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Modulation of lymphocyte proliferation by enzymes that degrade amino acids

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SUMMARY

In a previous study we demonstrated thirteen amino acids to be essential and two to be partially essential for lymphocyte proliferation. Arginine is one of the essential amino acids, and the highly purified arginase strongly inhibited lymphocyte proliferation. The modulation of lymphocyte growth by various amino acid-degrading enzymes was studied. Peripheral lymphocytes were cultured in RPMI 1640 with or without amino acid-degrading enzyme for 72 h. A total of 17 commercial L-amino acid-degrading enzymes were studied. At 10 μg/ml, both lysine decarboxylase and asparaginase completely inhibited lymphocyte proliferation, arginase resulted in 78% inhibition and tyrosinase 57% inhibition. Other enzymes inhibited less than 20% lymphocyte proliferation; they included alanine dehydrogenase, arginine decarboxylase, aspartase, glutamic decarboxylase, glutamic dehydrogenase, glutaminase, histidase, histidine decarboxylase, leucine dehydrogenase, phenylalanine decarboxylase, phenylalanine hydroxylase, tryptophanase, and tyrosine decarboxylase. All four enzymes that strongly inhibited lymphocyte proliferation degraded amino acids that are essential for lymphocyte growth.

Keywords amino acids degrading enzymes lymphocyte proliferation

INTRODUCTION

The ability of lymphocytes to synthesize new proteins is essential for the development and maintenance of humoral and cell-mediated immunity. Lymphocyte function is regulated by the availability of free amino acids for protein synthesis. Deprivation of selected amino acids serves as a good model for studying the amino acid modulation of lymphocyte proliferation.

We have shown that 13 amino acids (arginine, cysteine, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine) were essential for lymphocyte proliferation; two amino acids (serine and alanine) were partially essential (Chuang, Yu & Wang, 1990). However, highly purified arginase, which may degrade arginine, strongly inhibits lymphocyte proliferation (Su *et al.*, 1987a). It was suggested that the inhibition was related to the state of arginine depletion caused by the enzymatic effect of arginase (Su *et al.*, 1987b).

We extended these observations to study the effect of various enzymes that degrade amino acids on lymphocyte proliferation; it was our expectation that the addition of such enzymes to degrade the essential amino acids would cause a reduction in lymphocyte proliferation.

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MATERIALS AND METHODS

Lymphocyte preparation

Mononuclear cells (MNC) were separated from the heparinized peripheral blood of healthy donors by Ficoll–Hypaque density gradient centrifugation as previously described (Wang *et al.*, 1984). These cells were washed twice with HBSS and were suspended in RPMI 1640 (GIBCO, Grand Island, NY). The cells thus obtained consisted of 92±2% (2 s.d.) of MNC as determined by Wright stain. Cell viability determined by trypan blue dye exclusion was more than 96%.

Enzyme preparations

The enzymes used include alanine dehydrogenase, arginase, arginine decarboxylase, asparaginase, aspartase, glutamic decarboxylase, glutamic dehydrogenase, glutaminase, histidase, histidine decarboxylase, leucine dehydrogenase, lysine decarboxylase, phenylalanine decarboxylase, phenylalanine hydroxylase, tryptophanase, tyrosinase and tyrosine decarboxylase. They were all purchased from Sigma Chemical Co., St Louis, MO.

Their sources, activities and optimal pH are shown in Table 1.

Table 1. The enzymes used in this study

Enzyme	Source	Optimal pH	Activity (U/mg)
Alanine dehydrogenase	Bacillus subtilis	10	58
Arginase	Bovine liver	9·5	210
Arginine decarboxylase	*Escherichia coli*	5·2	4·5
Asparaginase	*E. coli*	8·6	150
Aspartase	*Hafnia alvei*	8·5	1·3
Glutamic decarboxylase	*E. coli*	5·0	28
Glutamic dehydrogenase	Bovine liver	7·3	33
Glutaminase	*E. coli*	4·9	6·1
Histidase	*Pseudomonas fluorescens*	9·0	1000
Histidine decarboxylase	*E. coli*	4·5	7
Leucine dehydrogenase	*Bacillus* spp.	10·5	33
Lysine decarboxylase	*B. cadaveris*	6·0	39
Phenylalanine decarboxylase	*Streptococcus frecaris*	5·5	0·05
Phenylalanine hydroxylase	Rat liver	7·2	0·075
Tryptophanase	*E. coli*	8·3	50
Tyrosinase	Mushroom	6·5	2125
Tyrosine decarboxylase	St. faecalis	5·5	0·7

Lymphocyte cultures

Lymphocytes (5×10^5/ml) were cultured in 96-well, flat-bottomed microculture plates (Costar, Cambridge, MA). There was 0·2 ml of RPMI 1640 in each well containing 10% (v/v) dialysed fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 1 μg/ml phytohaemagglutinin (PHA, Wellcome, Dartford, UK). Various commercial amino acid-degrading enzymes at various concentrations were added to the culture. All the cultures were set up in quadruplicate and incubated in a 5% CO_2/air humidified incubator for 72 h. during the final 4 h of the culture period, these cells were pulsed with 0·5 μCi/well of ^{3}H-thymidine (specific activity 6·7 Ci/mmole, New England Nuclear, Boston, MA). Cultures were then harvested onto glass/fiber filters with an automatic cell harvester (model M24, Brandel). Isotope incorporation was measured by a standard toluene-based counting technique (Wang & Zweiman, 1978). The results were expressed as the arithmetic means of ct/min for quadruplicate cultures.

RESULTS

Seventeen commercially available enzymes that degrade L-amino acids (Table 1) were studied. They were cultured separately with cells and the dose–response curves are shown in Fig. 1. Four enzymes caused marked inhibition of lymphocyte proliferation: lysine decarboxylase; asparaginase; arginase; and tyrosinase. Lysine decarboxylase was the most potent, with 50% inhibition at 0·8 μg/ml. At a concentration of 10 μg/ml, lysine decarboxylase caused 100% inhibition of lymphocyte proliferation; asparaginase caused 98%; arginase, 78%; and tyrosinase caused 57% inhibition. Other enzymes that inhibited less than 20% lymphocyte proliferation included alanine dehydrogenase; arginine decarboxylase; aspartase; glutamic decarboxylase; glutamic dehydrogenase; glutaminase; histidase; histidine decarboxylase; leucine dehydrogenase; phenylalanine decarboxylase; phenylalanine hydroxylase; tryptophanase; and tyrosine decarboxylase.

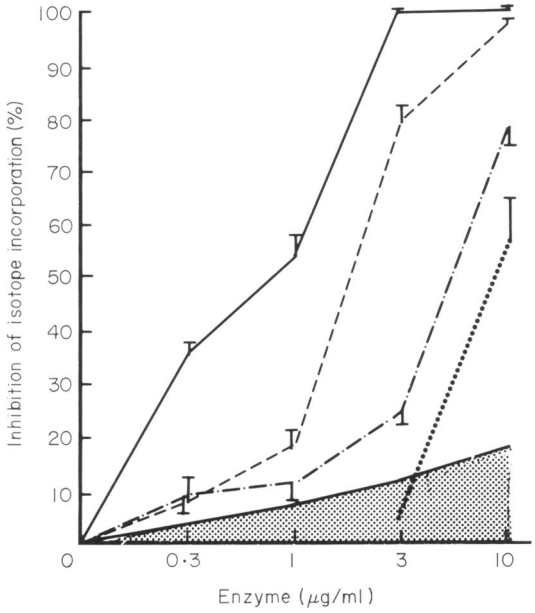

Fig. 1. The dose effects of the enzymes that degrade amino acids on lymphocyte proliferation. Lymphocytes were cultured in RPMI 1640 with 10% dialysed fetal bovine serum and various concentrations of lysine decarboxylase (——); asparaginase (– – –); arginase (·–·–·); or tyrosinase (· · · ·). The shaded area represents the other 13 enzymes that degrade amino acids. Bars are 1 s.d. The ct/min in RPMI 1640 with 10% dialysed fetal bovine serum (control) was 60430 ± 3525.

DISCUSSION

The present study demonstrated that four enzymes that degrade amino acids strongly inhibited lymphocyte proliferation. They were lysine decarboxylase, asparaginase, arginase and tyrosinase.

L-lysine and tyrosine are essential amino acids for humans (Rose, 1949). The deficiency of these essential amino acids is

harmful to the host as well as its immune system. It is conceivable that the lymphoid system of a host can not tolerate the deficiency of some amino acids that are depleted by their specific degrading enzymes. Experiments have shown that enzyme-mediated depletion of selected essential amino acids for a short period of time (2 or 3 weeks) appears to be relatively well tolerated by the host *in vivo* (Roberts, 1981), but is relatively intolerable by lymphocytes, as shown in this study.

Asparagine is an amino acid non-essential *in vivo* for humans (Rose, 1949) and *in vitro* for certain mammalian cells (Eagle, 1959a) and cell lines (Piez & Eagle, 1958). In these cells, L-asparagine is synthesized from L-aspartic acid, L-glutamine, ATP and Mg^{2+} by the action of an enzyme, L-asparagine synthetase (Levintow, 1957). There is low enzyme activity of L-asparagine synthetase in mouse spleen and lymph nodes (Horowitz *et al.*, 1968). However, in the condition of asparagine depletion, the asparagine synthetase activity in mouse lymphoid tissue can be rapidly induced (Prager & Bachynsky, 1968).

The discovery of immunosuppressive properties of the enzyme L-asparaginase has opened a new area in immunosuppression (Astaldi *et al.*, 1969). Ohno & Hersh (1970) have shown that L-asparaginase inhibits lymphocyte blastogenesis. Relatively non-cytotoxic suppression of both cellular and humoral immunity by L-asparaginase was first thought to result from the depletion of the non-essential amino acid L-asparagine (Eagle, 1959). Later study suggested that the immunosuppressive effects of asparaginase may be due to the capability of the enzyme to degrade L-glutamine (glutaminase activity) (Simberkoff & Thomas, 1970). *Escherichia coli* asparaginase degrades both asparagine and glutamine. The administration of this enzyme to mice causes rapid immunosuppression. Asparaginase from *Vibrio succinogenes*, which was not commercially available for this study, degrades only asparagine and does not cause any apparent impairment of the immune system (Durden & Distasio, 1980). Hence the depletion of glutamine rather than asparagine is related to immunosuppression. It is understandable that asparagine is non-essential for both humans *in vivo* and lymphocyte *in vitro*. However, although glutamine is non-essential for humans, it is essential for lymphocytes *in vitro* (Chuang *et al.*, 1990).

Two enzymes with glutaminase activity but without asparaginase activity have been isolated from soil organisms (Robert, Holcenberg & Dolowy, 1970). Both express an optimal activity at physiologic pH, but with a different K_m (0·65 and 0·008 mM) for glutamine. One of them (with K_m 0·008 mM) had no effect on lymphocyte proliferation because of low enzyme activity (6·1 U/ml; see Table 1) as shown in this study, and the other was not available commercially.

Arginine is not required for the adult human, rat, mouse, or dog. However, in young rats, arginine is required for optimal growth. In cell cultures, arginine is required for the growth of most cell types including lymphocytes.

The depletion of a non-essential amino acid from the circulation may not be harmful to the host, but may cause a functional impairment of lymphocytes, as in the case of arginine depletion. Lymphocytes may acquire under certain circumstances the ability to synthesize the missing amino acid and hence may tolerate its depletion. This phenomenon has been observed in asparaginase-treated leukaemia cells that change from being asparaginase-sensitive to being asparaginase-resistant.

Four enzymes (lysine decarboxylase; asparaginase; arginase; and tyrosinase) in the present study might degrade their corresponding essential amino acids, resulting in the inhibition of lymphocyte proliferation. The enzymes (alanine dehydrogenase; aspartase; glutamic decarboxylase; and glutamic dehydrogenase) that degraded non-essential amino acids caused no inhibition. It is of interest that nine enzymes degraded essential amino acids but caused no inhibition. Among them, three (histidase; histidine decarboxylase; and leucine dehydrogenase) had optimal pH at non-physiological condition, six (arginine decarboxylase; glutaminase; phenylalanine decarboxylase; phenylalanine hydroxylase; tryptophanase; and tyrosine decarboxylase) have low enzyme activity (Table 1).

There is great potential for the four enzymes with strong inhibitory activities on lymphocyte proliferation to be used as immunosuppressive agents or cytotoxic agents, such as asparaginase in the treatment of leukaemia.

We have shown that arginase causes a non-cytotoxic inhibition on lymphocyte proliferation, due to the arginine depletion from the enzyme effect (Su *et al.*, 1987b). It is conceivable that the other two enzymes (lysine decarboxylase and tyrosinase) capable of inhibiting lymphocyte proliferation operate by the same mechanism. Thus, besides asparaginase, lysine decarboxylase, arginase and tyrosinase may have a good potential to be used as immunosuppressive agents.

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