

Significance of Cerebrospinal Fluid Adenosine Deaminase Isoenzymes in Tuberculous (TB) Meningitis

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Adenosine deaminase (ADA) exists as two isoenzymes, ADA₁ and ADA₂. It appears that the ADA₂ isoenzyme originates mainly from monocytes and macrophages. In tuberculous pleural effusions most of the ADA activity consists of ADA₂. The aim of this prospective study was to analyse ADA isoenzymes in the CSF of patients with meningitis to investigate whether the expected rise of the ADA₂ isoenzyme would occur in tuberculous meningitis. ADA isoenzyme analysis was performed on the CSF of 15 patients with tuberculous and

11 patients with bacterial meningitis by an automated kinetic enzyme coupled assay in the presence and absence of a specific ADA inhibitor. The ratio of ADA₂/ADA_{Total} was >0.8 in 14/15 patients with tuberculous meningitis. In bacterial meningitis the ratio was ≤0.8 in 10/11 patients. The ADA₂ isoenzyme is the major contributor to increased ADA activity in the CSF of patients with tuberculous meningitis, probably reflecting the monocyte–macrophage origin of the ADA. *J. Clin. Lab. Anal.* 15:236–238, 2001. © 2001 Wiley-Liss, Inc.

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Adenosine deaminase (ADA) is the catalysing enzyme for the deamination of adenosine (or deoxyadenosine) to inosine (or deoxyinosine) and ammonia. There are two isoenzymes of ADA namely, ADA₁ and ADA₂ (1), each encoded by different gene loci. While the ADA₁ isoenzymes can be found in all cells with highest activity in lymphocytes and monocytes, ADA₂ is mainly present in monocytes (2). It is well known that total ADA activity is increased in pleural fluid of patients with tuberculous (TB) effusions, as well as in cerebrospinal fluid (CSF) of patients with TB meningitis. However, high ADA activity is also often found in the CSF in bacterial meningitis, limiting the diagnostic utility of ADA determination in CSF (3).

It has been shown previously that ADA₁ and ADA₂ isoenzymes contribute independently to ADA increase in tuberculous pleural effusions. In TB effusions most of the measured ADA activity is due to the ADA₂ isoenzyme, probably reflecting monocyte–macrophage origin (4).

In this prospective study the composition of ADA enzymes in the CSF of patients with TB and bacterial meningitis was investigated to determine whether the trend found in tuberculous effusions was also present in CSF of patients with TB meningitis.

MATERIALS AND METHODS

Patients

The CSF of 15 consecutive patients with TB meningitis and 11 consecutive patients with bacterial meningitis present-

ing at the Pretoria Academic Hospital was investigated. The laboratory performing the ADA determination was unaware of the clinical diagnosis of the patients. Of the patients with TB meningitis, 7 were male and 8 female; the ages ranged from 15 to 45 years. All patients were Black. The time elapsed since beginning of symptoms (headache, neck pain, fever) and first ADA analysis at admission ranged from 5 days (one patient) to more than 2 weeks (5 patients).

Of the patients with bacterial meningitis, 9 were male and 2 were female; the ages ranged from 18 to 65 years, and all patients were Black. The time elapsed from beginning of symptoms to ADA analysis ranged from 2 days (6 patients) to 5 days (1 patient).

The activity of ADA and its isoenzymes was determined by an automated kinetic enzyme-coupled assay in the presence and absence of a specific ADA₁ inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine (5). The Mann–Whitney *U*-test was used to determine whether the two groups of CSF differed significantly. Linear association tests (Pearson) were performed to establish whether correlations between ADA₁ and ADA₂ and lymphocyte/neutrophil counts in the CSF, respectively, were present.

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RESULTS

The diagnosis of TB was made according to standard criteria including typical CSF findings, clinical findings, histology, and positive cultures of *Mycobacterium tuberculosis*. The criteria for the diagnosis of tuberculous meningitis were as follows: presence of *M. tuberculosis* on CSF examination; positive culture and/or PCR of *M. tuberculosis* from CSF; post mortem examination confirming tuberculosis; CSF findings of a predominantly lymphocytic pleocytosis with CSF-protein > 0.5 g/l and CSF-serum-glucose ratio of <50% together with typical clinical findings and/or presence of tuberculosis in other organs and response to anti-tuberculous treatment. The laboratory evaluation of the CSF findings is shown in Table 1. In 4 patients the diagnosis of TB was confirmed at post mortem; in 3 patients histologic evidence for TB was found on lymph node biopsies as previously described (6); in one patient CSF cultures were positive; and in 7 patients the diagnosis was made by typical clinical and CSF findings with clinical response to anti-tuberculous treatment.

In the patients with bacterial meningitis *Streptococcus pneumoniae* was cultured from the CSF in 8, while 2 patients had meningococcal meningitis, and one had streptococcal Group B infection. The laboratory evaluation of the CSF findings is shown in Table 2.

The results of the ADA activity studies are also shown in Tables 1 and 2. The ratio of ADA₂/ADA_{Total} was >0.8 in 14/15 patients with TB meningitis and ≤0.8 in 10/11 patients with bacterial meningitis. Statistical analysis shows a significant difference between the two groups ($P = 0.0001$). The one patient with TB meningitis with an ADA₂/ADA_{Total} ratio of <0.8 had marked hydrocephalus, and CSF was obtained from the ventricles when an external drain was placed. Two pa-

TABLE 1. CSF findings in TB meningitis^a

N	L	CSF Glu-S Glu	P	ADA _T	ADA ₂	ADA ₂ /ADA _{Total}
270	42	0.9–6.2	3,400	3.5	3.2	0.91
55	128	0.7–5.5	2,960	7.5	6.1	0.81
77	108	0.8–5.7	1,850	10.5	9.9	0.94
12	470	1.5–5.2	2,300	10.5	9.7	0.92
0	244	6.4–1.6	3,000	16.5	14.3	0.87
0	385	1.0–3.43	2,190	22.2	20.6	0.93
24 ^b	134	1.9–7.8	200	6.4	4.4	0.7
385	275	0.8–12.1	2,300	15.5	13.1	0.85
0	647	2.2–7.9	1,400	10.4	10.2	0.98
36	18	1.1–4.5	3,645	14.8	14.0	0.94
10	251	1.4–6.8	2,060	17.7	17.6	0.99
61	97	1.0–?	3,649	38.67	37.0	0.95
85	12	2.5–4.6	6,415	14.9	13.7	0.92
323	293	2.6–6.3	4,177	27.2	23.9	0.88
48	256	2.1–6.8	2,434	22.0	21.9	0.99

^aN, neutrophils (/mm³); L, lymphocytes (/mm³); CSF Glu-S Glu, CSF glucose-serum glucose (mmol/l); P, protein (mg/l); ADA_{Total}, ADA total (U/l); ADA₂, ADA₂ isoenzyme (U/l).

^bCSF is ventricular fluid collected when external drain was inserted; diagnosis of TB confirmed at postmortem.

TABLE 2. CSF findings in bacterial meningitis^a

N	L	CSF Glu-S Glu	P	ADA _{Total}	ADA ₂	ADA ₂ /ADA _{Total}
672	250	0.1–8.9	4,298	10	5.2	0.52
684	24	2.1–7.1	4,991	6.7	2.2	0.33
138	0	0.1–13.5	17,200	9.8	8.6	0.88
1,311	30	0.3–16.4	17,700	9.4	7.5	0.79
600	75	1.6–8.3	1,310	56.4	25.4	0.45
5,894	507	1.3–5.1	5,720	4.6	2.1	0.45
2,200	110	0.1–17.2	5,870	3.8	1.5	0.39
2,536	208	1.9–5.5	2,370	27.5	7.3	0.26
6,313	12	0.1–6.3	3,910	5.1	3.9	0.76
12,100	22	0.2–9.5	5,810	6.3	2.5	0.39
4,620	110	1.3–4.8	2,120	15.8	9.9	0.63

^aN, neutrophils (/mm³); L, lymphocytes (/mm³); CSF Glu-S Glu, CSF glucose-serum glucose (mmol/l); P, protein (mg/l); ADA_{Total}, ADA total (U/l); ADA₂, ADA₂ isoenzyme (U/l).

tients with bacterial meningitis had relatively high ratios of ADA₂/ADA_{Total} (0.88 and 0.79)—in both patients the CSF protein values were exceptionally high (17,200 and 17,700 mg/l, respectively), which possibly could have affected measurement of ADA. On statistical analysis no significant correlations, i.e., linear associations were present either between ADA₁ (or transformation of ADA₁) and CSF lymphocytes and neutrophils, respectively, or between ADA₂ (or transformations of ADA₂) and CSF lymphocytes and neutrophils, respectively. Biplots of ADA₁ and ADA₂ versus both neutrophils and lymphocytes also reflected no correlations.

DISCUSSION

The ADA₂ isoenzyme was found to be the major contributor to total ADA activity in the CSF of patients with TB meningitis, with a median contribution of 90%. In bacterial meningitis, the median ADA₂ isoenzyme contribution was 51%.

The origin of ADA activity in the CSF of patients with TB meningitis is uncertain, but studies based on isoenzyme occurrence in body fluids suggest a monocyte-macrophage origin (7). In another recent study it was found that the ADA₁ isoenzyme was responsible for all the ADA activity in lymphocytes while ADA₂ was present only in monocytes (2). The increased ADA activity in CSF in TB meningitis therefore is probably due to monocyte-macrophage activation. In the bacterial meningitis group the ADA activity probably originates from neutrophils—the most abundant cell present in the CSF in bacterial meningitis—and lymphocytes. Total CSF ADA activity is often also increased in bacterial meningitis, decreasing the specificity of ADA determination in the diagnosis of TB. However, as this study shows, measurement of the ADA isoenzymes could help to distinguish between bacterial and TB infections in the CSF.

In conclusion, the ADA₂ isoenzyme is the major contributor to increased ADA activity in the CSF of patients with TB meningitis, probably reflecting monocyte-macrophage origin of the ADA. Thus, the same trend found in studies of

tuberculous pleural effusions where the ADA₂ isoenzyme is the major contributor to increased ADA activity is also found in cerebrospinal fluid of patients with TB meningitis.

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