopterin concentrations demonstrated in CSF in our patients reflect an intrathecal response.

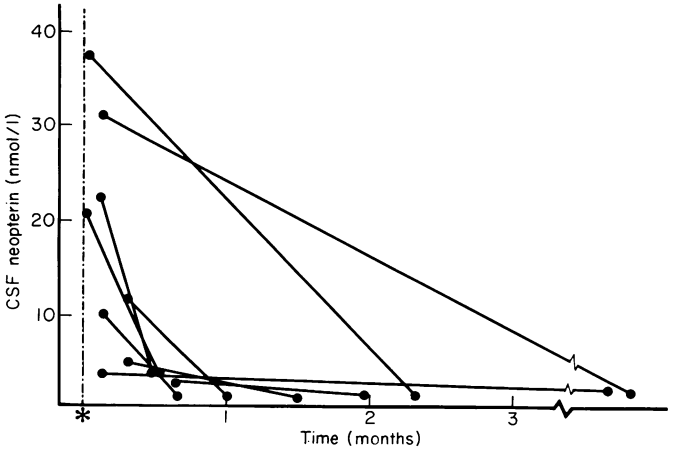

Fig. 2. Concentrations of neopterin in CSF (nmol/l) in patients with acute aseptic meningo-encephalitis during the acute phase and at the time of a second lumbar puncture where all patients had recovered clinically. Asterisk shows onset of clinical symptoms.

The neopterin levels in CSF from patients with AM were higher during early compared to later phases of the disease. This observation agrees with previous findings of a rapidly normalizing neopterin excretion reported in systemic viral diseases (Reibnegger *et al.* 1984).

When discussing neopterin levels in CSF it should be remembered that neopterin is produced as a by-product in neurotransmitter synthesis, which might influence neopterin levels within the CNS–CSF compartment. This could not, however, explain the elevated neopterin levels selectively in CSF from patients with AM.

Normal neopterin levels were encountered in the CSF from a majority of our patients with MS despite the fact that several of them were examined close to an exacerbation and thus during active disease. It might be, however, that an elevation in a diffusable substance such as neopterin persists for only days or hours. Therefore we are currently examining individual patients over the course of MS for possible fluctuations of neopterin levels in CSF and serum.

It has been suggested that T8 positive lymphocytes play a crucial role in the release of neopterin in certain viral infections (Kern *et al.*, 1984). A positive correlation between T8 positive cells and neopterin levels has also been reported (Rokos *et al.*, 1983). Since these cells are increased in numbers in diseases which are accompanied by elevated neopterin concentrations, e.g. infections caused by cytomegalovirus or Epstein-Barr virus, hepatitis and AIDS, this should be considered together with our results in the patients with MS. Decreased numbers of T8 positive lymphocytes have been demonstrated in active MS (Bach *et al.*, 1980; Reinherz *et al.*, 1980). Others have questioned this observation (Rice *et al.*, 1984; Zabriskie *et al.*, 1985). Considering neopterin as a biochemical marker reflecting the functional status of lymphocytes, a lack of more pronounced increase of neopterin levels in CSF in our patients with MS, despite increased numbers of mononuclear cells and the fact that activated cells in CSF have been reported (Noronha, Richman & Arnason, 1980; Hafler *et al.*, 1985) might be explained in terms of dysfunction of the lymphocyte-monocyte interaction.

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REFERENCES

BACH, M.A., TOURNIER, E., PHAN-DINH-TUY, F., CHATENOUD, L., BACH, J-F., MARTIN, C. & DEGOS, J-D. (1980) Deficit of suppressor T cells in active multiple sclerosis. *Lancet* **i,** 1221.

FUCHS, D., GRANDITSCH, G., HAUSEN, A., REIBNEGGER, G. & WACHTER, H. (1983) Urinary neopterin excretion in coeliac disease. *Lancet* **i,** 463.

HAFLER, D.A., FOX, D.A., MANNING, M.E., SCHLOSSMAN, S.F., REINHERZ, E.L. & WEINER, H.L. (1985) In vivo activated T lymphocytes in the peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. *N. Engl. J. Med.* **312,** 1405.

HAUSEN, H., FUCHS, D., REIBNEGGER, G., WACHTER, H., EGG, D. & GUNTER, R. (1983) Neopterin as index for activity of disease in patients with rheumatoid arthritis. In: *Biochemical and Clinical Aspects of Pteridines*, Vol. 2, p. 245. Walter de Gruyter, Berlin.

HUBER, C., FUCHS, D., HAUSEN, A., MARGREITER, R., REIBNEGGER, G., SPIELBERGER, M. & WACHTER, H. (1983) Pteridines as a new marker to detect human T cells activated by allogeneic or modified cells for major histocompatibility complex (MHC) determinants. *J. Immunol.* **130,** 1047.

HUBER, C., BATCHELOR, J.R., FUCHS, D., HAUSEN, A., LANG, A., NIEDERWIESER, D., REIBNEGGER, G., SWETLY, P., TROPPMAIR, J. & WACHTER, H. (1984a) Immune response associated production of neopterin, release from macrophages primarily under control of interferon gamma. *J. exp. Med.* **160,** 310.

HUBER, C., FUCHS, D., NIEDERWIESER, D., HAUSEN, A., REIBNEGGER, G., NILSSON, K. & WACHTER, H. (1984b) Neopterin, eine neue biochemischer Marker zur klinischen Erfassung Zellulärer Immunreaktion. *Klin. Wossenschr.* **62,** 103.

KERN, P., ROKOS, H. & DIETRICH, M. (1984) Raised serum neopterin levels and imbalances of T lymphocyte subsets in viral diseases, acquired immune deficiency and related lymphadenopathy syndromes. *Biomed. Pharmacother.* **38,** 407.

KERN, P. & DIETRICH, M. (1985) Increase in serum neopterin levels in patients receiving recombinant interleukin-2 and recombinant interferon α. In: *Biochemical and Clinical Aspects of Pteridines*, Vol. 4, p. 335, Walter de Gruyter, Berlin.

KERN, P., HORSTMANN, R. & DIETRICH, M. (1985) Clinical resolvement of malaria infection parallels decreases of highly elevated serum neopterin level. In: *Biochemical and Clinical Aspects of Pteridines*, Vol. 4, p. 341. Walter de Gruyter, Berlin.

LINK, H. & TIBBLING, G. (1977) Principles of albumin and IgG analyses in neurological disorders. III.

Evaluation of IgG synthesis within the central nervous system in multiple sclerosis. *Scand. J. clin. Lab. Invest.* **37,** 397.

LINK, H. & KOSTULAS, V. (1983) Utility of isoelectric focusing of cerebrospinal fluid and serum on agarose elevated for neurological patients. *Clin. Chem.* **29,** 810.

LINK, H., KAM-HANSEN, S., FORSBERG, P. & HENRIKSSON, A. (1983) Humoral and cellular immunity in patients with acute aseptic meningitis. In: *Immunology of Nervous System Infections. Progress in Brain Research.* (ed. P. O. Behan, V. ter Meulen & F. Clifford Rose), Vol. 59, p. 29. Elsevier Science Publishers, Amsterdam.

MARGREITER, R., FUCHS, D., HAUSEN, A., HUBER, C., REIBNEGGER, G., SPIELBERGER, N. & WACHTER, H. (1983) Neopterin as a new biochemical marker for diagnosis of allograft rejection. *Transplantation* **36,** 650.

NORONHA, A., RICHMAN, D. & ARNASON, B. (1980) Detection of *in vivo* stimulated cerebrospinal fluid lymphocytes by flow cytometry in patients with multiple sclerosis. *N. Engl. J. Med.* **303,** 713.

REDER, A.T. & ARNASON, B.G.W. (1985) Immunology of multiple sclerosis. In: *Handbook of Clinical Neurology Vol. 3 (47): Demyelinating Diseases.* (eds. J. C. Koestler), p. 337 Elsevier Science Publishers, Amsterdam.

REIBNEGGER, G., FUCHS, D., GRUBAUER, G., HAUSEN, A. & WACHTER, H. (1984) Neopterin excretion during incubation period, clinical manifestation and reconvalescence of viral infection. In: *Biochemical and Clinical Aspects of Pteridines* Vol 3, p. 433, Walter de Gruyter, Berlin.

REINHERZ, E.L., WEINER, H.L., HAUSER, S.L., COHEN, J.A., DISTASO, J.A. & SCHLOSSMAN, S.F. (1980) Loss of suppressor T cells in active multiple sclerosis. Analysis with monoclonal antibodies. *N. Engl. J. Med.* **303,** 125.

RICE, G.P.A., FINNEY, D., BRAHENY, S.L., KNOBLER, R.L., SIPE, J.C. & OLDSTONE, M.B.A. (1984) Disease activity markers in multiple sclerosis, another look at suppressor cells defined by monoclonal antibodies OKT4, OKT5 and OKT8. *J. Neuroimmunol.* **6,** 75.

ROKOS, H., ROKOS, K. & ZIEGLER, I. (1983) Configuration of biopterin and neopterin excreted by patients with various diseases. In: *Biochemical and Clinical Aspects of Pteridines* Vol 2., p. 293. Walter de Gruyter, Berlin.

ROKOS, H., ROKOS, K., KERN, P. & DIETRICH, M. (1984) Radioimmunoassay for neopterin serum. Levels in patients with viral infections, lymphadenopathy syndrome, AIDS, leprosy and in normals after hepatitis vaccination. In: *Biochemical and Clinical Aspects of Pteridines* Vol. 3, p. 503. Walter de Gruyter, Berlin.

ROKOS, H., BIENHAUS, G., GADOW, A. & ROKOS, K. (1985) Determination of neopterin and reduced neopterins by radioimmunoassay. In: *Biochemical and Clinical Aspects of Pteridines.* Vol. 4, p. 73. Walter de Gruyter, Berlin.

SCHUMACHER, G., BEEBE, G., KIBLER, R., KURLAND, L., KURTZKE, J., MCDOWELL, F., NAGLER, B., SIBLEY, W., TOURTELLOTTE, W.W. & WILLMON, T.L. (1965) Problems of experimental trials of therapy in multiple sclerosis: Report by the panel on the evaluation of experimental trials of therapy in multiple sclerosis. *Ann. N.Y. Acad. Sci.* **122,** 552.

TIBBLING, G., LINK, H. & ÖHMAN, S. (1977) Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference values. *Scand. J. clin. Lab. Invest* **37,** 385.

WACHTER, H., HAUSEN, A. & GRASSMAYR, K. (1979) Erhöhte Ausscheidung von Neopterin im Harn von Patienten mit malignen Tumoren und mit Viruserkrankungen. *Hoppe-Seyler's Z. Physiol. Chem.* **360,** 1957.

WAKSMAN, B.H., REYNOLDS, W.E. (1984) Multiple sclerosis as a disease of immune regulation. *Proc. Soc. exp. Immunol. Med.* **175,** 282.

ZABRISKIE, J.B., MAYER, L., FU, S.M., YEADON, C., CAM, V. & PLANK, C. (1985) T cell subsets in multiple sclerosis. Lack of correlations between helper and suppressor T cells and the clinical state. *J. Clin. Immunol.* **5,** 7.