EDITORIAL

Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biological role

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The isoenzymes ADA1 and ADA2 of the enzyme adenosine deaminase (ADA 3.5.4.4) deaminate mainly two nucleosides: adenosine and 2'deoxyadenosine, producing inosine and 2'deoxyinosine. Adenosine and 2'deoxyadenosine are molecules with many effects on human cells [1–4]. Thus, the homeostasis of these substances and the activity of the isoenzymes ADA1 and ADA2 in human cells are of extreme importance.

Isoenzyme ADA1

In humans, the isoenzyme ADA1 is ubiquitous and guarantees the downregulation of the substrates adenosine and 2'deoxyadenosine [1]. This homeostasis is particularly important because a low level of 2'deoxyadenosine is essential for a proper function in immune cells [2–5]. The isoenzyme ADA1 is also present in red cells, which are equipped with an efficient mechanism to capture and internalize 2'deoxyadenosine. Therefore, in humans, red cells form a "dialysis" system for circulating 2'deoxyadenosine and, in this way, preserve the nucleated cells from an excessive external supply of 2'deoxyadenosine [6].

The importance of ADA1 in cells is revealed by the disfunction of the immune response in subjects congenitally lacking ADA1 due to inadequate homeostasis of 2'deoxyadenosine in their immune cells [2, 4, 6]. There are several other important effects of the substrate adenosine which also bear witness to the importance of the presence of ADA1 in the human body [7]; these will not be discussed here.

The Km of ADA1 is 5.2×10^{-5} M. ADA1 has an optimal pH of 7–7.5 and a similar affinity for both adenosine and 2'deoxyadenosine (2'deoxyadenosine/adenosine deaminase ratio of 0.75) [1, 8].

These features make ADA1 highly efficient in deaminating the substrates (adenosine and 2'deoxyadenosine) in biological sites where the pH is optimal for this isoenzyme even though the substrate concentration is very low.

Isoenzyme ADA2 (ADA1-ADA2 isoenzymatic system)

The isoenzyme ADA2 is not ubiquitous, but coexists with ADA1 only in monocytes-macrophages. ADA2 and ADA1 are coded by different gene loci [1, 4, 9, 10].

The Km of ADA2 is 200×10^{-5} M; ADA2 has an optimum pH of 6.5 and a weak affinity for 2'deoxyadenosine (2'deoxyadenosine/adenosine deaminase ratio of 0.25)

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[1, 8, 9]. These features make ADA2 inefficient in deaminating 2'deoxyadenosine in biological sites where the pH is higher than the optimum for this isoenzyme and the concentration of substrates is too low.

Why, therefore, should ADA2 be present in monocytes-macrophages, where conditions are not suitable for its optimum function? To answer this question, which is extremely important from a biological point of view, it is necessary to consider ADA1 and ADA2 as a system which acts to guarantee the homeostasis of adenosine and 2'deoxyadenosine in monocytes-macrophages. This homeostatic mechanism involves two substrates and two isoenzymes. Both isoenzymes have similar affinity for the substrate adenosine, whilst ADA2 has a different affinity (very weak) for the substrate 2'deoxyadenosine [1, 8].

Through the simulation model STELLA II (High Performance System Inc., 1993), we have demonstrated that in monocytes-macrophages the adenosine level is always low, whilst, in certain conditions (see below), the 2'deoxyadenosine level rises dramatically owing to an increase of ADA2. This results in a homeostatic mechanism for the upregulation of 2'deoxyadenosine inside monocytes-macrophages [11].

Taking into account that the increase of ADA2 in monocytes-macrophages occurs when these cells are infected by intracellular micro-organisms and whilst the parasite is still alive [8, 12–14], and the fact that: 1) monocytes-macrophages, especially in an activated state, tolerate high levels of 2'deoxyadenosine [3, 5]; 2) monocytes-macrophages are crucial cells in immune defence; and 3) 2'deoxyadenosine is deleterious for nucleic acid [15–18], leads us to infer that the ADA1-ADA2 homeostatic system may be a tool in the production of a "weapon" (2'deoxyadenosine) of monocytes-macrophages against offending parasites. Although this question is still open, we think that it deserves attention.

Diagnostic role of ADA1 and ADA2 and the 2'deoxyadenosine/adenosine deaminase ratio

Whatever the biological role of ADA1 and ADA2, it has been demonstrated that the presence (low or high) of these isoenzymes in biological fluids has diagnostic relevance [9, 10, 12–14, 19–22]. The paper by VALDES et al. [23] in this issue of the Journal confirms the above statement. Therefore, measuring ADA1 and ADA2 activity in biological fluids is useful in clinical practice. However, since ADA activity in body fluids is due to a mixture of ADA1 and ADA2, to estimate the precise quantity of each of these isoenzymes in a specimen, one

should separate the two isoenzymes. This is highly complicated and impractical.

Therefore, to interpret the findings of the values of ADA activity in biological fluids correctly one must be aware of the concept and the significance of the "2'deoxyadenosine/adenosine deaminase ratio" [1, 8–10, 14, 21, 22]. This is the ratio between the rate of deamination of 2'deoxyadenosine and adenosine, independently performed in two separate test tubes [9, 10, 21, 22]. This ratio, due to the fact that ADA1 and ADA2 have different affinities for the substrate 2'deoxyadenosine, will have different values according to the relative percentages of the two isoenzymes in the specimen. In fact, it is a practical way to obtain an approximate measurement of the relative percentage of ADA1 and ADA2 activity in the sample [21].

Conclusions from our experience in this field

Low ratio plus ADA value (high or low) distinguish those infectious diseases due to intracellular microorganisms (high ADA value) from those due to other bacteria (low ADA value) [21, 22]. We have established by experience that a ratio <0.45 in a biological fluid specimen with high ADA activity is indicative of an infection by intracellular micro-organisms, which induce the increase of ADA2 [14, 21, 22]. On the contrary, a ratio >0.45, which means an increase of ADA1 with high ADA activity, is indicative of malignancy or empyemas [14, 21, 22].

The value of ADA activity must be at least 2.5 times the normal value to have a clear diagnostic relevance [22]. Briefly, in serum: 1) high ADA activity (normal value 18±7 UI·L^{-1}) with a ratio <0.45 indicates: typhoid fever, brucellosis, acute toxoplasmosis, visceral leishmaniasis, rickettsiosis, B and A hepatitis, chicken-pox, mononucleosis, and others; 2) low ADA activity (near the normal value) in almost all cases excludes these diseases; and 3) high ADA activity with a ratio >0.45 is indicative of malignancy [9, 14, 21, 22].

In fluids (pleural, peritoneal and pericardic effusions): 1) high ADA activity (>40 UI·L^{-1}) with a ratio <0.45 is highly indicative of tuberculous disease; 2) low ADA value excludes this disease; and 3) high ADA activity with a ratio >0.45 is indicative of malignancy or empyemas [13, 19–22].

In CSF: 1) high ADA activity (>8–9 UI·L^{-1}, normal value 0–3 UI·L^{-1}) is indicative of tuberculous meningitis; and 2) low ADA activity excludes this disease [12, 14, 21, 22].

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