PURINE NUCLEOSIDE PRODUCTION IN MATURE AND IMMATURE HUMAN LYMPHOCYTES

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Received 12 February 1982; revision received 12 March 1982

1. Introduction

Adenosine and adenosine deaminase appear to play a crucial role in lymphocyte maturation. 5′-Nucleotidase, an ecto-enzyme which produces adenosine from extracellular 5′-AMP [1], displays very low activity in immature lymphocytes [2–5]. In the human, adenosine can induce lymphocyte maturation [6]. However, high levels of adenosine deaminase (ADA), which leads to inosine, were found in immature lymphocytes [7]. It was postulated that adenosine deaminase is involved in lymphocyte maturation [8]. ADA deficiency leads to severe combined immunodeficiency, characterized by the lack of mature T and B lymphocytes [9].

Very low 5′-nucleotidase (5′N) activities were reported in thymocytes from various species [10–14]. We have shown that in mouse this low level was due to the high percentage of peanut agglutinin (PNA)-positive, hydrocortisone-sensitive thymocytes, which almost lack 5′N activity [14]; this population is thought to represent immature cortical cells; on the contrary, PNA-negative thymocytes, which are immunocompetent, hydrocortisone-resistant and could represent the medullary population, display a 10-fold higher 5′N level [14]. This result is conflicting with [15] where no difference between 5′N activities in rat medullary and cortical thymocytes was found.

T Human lymphoblastoid cell lines of acute lymphocytic leukemia (ALL) origin lack also 5′N activity [16]. These cells display very high terminal deoxynucleotidyl transferase (TdT) activity and were described as immature [17].

In [18] 5′N activity of unseparated human thymocytes increased after incubation of these cells with factors known to induce thymocyte maturation. This result prompted us to verify if immature human thymocytes lack 5′N activity, like immature mouse thymocytes [14], and if 5′N levels can be used as a marker of human cell maturation as postulated in [2–5]. We also measured ADA activities of human mature and immature thymocytes.

Human lymphoblastoid cell lines are often used as models of lymphoid cell subpopulations, as they possess some of the characteristics of normal cells. We determined 5′N and ADA activities in some of these cell lines displaying mature or immature properties.

2. Materials and methods

Normal peripheral blood lymphocytes were purified from heparinized blood by centrifugation of Ficoll-Hypaque [19]. Tonsil lymphocytes were purified as in [20]. Normal human thymuses were obtained from children undergoing cardiac surgery; thymuses were minced in PBS (pH 7.4) then passed through a fine stainless steel mesh to give a single cell suspension. When necessary erythrocytes were selectively killed by 0.14 M ammonium chloride. Cell viability was always >92%.

Mature and immature thymocyte populations were isolated by agglutination with PNA as in [21]. Thymocytes, 10^8 in 0.25 ml PBS, were incubated with 0.5 mg PNA in 0.25 ml PBS for 15 min at 25°C; the mixture was then layered on 8 ml 50% fetal calf serum (FCS) in PBS. After 30 min sedimentation at $1 \times g$, agglutinated (immature) cells (bottom layer) were removed from non-agglutinated (mature) cells (top layer), dissociated into single cells by 10 min incubation at 37°C with 5 ml 0.2 M D-galactose in PBS, washed twice with 5 ml 0.2 M D-galactose and twice with

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00145793/82/0000–0000/$02.75

PBS. Unagglutinated cells (5–10% of total thymocytes) received the same treatment.

The malignant T lymphoblastoid cell lines MOLT-3, MOLT-4, JM, H-SB2, the B cell line CCRF-SB, and the non-T non-B leukemia cell lines REH and K 562 were provided by Clin-Midy Research Center (Montpellier). 4 B lymphoblastoid cell lines were established by Dr Malissen (Institut d'Immunologie, Marseille Luminy) after infection of normal circulating lymphocytes with Epstein-Barr virus. Cells maintained in RPMI 1640, 10% FCS, were collected when they reached 10^6 cells/ml.

5′-Nucleotidase activity was determined using 5′-[^{32}P]AMP as substrate [16]. Measures were performed as in [22] with intact cells, the specificity of 5′-AMP hydrolysis was assessed by several controls: specific inhibition by anti-5′N [23] and concanavalin A [24], lack of inhibition by the substrates of non-specific phosphatases [1].

Adenosine deaminase was measured using [^{3}H]-adenosine as substrate, on lymphocytes permeabilized with Lubrol PX, as in [16].

5′N activity was expressed as nmol P_i . h^{-1} . mg protein^{-1}, ADA activity as nmol inosine . min^{-1} . mg protein^{-1}. The amount of proteins corresponding to the cells was determined by Lowry's method.

3. Results and discussion

3.1. *5′N and ADA activities of human lymphocytes*

Like for mouse thymocytes [25] the major population of human thymocytes (80–90% of cells) binds PNA and can be separated from thymocytes which do not bind the lectin. As reported in [21] the PNA$^+$ subpopulation did not respond to PHA, while the minor subpopulation (10–15% of total cells) did exhibit this response exactly like blood circulating lymphocytes (PBL): our results (not shown) were identical to those in [21] where the immature character of PNA$^+$ cells and the mature character of PNA$^-$ cells was shown.

5′N activities of both populations are reported in table 1; for 3 different experiments the 5′N activity of PNA$^-$ thymocytes is 5-fold higher than that of PNA$^+$ thymocytes. We have shown [14] that PNA or D-galactose did not inhibit 5′N activity. The low 5′N activity of PNA$^+$ thymocytes is not related to an inhibition process but to the lack of 5′N active sites, as evidenced by the fact that 5′N of unseparated cells reflects the percentage of PNA$^-$ and PNA$^+$ populations.

The high 5′N level of mature PNA$^-$ thymocytes is similar to that of tonsil lymphocytes and slightly lower than that of peripheral blood lymphocytes

Table 1
5′N and ADA activities of human lymphocytes

Cells		5′N (nmol . h^{-1} . mg^{-1})	ADA (nmol . min^{-1} . mg^{-1})	5′N / ADA
Thymocytes:				
Exp. 1	unseparated	64	n.d.[a]	
	PNA$^+$	45	470	0.095
	PNA$^-$	204	200	1
Exp.2	unseparated	65	n.d	
	PNA$^+$	40	490	0.08
	PNA$^-$	280	200	1.4
Exp. 3	unseparated	72	n.d	
	PNA$^+$	60	450	0.13
	PNA$^-$	300	210	1.5
PBL:		274 ± 82	70 ± 17	3.9
Tonsil lymphocytes:		246 ± 30	100 ± 20	2.5

[a] Not determined

5′N and ADA activities of unseparated, PNA$^+$ and PNA$^-$ thymocytes were measured in 3 different expt as in section 2. Each measure was carried out in triplicate and the precision is >5%. 5′N and ADA activities of peripheral blood lymphocytes (PBL) and tonsil lymphocytes were measured in five different experiments; the mean value of the activity and the standard deviation are reported

(table 1). We did not measure the 5′N activity of T and B PBL separated by the rosetting technique because the 2 populations are submitted to very different treatments (hemolysis, different number of centrifugations), which might differently affect their membrane enzymatic activities and especially their 5′N activity which can easily be detached from the plasma membranes [16]; it could explain the opposite results reported for circulating T and B cells [26,27].

We also reported ADA activities of mature and immature thymocytes (table 1): PNA^+ cells display 2–2.5-fold higher ADA activity than PNA^- cells; the same result was observed for mouse thymocytes [16]. It is consistent with the data in [7] where immature rat thymocytes, separated by density gradient centrifugation, displayed ADA activity 2–3-fold higher than that of mature thymocytes. The ADA activity of human PNA^- thymocytes was found 2-fold higher than that of unseparated PBL and of tonsil lymphocytes.

The ratio 5′N/ADA is >10-fold higher in PNA^- than in PNA^+ thymocytes. This ratio is even greater in PBL or tonsil lymphocytes: 2-times with respect to PNA^- thymocytes and >20-times with respect to PNA^+ thymocytes.

3.2. *5′N and ADA activities of human lymphoblastoid cell lines*

Several authors found undetectable 5′N levels in various lymphoblastoid cell lines [28–31], using 5′-AMP radiolabelled on the adonosine moiety as substrate [28,32]; in [16] such substrates were shown unsuitable for the determination of 5′N activity on intact cells. We measured 5′N activities of several human lymphoblastoid cell lines using 5′-[^{32}P]AMP as substrate [16]: we found very low but detectable 5′N levels in T-ALL cell lines, with the exception of MOLT-3 and H-SB2 cells which almost completely lack the activity (table 2). EBV-induced B lymphoblasts displayed higher 5′N activity, close to that of normal PBL. H-SB2 and CCRF-SB lymphoblasts further proved this point: these 2 cell lines are issued from the same patient [33]; H-SB2 are T-ALL cells and lack completely 5′N activity while CCRF-SB of mature B origin have relatively high 5′N level (110 nmol . h^{-1} . mg protein^{-1}). At the same time ADA activity was high in T-ALL cells and low in B lymphoblasts.

The non-T non-B (null) leukemia line REH showed also low 5′N and high ADA levels. Another null cell line K562 [17] had low ADA and almost normal 5′N activities; however, this cell line, of chronic myeloid leukemia origin is properly considered an erythromyeloid cell line rather than a lymphoblast [34]. A low TdT level for K562 cells and high TdT level for REH cells was reported in [17].

B-ALL lymphoblasts behave like T-ALL cells – low 5′N and high ADA activities [35]. On the contrary EBV-induced lymphoblasts have 5′N and ADA levels similar to those of normal human PBL.

Table 2
5′N and ADA activities of human lymphoblastoid cell lines

Cell lines	Origin	TdT level[a]	5′N (nmol . h^{-1} . mg^{-1})	ADA (nmol . min^{-1} . mg^{-1})	5′N / ADA
MOLT-3	T-ALL[b]	H	0	803 ± 200	0
MOLT-4	T-ALL	H	11 ± 4	740 ± 100	0.01
JM	T-ALL	H	35 ± 12	240 ± 50	0.15
H-SB2	T-ALL	H	0	360 ± 30	0
REH	Null[c]-ALL	H	41 ± 6	210 ± 20	0.40
K562	Null-CML[d]	L	73 ± 18	39 ± 10	1.88
CCRF-SB	B cell[e]	L	110 ± 32	35 ± 12	3.10
EBV-B lymphoblasts [4]	Normal PBL	L	165 ± 36	62 ± 13	2.64

[a] TdT levels as reported by [17]: low (L) or high (H)
[b] Acute lymphocytic leukemia; [c] Non-T non-B; [d] Chronic myeloblastic leukemia
[e] Apparently normal B cell deriving from the same ALL patient who gave H-SB2 cell lines [33]

The mean values and standard deviations are reported (3 expt)

4. Conclusion

These data show that different levels of 5′N and ADA are associated with different stages of human lymphocyte maturation. Like for mouse lymphocytes [16] we found an inverse relationship between 5′N and ADA activities, with low 5′N and high ADA values in PNA^+ thymocytes, high 5′N and low ADA values in PNA^- thymocytes, tonsil lymphocytes and PBL.

The result concerning 5′N is not consistent with that in [15] where similar 5′N values were reported in mature and immature rat thymocytes, but it agrees with our data on mouse thymocytes [14] and with the demonstration of an increase of 5′N activity during in vitro human thymocyte maturation [18].

We also found very low 5′N and high ADA activities in several lymphoblastoid cell lines from acute lymphocytic leukemia (T or null) (table 2 and [16]). A similar result was reported for a B-ALL patient [34]. Very low 5′N/ADA ratios characterize all kinds of ALL lymphoblasts and not their T, B or null character. These cells are known to possess immature characteristics such as high TdT activity [17].

Two different cell lines (REH and K562) described as non-T non-B leukemia lines [17] display very different 5′N/ADA ratios. REH cells of ALL origin have low 5′N/ADA ratio (table 2) and high TDT activity [17], KR62 (chronic myeloblastic leukemia [34]) show high 5′N/ADA ratio and low TdT activity [17].

B lymphoblastoid cell lines deriving from normal mature B lymphocytes transformed by Epstein-Barr virus display low TdT activity, like mature cells [17]. We found in 4 such lines high 5′N and low ADA levels. It is often postulated that the differences of 5′N and ADA activities between T and B lymphoblastoid cell lines are related to their T or B character. But this hypothesis is not supported by the low 5′N activity of B lymphoid cells arrested in an early stage of maturation [5] and by the data on B-ALL lymphocytes [35] and on T and B normal lymphocytes [27]; B and T cord blood lymphocytes are also claimed to have low similar 5′N activity [3].

A good correlation appears between high TdT activity and low 5′N/ADA ratio and vice-versa. If we consider that TdT is one of the features of immature cells, it might be the same for the 5′N/ADA ratio. Lymphocytes from peripheral blood and tonsils which include a majority of immunocompetent cells show high 5′N/ADA ratios unlike PNA^+ (immature) thymocytes. However, further investigations should be necessary to confirm the relationship between 5′N/ADA ratio and the stage of cell maturation.

The physiological meaning of the increase of this ratio during cell maturation is poorly understood. 5′N is a glycoprotein and one of the high affinity receptor of concanavalin A [24]; it is inhibited by the binding of this lectin mitogen. It appears involved in the uptake of adenosine from extracellular nucleotides [36] and in the regulation of cyclic AMP levels [37] through adenosine receptors. In [38] we showed the presence of such receptors on immature thymocytes, which is consistent with the possible involvement of cyclic AMP in adenosine-induced thymocyte maturation [6].

The high ADA level of immature lymphocytes might be an adaptation mechanism because of the low availability of adenosine in cells with low 5′N levels. The fact that ADA deficiency prevents cell maturation and leads to immunodeficiency emphasizes the importance of adenosine metabolism in lymphocyte maturation and function.

Acknowledgements

This work received financial support from the Centre National de la Recherche Scientifique and the Fondation pour la Recherche Médicale Française. The authors acknowledge skillful technical assistance of N. Bernad and R. Larguier.

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