Adenosine deaminase activity in the supernatant of monocytes infected with *Mycobacterium tuberculosis*

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Submitted: 27 Aug. 2007; Accepted: 2 Sep. 2007

**Abstract**

Adenosine deaminase (ADA) estimation is useful in the tuberculosis (TB) diagnosis. There is no information available for ADA activity source in tuberculosis (TB) infection. In the present study we aimed to evaluate whether cells of the monocyte-macrophage lineage are the source of adenosine deaminase. ADA activity in supernatant of mononuclear cell line (THP-1) was determined according to a method based on the Berthlot reaction, which is the formation of a colored indophenols complex from ammonia liberated from adenosine, and quantified spectrophotometrically. Increased ADA activity was noted in supernatant of monocytes after exposure to *Mycobacterium tuberculosis* as compared to non-infected cells. This study shows the evidence that monocytes are the main source of enzyme ADA due to interaction between *M. tuberculosis* bacilli and host monocytes during TB infection.

**Keywords:** Adenosine deaminase, Monocytes, *M. tuberculosis*

**INTRODUCTION**

Adenosine deaminase (ADA) is an enzyme that catalyzes the deamination of adenosine, forming inosine in the process (Fox *et al*., 1978). The chief physiological function of ADA is related to lymphocytic proliferation and differentiation (Erel *et al*., 1998). As a marker of cellular immunity, activity is found to be elevated in those diseases in which there is a cell-mediated immune response (Galanti *et al*., 1981). Numerous previous studies have demonstrated that ADA estimation is useful in the diagnosis of pulmonary and extra pulmonary tuberculosis (TB) and can be useful as a supporting test.

Although, measurement of ADA levels is a widely accepted complementary diagnostic test for pulmonary and extra pulmonary TB, the source of this enzyme has not carefully studied. Earlier José Luis Bañales *et al*. evaluated ADA activity as described by Giusti in the culture supernatants of eight *Mycobacterium tuberculosis* isolates and demonstrated that mycobacterial culture supernatants are unable to generate increased ADA activity indicating *M. tuberculosis* is not the source of ADA activity (Banales *et al*., 1999).

An interesting point remains to be elucidated with respect to the type of interaction between mycobacterial products and host factors in TB that produce increased ADA activity, however no experimental evidences was reported so far to confirm the same. In the present study we aimed to evaluate whether cells of the monocyte-macrophage lineage are the main source of ADA. We have evaluated ADA activity in supernatant of monocytes after exposure to *M. tuberculosis* and non-infected cells.
ADA activity in infected monocytes

Figure 1: Cultured monocytes (THP-1 cells) were subjected to Mycobacterium tuberculosis exposure at different time intervals. ADA activity (±SEM) was measured using Guisti and Galanti method. THP-1 cells incubated with Mycobacterium tuberculosis shows high ADA activity (filled bar) as compared with untreated cells (filled bar) (P=0.004). The experiment was repeated five times.

MATERIALS AND METHODS

Monocytes culture and in vitro M. tuberculosis infection

M. tuberculosis H37Rv was obtained from JALMA, Agra and grown in Bio FM liquid broth (BIO-RAD, France). All bacterial cultures were used in the log phase of growth. Monocytic (THP-1) cell line (2 × 10⁵ cells) were cultured in 100 ul of medium RPMI-1640 medium supplemented with 10 % PHS plus 2 mM L-glutamine and 10 mM HEPES for 24 h in 96-well plates. The cells were infected with M. tuberculosis at 10⁴ bacilli / well. The viability of the resulting cell cultures was assessed by trypan blue exclusion, and expressed as a percentage of the total number of cells observed. Each of these cell culture suspensions were incubated for 0, 2, 4, 24 & 48 hrs. At designated incubation periods, culture supernatant were harvested as per experiment and analyzed for ADA activity. The experiment was repeated five times and was carried out under appropriate containment conditions in a biological safety cabinet. Pilot experiments were conducted before final experiments to standardize the experiments. Trypan blue dye exclusion test was used to monitor the cell number. Trypan blue solution (0.5 % in double distilled water) was mixed with an equal volume of cell suspension and transferred into a hemocytometer. Then, the number of viable (unstained) cells per culture flask was calculated. This test evaluated the capacity of cells to proliferate. Other measured activities were then normalized for cell number.

ADA activity

ADA activity in supernatant was determined at 37 °C according to the method of Guisti and Galanti (Guisti G & Galanti B et al., 1984) based on the Berthlot reaction, that is the formation of colored indophenol complex from ammonia liberated from adenosine and quantified spectrophotometrically (UV visible spectrophotometer. Remi Model C - 24). One unit of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia/min from adenosine at standard assay conditions. Results were expressed as units per litre per minute (U/L/min). The assays were performed in triplicate.

Statistical analysis

Paired Sample T-Test was done to calculate the ADA activity and cell viability between Monocytes incubated at different intervals. P value was considered <0.05.

RESULTS

Fig.1 shows the ADA activity in supernatant of THP-1 cells with and without exposure of M. tuberculosis collected at different time interval (0, 2, 4, 24, 48 hrs). THP-1 cells incubated with M. Tuberculosis shows high ADA activity as compared with untreated cells (P = 0.012). The experiment was repeated five times.
ADA activity in infected monocytes

*M. tuberculosis* induced death in THP-1 cells was evaluated by Trypan blue dye exclusion test. Cell viability in percentage was determined after infection with *M. tuberculosis* at 0, 2, 4, 24, 48 hrs. Neither *M. tuberculosis* infected nor control group induced any significant loss in cell viability at the earlier 4 hrs time interval studied. After 24 hrs of culture not much cell death (loss of viability) was evident in the control group however cell death was noted in THP-1 cells infected with *M. tuberculosis* (As shown in Fig. 2). At 48hrs of culture slightly increase in cell death was noted *M. tuberculosis* infected cells (P=0.004).

**DISCUSSION**

Earlier several studies have indicated that measurement of ADA activity in body fluid is useful in the diagnosis of pulmonary and extra pulmonary TB (Titarenko *et al.*, 2006; Tuon *et al.*, 2006; Mishra *et al.*, 2006; Kashyap *et al.*, 2006). However no experimental evidence was available to confirm the source of elevated level of ADA in *M. tuberculosis* infection. In the present study THP-1 cell line were infected with *M. tuberculosis* and extracellular ADA activity was estimated in the medium at different time intervals. THP-1 cell line is widely used for *in vitro* infection of *M. tuberculosis* and other intracellular pathogen like *Listeria monocytogenes* (Carryn *et al.*, 2002) and *Legeionella* (Takemura *et al.*, 2000).

Our result shows elevated level of ADA activity in medium released from monocytes which were infected with *M. tuberculosis*. This experimental finding suggest these cells are the primary source of releasing ADA activity in *M. tuberculosis* infection. Earlier studies have demonstrated that *M. tuberculosis* infection induces monocyte/macrophage cell death (Sánchez *et al.*, 2006). It would be important to report the viability of the THP-1 cells in their cultures and at different time interval. Cell death was evaluated by Trypan blue dye exclusion test and results show at 48 hrs of culture slightly increase in cell death was noted *M. tuberculosis* infected cells as compared to the control group.

Human monocytes cells are the primary target for *M. tuberculosis* infection and their innate capacity to deal with *M. tuberculosis* may define the early progression of the TB infection. Earlier some studies have reported functional and phenotypic changes in monocytes from patients with TB but their significance is poorly understood (Sánchez *et al.*, 2006). Our data support the hypothesis that the release of ADA activity by the monocytes into their surroundings contributes to the relative elevation of serum ADA activity seen following an inflammatory response in tuberculosis patients. This is to our knowledge the first study to investigate the source of ADA using THP-1 cell line model. We hope these results spur interest in understanding the source of ADA in the TB patients. However a more detailed characterization of the monocyte function in TB patients is needed to better understand the source of ADA from infected monocytes.

**Figure 2**: THP-1 cells (2x10^5) incubated with M.tuberculosis (filled bar) and without M.tuberculosis (open bar) in RPMI 1640 medium at different time interval. Cell viability of THP-1 cells incubated with *Mycobacterium tuberculosis* induction decreases cell viability of THP-1 cell line at 48 hrs.
References


