Volume 226, number 2, 303-306 FEB 05455 January 1988

Tuftsin stimulates growth of HL60 cells

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Received 12 November 1987

Many functions of monocyte/macrophage and granulocyte are activated by tuftsin; principally phagocytosis, motility, immunogenic stimulation, antibacterial and antineoplastic activities. Here it is shown that tuftsin stimulates HL60 growth to twice the control rate. The uptake of [^{3}H]uridine and [^{3}H]leucine in a pulse of 30 min was also double that of the control. The uptake of thymidine was not stimulated.

Tuftsin; Growth; (HL60 cell)

1. INTRODUCTION

Tuftsin (Thr-Lys-Pro-Arg) was discovered in this laboratory several years ago [1–3]. It is a highly active tetrapeptide that activates phagocytic cells [4]. It is a part of a carrier molecule, a leukophilic γ-globulin that binds specifically to PMN and macrophage/monocyte cells [5–7]. It is active only as the free tetrapeptide. Consequently, it requires two enzymes to cleave it off the carrier molecule [1–4]; a splenic enzyme, tuftsin-endocarboxypeptidase and a cell membrane enzyme leukokininase. The latter is present on the outer surface of the membrane and is active in the intact viable cell, as well as in broken cell membranes. In instances where the spleen is removed, the tetrapeptide remains covalently anchored at the arginine carboxy-end, and as such is inactive. Such a state of affairs results in an 'acquired tuftsin' deficiency with its attendant severe infections [8,9]. We have also discovered another type of deficiency 'congenital familial tuftsin deficiency' [10,11] in which the active tetrapeptide tuftsin, Thr-Lys-Pro-Arg, is replaced by the inactive and inhibitory tetrapeptide, Thr-Glu-Pro-Arg. Here a transition mutation, A⟶G, results in the replacement of lysine with the glutamate residue in either of the two lysine triplets. We have identified 17 patients with this deficiency and 2 were described in Japan [12]. All showed repeated severe infections. The presence of this mutation renders tuftsin a true biological entity with a defined physiological function.

The activation of phagocytic cells, macrophage/monocyte, and PMN by tuftsin results in the stimulation of phagocytosis [1–3], motility [3], immunogenic response [13], reversal of age depressed activities [14,15], bactericidal [16] and tumoricidal activities [17]. Specific tuftsin receptors are found on phagocytic cells. We have purified them to apparent homogeneity from rabbit peritoneal granulocytes [17]. They are composed of two subunits, an α of 62 kDa and a β 52 kDa subunit. Similarly, receptors on HL60 cells, a promyelocytic human cell line, showed an α-subunit of mass 67 kDa and a β-subunit of 55 kDa [18].

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Abbreviations: ATCC, American Type Culture Collection; FCS, fetal calf serum; PBS, phosphate buffered saline; PMN, polymorphonuclear leukocyte; TFMS, trifluoromethanesulfonic acid

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

From the standpoint of lethal effects of the phagocyte on target cells, both mammalian and bacterial, we have shown substantial tuftsin stimulation of the formation of tumor necrosis factor, cachectin, in mice both in vitro and in vivo, as well as in the cell line HL60 [19]. Similarly, the large tuftsin stimulation of the superoxide anion and hydrogen peroxide formation [20,21] may well add to the lethal effects of the phagocytic cell.

Because of the increased and varied biological expressions of phagocytic cells upon activation by tuftsin, it became of interest to determine whether tuftsin has any effect on cell growth in complete RPMI 1640 medium with added FCS. Of equal interest is the possible stimulation of the rate of incorporation, during a short pulse, of labeled thymidine, uridine and leucine into DNA, RNA and protein, respectively, in the absence of growth, in RPMI lacking FCS.

This article shows that indeed the stimulation of the rate of growth of HL60 cells by 1 μg/ml of tuftsin and the stimulation of uridine and leucine incorporation by 0.1 μg/ml was substantial.

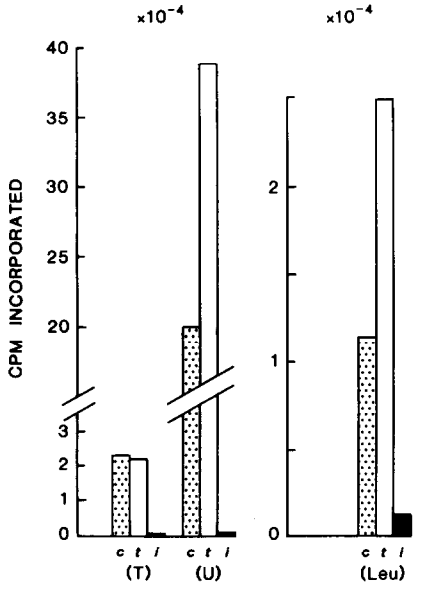

Fig.1. HL60 cells were grown in a basal medium consisting of RPMI 1640, 2 mM glutamine, 100 U penicillin and 100 μg of streptomycin/ml in 5% CO_2 at 37°C with added 10% FCS. Cells were washed with basal medium without FCS and allowed to equilibrate for 1 h at 37°C. To a sample containing 4×10^5 cells/ml, tuftsin (t) was added to 0.1 μg/ml. Control (c) received no tuftsin. After 1 h of tuftsin activation at 37°C, 1 ml of (c) cells and 1 ml of (t) cells were transferred each to a separate falcon dish 35 × 100 mm containing 10 μCi [^{3}H]leucine (Leu). A similar dish was prepared containing in addition 6 μM cycloheximide as inhibitor of leucine incorporation. After 30 min incubation, dishes were washed in cold PBS and washed twice in 7% trichloroacetic acid. Pellet was dissolved in 0.1 N NaOH and counted. The method used for the incorporation from 10 μCi [^{3}H]thymidine (T), 10 μCi [^{3}H]uridine (U) was exactly as for leucine except for the use of 6 μM actinomycin D and mitomycin as inhibitors of thymidine and uridine incorporation, respectively.

2. MATERIALS AND METHODS

HL60 human cell line (ATCC, Rockville, MD); RPMI 1640 culture medium (Tufts University School of Medicine, Boston, MA); FCS (Hy-Clone

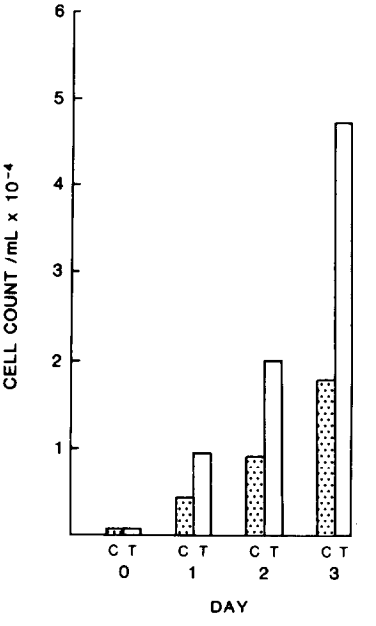

Fig.2. All growth experiments were done in RPMI 1640 complete medium as in fig.1, but with added 10% FCS. Aliquots of growing cells usually around 1×10^5, were transferred into flasks containing 100 ml of complete medium with and without tuftsin (t) 1 μg/ml. Since tuftsin is unstable in serum [26], it was added once daily to obtain a fresh concentration of 1 μg/ml. Flasks were incubated at 37°C in 5% CO_2. The pH was maintained by the addition of the necessary amount of fresh medium. All cells were counted each day. The number of cells are plotted against days of incubation.

Laboratories, Logan, UT); [^{3}H]thymidine (25 Ci/mmol), [^{3}H]uridine (50 Ci/mmol), [^{3}H]leucine (50 Ci/mmol) (Amersham, Arlington Heights, IL); mitomycin, actinomycin D and cycloheximide (Sigma, St. Louis, MO); glutamine, penicillin and streptomycin (Irvine Scientific, Santa Ana, CA).

Tuftsin was synthesized by the solid-phase method [22] and the use of TFMS for deblocking [23] protective groups, and in addition cleavage of the arginyl-resin bond [24].

3. RESULTS AND DISCUSSION

Fig.1 illustrates the results obtained in over 10 experiments. In each case, washed cells were equilibrated in RPMI 1640 for 1 h without FCS. Following this, a 30 min pulse was performed with 10 μCi each of [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]leucine in the presence and absence of 0.1 μg tuftsin/ml. This resulted in a substantial and reproducible tuftsin stimulation of incorporation of both [^{3}H]uridine and [^{3}H]leucine. However, [^{3}H]thymidine in this FCS-deficient medium showed no increased incorporation. This was expected as no growth occurs in the absence of FCS [25]. The presence of specific inhibitors for DNA, RNA and protein showed almost complete inhibition of incorporation.

Growth experiments with HL60 in RPMI 1640 with 10% FCS were carried out with and without 1.0 μg/ml of tuftsin. They were allowed to grow at 37°C in 5% CO_2 in air and an aliquot counted every 24 h. The pH was maintained by the addition of fresh medium. Because of the instability of tuftsin in the presence of serum, fresh tuftsin was added each day to replenish the level of 1 μg/ml of culture medium [26].

Fig.2 illustrates the growth in the absence and presence of tuftsin. The stimulation of growth of HL60 cells, in an otherwise complete medium [25], was particularly pronounced during the first 24 h of growth. It is possible that this is due to the internalization of the receptors following exposure to tuftsin; a process of down-regulation. The generation time of HL60 cells in this complete medium is approx. 24 h [27]. In the presence of tuftsin this was considerably reduced.

The mechanism of stimulation of growth by tuftsin is unknown. However, the stimulation of pinocytic activity by tuftsin [2,3] should be considered in that it may result in an increase in nutrient uptake that might influence the rate of growth, if indeed this is a rate limiting step. This does not seem to be a likely possibility.

ACKNOWLEDGEMENTS

This work was supported by the March of Dimes Birth Defects Foundation Grant no.1-556 and the American Cancer Society Grant no.RDP 32E.

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