Biochemical Pharmacology, Vol. 28, pp. 3131–3137

0006-2952/79/1015-3131 $02.00/0

ACTIVATION OF DNA METABOLISM IN T-CELLS BY BESTATIN *

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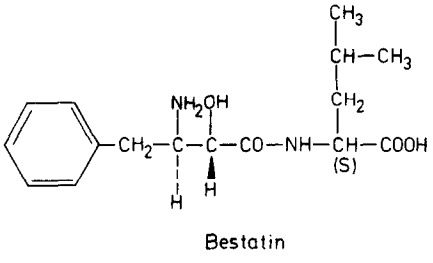
(*Received* 31 *January* 1979; *accepted* 30 *April* 1979)

Abstract—Bestatin, [(2*S*, 3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine, is a microbial product which selectively influences the DNA metabolism of lymphoid tissues *in vivo*. The present studies, using CBA/J mice, revealed that bestatin increases the incorporation rate of dThd into DNA in spleen, thymus and bone marrow, but not in liver, kidney, spinal cord and lung. The stimulation was found to be dose-and time-dependent, and it occurs only in T-cells, not in B-cells, from spleen and thymus. In addition, it is shown that bestatin causes a several-fold induction of DNA polymerase α only in T-cells from spleen and thymus, while the level in B-cells remains constant. Bone marrow cells respond to bestatin treatment with an increase of the DNA polymerase α activity and with an 8-fold induction of terminal deoxynucleotidyl transferase.

Numerous studies seem to indicate that profound disturbances in immune function may accompany human cancer (review: [1,2]). Because most of the compounds used as anticancer drugs cause strong immunosuppression in the patients [2], controlled experimental and clinical trials of combined immunotherapy and chemotherapy with cancer patients appear to be an urgent need. One of the most widely recommended forms of immunotherapy is the active immunotherapy with the following agents: bacillus Calmette Guérin (BCG), corynebacterium parvum, levamisole and some synthetic polynucleotides (survey: [2]). The mode of action of these compounds is known only to a small extent due to the fact that the mentioned preparations are chemically not well defined. Hence a biochemical elucidation of the effect of agents stimulating the immuncompetence of T- as well as of B-cells can only be performed if chemically well defined agents exhibiting an enhancement of T- or B-cell function are available. With the discovery of the thymic hormone, thymosin [3], it was possible to study the mode of action of an agent which increases the cell-mediated immunity in cancer patients [4] on the biochemical level [5].

With the discovery of enzymes present on the outer cell surface membrane of eukaryotic cells [6,7–8], the search for specific inhibitors of those enzymes was successful [9]. Among them bestatin, isolated from culture filtrate of *Streptomyces olivoreticuli* [10], was found to be a strong inhibitor of the cell surface associated aminopeptidase [11]. The chemical structure of this microbial product was determined as [(2*S*, 3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine [10] (Fig. 1). Bestatin was found to enhance cell-mediated immunity [12]. On the biochemical level, it is known



Bestatin

Fig. 1. Chemical structure of bestatin.

that bestatin stimulates polysome assembly in T-cell lymphoma (growing in suspension) [13] and causes induction of DNA polymerase α in the same cell culture system [14].

In the present experiments we have extended these observations and determined the ability of bestatin to activate *in vivo* (mice) DNA polymerase α and terminal deoxynucleotidyl transferase in T-cells from bone marrow, thymus and spleen.

MATERIALS AND METHODS

Compounds. The following materials were used: sterile nylon wool in LP-1 Leuko-Pak Leukocyte Filters from Fenwal Laboratories, Morton Grove, Fl, U.S.A.; [methyl-^{3}H]dThd (specific activity 2 Ci/mole), [^{3}H]dCTP (sp. act. 30 Ci/mmole) and [^{3}H]dGTP (sp. act. 12 Ci/mmole) from the Radiochemical Centre, Amersham, England; unlabeled deoxyribonucleoside triphosphates, poly dA from Boehringer-Mannheim, Tutzing, Germany.

Bestatin was isolated from a fermentation broth of *Streptomyces olivoreticuli* and determined to be chromatographically pure [15]. Herring sperm DNA was isolated according to Zahn *et al.* [16].

Mice. Male mice of inbred CBA/J were used at an age of 10–15 weeks; they were purchased from Ivanovas, Kisslegg, Germany.

* Supported by grants from the Akademie der Wissenschaften und der Literatur, Mainz and the Deutsche Forschungsgemeinschaft.

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In vivo incorporation of [^{3}H]dThd into DNA of various organs. If not mentioned otherwise, 15 hr after pretreatment with bestatin the animals received intraperitoneally 200 μCi [^{3}H]dThd (= 100 nmoles, dissolved in 0.5 ml saline). After 5 hr the animals were killed and the different organs were removed. Subsequently the organs were either dissociated into single cell suspensions or they were directly worked up to determine the acid insoluble radioactivity. In the latter case 50 mg of tissue was homogenized (Dounce potter S) in 5% trichloroacetic acid and processed as previously described [17]. The samples were counted in a Mark III scintillation counter; quenching correction was performed by use of an automatic program. The acid insoluble radioactivity of the single cell suspension was determined as previously described [18].

Cells. Minced thymus and spleen from mice was forced through a 40 nylon mesh screen to obtain a single cell suspension. T-cells were separated from B-cells and macrophages by passing the cell suspension through a nylon wool column [19]. The viabiltiy of the T-cells in the effluent was greater than 95%. B-cells and macrophages attached to the nylon fibers were obtained by shaking the nylon wool in a beaker containing medium; the viable cells (> 85%) were obtained by centrifugating them (400 *g*, 5 min, 2°) into a cushion consisting of undiluted horse serum. The suspension obtained contained approx 5% macrophages (as assessed by neutral red staining) and approx 95% B-cells; because of the high percentage of B-cells in the preparation, it is designated in the following as the B-cell fraction.

Bone marrow was flushed from femurs using a 1 ml syringe filled with saline and attached to a 25 gauge needle. The cells were transferred into warm (37°) Fisher's medium for leukemic cells, supplemented with 10% horse serum.

Viable cells excluding trypan blue were counted in a Coulter Counter.

Enzyme preparation. DNA polymerases α and β were extracted from 2×10^8 cells in 1 vol of a buffer containing 50 mM Tris–HCl (pH 7.6), 25 mM KCl, 5 mM $MgCl_2$ and 0.25 M sucrose. The cells were sonically disrupted (Branson sonicator with seven 5-sec bursts at setting 4). After centrifugation (1 hr, 2°, 150,000 *g*) the extract was dialyzed (5 hr, 2°) against 0.5 M NaCl, 50 mM Tris–HCl (pH 7.0) and 1 mM EDTA. A 0.5 ml aliquot of the dialyzed fraction was layered on a 5 ml 5–20% sucrose gradient in 0.5 M NaCl, 50 mM Tris–HCl (pH 7.0), 1 mM EDTA and 1 mM 2-mercaptoethanol. Centrifugation was carried out for 16 hr at 100,000 *g* and 2° [20, 21]. Fractions of 0.2 ml were collected from the top.

Terminal deoxynucleotidyl transferase was extracted from 10^9 cells in 0.25 M K-phosphate buffer (pH 7.5) containing 1 mM 2-mercaptoethanol. The cells were sonically dirupted (see above). The sonicates were centrifuged (1 hr, 2°, 150,000 *g*) and used for enzyme assays.

Enzyme assays. The assays for DNA polymerase α and β were performed as described [22]. Briefly, activated herring sperm DNA was used as template, the pH in the polymerase α mixture was adjusted to 7.2 and in the case of the β enzyme to pH 8.8, [^{3}H]dCTP with a sp. act. of 50 c.p.m./pmole, dATP, dGTP, dTTP and $MgCl_2$ were used. One unit of enzyme activity is defined as 1 nmole of radioactive deoxyribonucleotide incorporated per hr. The reaction mixture for determination of terminal deoxynucleotidyl transferase was essentially as previously described [23], using $d(pA)_{\overline{12-18}}$ as initia-

Table 1. Incorporation rate of [^{3}H]dThd into DNA of different organs in dependence on bestatin pretreatment

Organ	Bestatin pretreatment Days	Dose (mg/kg)	Incorporation rate (d.p.m./5 hr × 50 mg tissue)
Spleen	—	0	12,570
	1	5	28,360
	1	50	34,690
	3	5	65,120
	3	50	69,170
	5	5	85,950
	5	50	83,390
Thymus	—	0	21,400
	1	5	43,450
	1	50	57,280
	5	5	64,800
	5	50	78,350
Liver	—	0	4790
	5	50	5640
Kidney	—	0	3260
	5	50	3570
Spinal cord	—	0	1900
	5	50	2200
Lung	—	0	7420
	5	50	7880

Fifteen hours after the last bestatin injection [^{3}H]dThd was administered for 5 hr. The acid insoluble radioactivity was then determined. Values represent means of 10 parallel experiments. The S.D. does not exceed 8%.

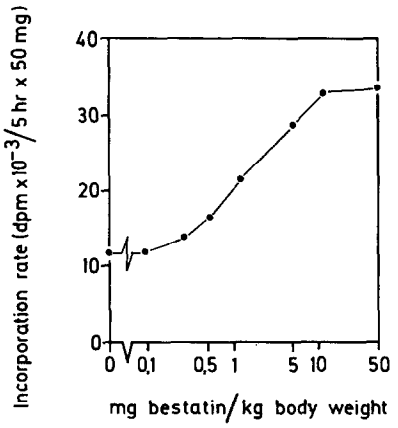

Fig. 2. Effect of bestatin on the incorporation rate of [^{3}H]dThd into DNA of spleen. A single dose of bestatin of different amounts was administered. For further data see legend to Table 1.

tor, K-cacodylate (pH 7.2), [^{3}H]dGTP with a sp. act. of 500 c.p.m./pmole and $MgCl_2$. After incubation, acid insoluble radioactivity was determined on GF/C filters [24,25]. One unit of enzyme is equal to an incorporation of 1 nmole of the deoxyribonucleotide during a 1 hr incubation period.

Miscellaneous. Native DNA was activated according to Aposhian *et al.* [26] with the characteristics described earlier [21]. Oligo $d(pA)_{\overline{12-18}}$ was prepared in this laboratory according to Bollum [27].

RESULTS

In the following study, the influence of bestatin on DNA-synthesis *in vivo* (CBA/J mice) was traced by measuring the incorporation rate of [^{3}H]dThd into DNA, by determination of the level of the key enzyme for DNA replication (the DNA polymerase α), and by examination of the T-cell-specific DNA-synthesizing enzyme (terminal deoxyribonucleotidyl transferase), present in bone marrow of adult animals [28].

Effect of bestatin pretreatment on the incorporation rate of [^{3}H]dThd in lymphoid- and non-lymphoid tissues. Pretreatment of the animals with bestatin stimulates the incorporation rate of [^{3}H]dThd exclusively in lymphoid tissues such as spleen and thymus, while in non-lymphoid tissues (liver, kidney, spinal cord and lung) no change is observed (Table 1). Two doses were applied daily (either 5 or 50 mg/kg). It was found that the stimulation of the incorporation rate in lymphoid tissues is only slightly enhanced if the higher dose of 50 mg/kg is administered. In a dose–response experiment with spleen (Fig. 2) is was found that doses higher than 0.5 mg bestatin/kg body wt cause an increase of [^{3}H]dThd incorporation. The stimulation of the incorporation rate is 3-fold, from 12,500 determined in untreated animals to 34,700 d.p.m. per 50 mg tissue under the labelling conditions used. At higher bestatin concentrations (500 mg/kg) no further increase occurs (data not shown).

The incorporation rate of [^{3}H]dThd into DNA of bone marrow cells is also dramatically enhanced (Fig. 3). In the absence of bestatin only an incorporation rate of 32,000 d.p.m. is observed, while after a bestatin pretreatment an increase to 580,000 d.p.m./5 hr × 10^8 cells occurs. Figure 4 shows the kinetics of the increase of the incorporation rate using 5 mg bestatin/kg. Again it is observed that in non-lymphoid tissue (liver), no change occurs. In the case of spleen and thymus the induction *in vivo* is rapid; within 5 hr an increase of the incorporation rate is already evident. The maximum is reached 15 hr after the bestatin injection; at this time point the stimulation is 2.5-fold in spleen and 2.4-fold in thymus.

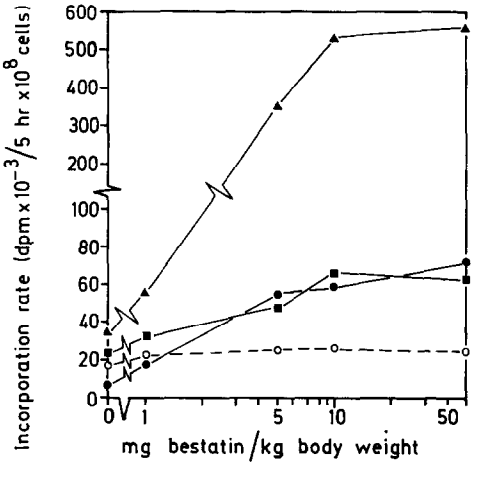

Fig. 3. Alteration of [^{3}H]dThd incorporation rate into DNA of T- and B-cells in relation to bestatin dose. Fifteen hours after a single bestatin administration the animals were injected with [^{3}H]dThd. Five hours later spleen, thymus and bone marrow were taken and these tissues were dissociated into single cells. After fractionation into T- and B-cells the acid insoluble radioactivity was determined. (●——●), T- and (○- - -○), B-cells from spleen; (■——■), T-cells from thymus and (▲——▲), bone marrow cells. Note the change of the scale on the ordinate. Values represent means of 10 parallel experiments. The S.D. does not exceed 8%.

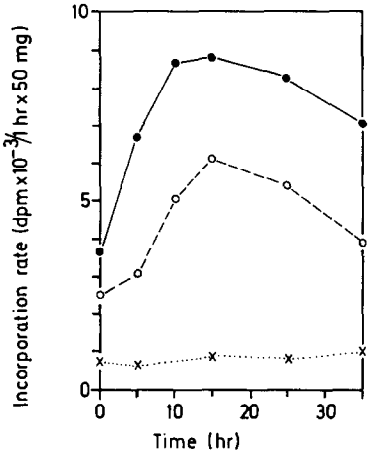

Fig. 4. Time dependence of the amount of [^{3}H]dThd incorporation rate into DNA by bestatin. The animals were treated with a single dose of bestatin (5 mg bestatin/kg). At the indicated intervals 200 μCi [^{3}H]dThd (= 100 nmoles) were injected. One hour later tissue samples (50 mg) from thymus (●) spleen (○) and liver (×) were taken and the acid insoluble radioactivity was determined. Values represent means of 5 parallel experiments. The S.D. does not exceed 15%.

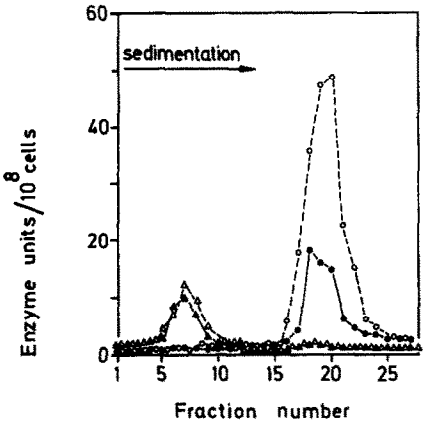

Fig. 5. DNA polymerase α and β activities in extracts from T-cells isolated from spleen of mice which received either 0 or 50 mg bestatin/kg. Fifteen hours after the bestatin injection the spleens were removed, dissociated into single cells and subsequently fractionated on nylon fibers to obtain T-cells. After extraction the DNA polymerase α was separated from the β enzyme by sucrose density gradient centrifugation. ●, α Enzyme from untreated mice; ○, α enzyme from bestatin-treated animals; ▲, β enzyme from untreated animals; △, β enzyme from bestatin-treated animals.

Influence of bestatin pretreatment on the incorporation rate of [^{3}H]dThd into fractionated lymphocytes. From the data mentioned above it is evident that a bestatin-caused stimulation of the [^{3}H]dThd incorporation rate takes place only in lymphoid tissues. Because these tissues consist primarily of T- and B-cells (of different maturation states) [29] it seemed promising to check in which population of lymphocytes the alteration of the incorporation rate occurs. Spleen and thymus cells were fractionated by the nylon fiber technique into T- and B-cells. When this method was applied 95% of the total thymus cells were recovered in the T-cell fraction, while from the spleen cell suspension only 45% of the total cells were counted in the T-cell fraction. In the case of bone marrow cells, the nylon fiber technique failed to separate T- from B-cells; therefore only the overall incorporation of the different cell population could be determined.

The results illustrated in Fig. 3 show that it is the T-cells which respond to bestatin. This conclusion must be drawn especially from the data obtained with T- and B-cells from spleen; while the incorporation rate of the B-cells is not influenced at all, the incorporation rate of T-cells increases from 8500 d.p.m. (at 0 mg bestatin/kg) to 72,000 d.p.m. (at 50 mg bestatin/kg). The incorporation in the T-cells from thymus is stimulated 2.7-fold (comparing untreated animals with mice receiving 50 mg bestatin/kg). This latter value corresponds well with that determined in unfractionated thymus tissue (see above), indicating that approximately all thymus cells (which are of the T-cell type; [29]) are stimulated by bestatin. The most dramatic increase of the incorporation rate in response to bestatin is observed in bone marrow cells; a stimulation from 32,000 d.p.m. (0 mg bestatin/kg) to 580,000 d.p.m./10^8 cells (50 mg bestatin/kg) was determined.

In vivo induction of DNA polymerase α in lymphocytes by bestatin. The two DNA polymerases α and β were extracted and separated by sucrose density gradient centrifugation; as an example the activity profiles of the two isoenzymes of spleen T-cells from untreated as well as from bestatin-treated mice are shown in Fig. 5. The data plotted in this figure show that the activity of DNA polymerase β (fractions 5–9) remains unaltered, while in the case of DNA polymerase α (fractions 17–22) the activity in T-cells from untreated spleens is markedly lower than the one determined in T-cells from spleens of bestatin-treated animals.

Table 2 summarizes the levels of the two DNA polymerases of lymphocytes from spleen, thymus and bone marrow after *in vivo* administration of bestatin. In the case of spleen and thymus the lymphocytes were separated into T- and B-cells by the nylon fiber technique. The first conclusion drawn from the data shown is that the DNA polymerase β activity in the cells from

Table 2. DNA polymerase α and β levels of lymphocytes isolated from spleen, thymus and bone marrow.

Organ	Bestatin pretreatment		DNA polymerase α activity (units/10^8 cells)			DNA polymerase β activity (units/10^8 cells)		
	Days	Dose (mg/kg)	T-cells	B-cells	Unfractionated	T-cells	B-cells	Unfractionated
Spleen	—	0	75	84		32	55	
	1	5	193	89		42	48	
	1	50	225	72		35	49	
	5	5	221	89		44	56	
	5	50	205	83		47	44	
Thymus	—	0	42			25		
	1	5	89			31		
	1	50	92			22		
	5	5	94			28		
	5	50	107			24		
Bone marrow	—	0			81			38
	1	5			107			44
	1	50			115			46
	5	5			109			33
	5	50			133			42

The animals were treated with bestatin as indicated. Fifteen hours later the mice were killed, and the organs taken and dissociated into single cells. In the case of spleen and thymus, lymphocytes were fractionated into T- and B-cells. The cells were extracted and the DNA polymerases were isolated as shown in Fig. 5. The values represent means of 5 parallel experiments. The S.D. does not exceed 10%.

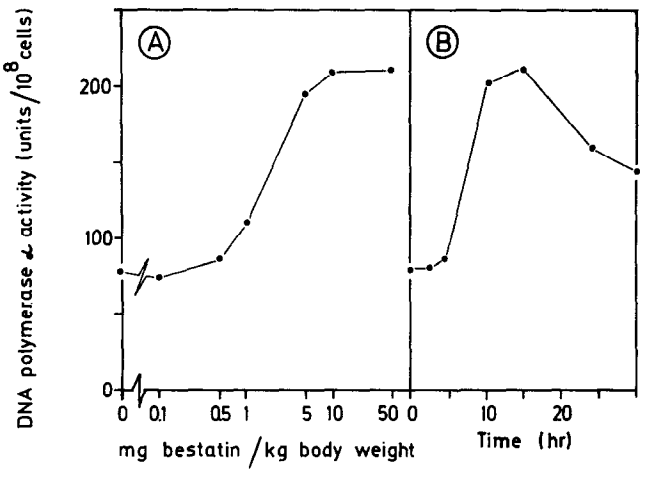

Fig. 6. Activation of DNA polymerase α *in vivo* in T-cells from spleen as functions of bestatin concentration (A) and time (B). (A) Mice were pretreated with different amounts of bestatin; 15 hr later spleens were taken and DNA polymerase α activity was determined in their T-cells. (B) Mice were pretreated with 5 mg bestatin/kg; at the indicated intervals spleens were taken and DNA polymerase α level in T-cells was measured. Values represent means of 5 parallel experiments. The S.D. does not exceed 10%.

the various organs remains almost constant even after prolonged bestatin administration. Only the base level of the enzyme shows organ-specific differences; it is lowest in lymphocytes from thymus and somewhat higher in the cells from spleen and bone marrow. The second result from this series of experiments is the finding that the DNA polymerase α activity in B-cells from spleen is independent of the pretreatment of the animals with bestatin.

The third and most important finding, documented in Table 2, is the fact that in T-cells from both spleen and thymus the DNA polymerase α is induced in response to an *in vivo* pretreatment of animals with bestatin. In the case of T-cells from spleen the bestatin-caused induction is 2.5-fold higher after a single administration of bestatin and 2.8-fold higher after an injection of bestatin for 5 days.

In addition, it is obvious that, under the conditions used for these experiments, the activation of the enzyme occurs irrespective of the dose of bestatin (5 or 50 mg/kg) used. The DNA polymerase α is also activated in T-cells from thymus after *in vivo* adminstration of the compound. The amount of enzyme determined after a single administration is 2.1-fold, and after five injections 2.4-fold higher compared with the levels present in control T-cells (from untreated animals). Bone marrow cells which were not fractionated show an activation of the DNA polymerase α after pretreatment of the animals with bestatin (Table 2); the extent of activation was found to be 1.3–1.6-fold, depending on the dosage and the duration of treatment.

In the case of T-cells from spleen the kinetics of DNA polymerase α activation was studied in more detail. The extent of activation is both dose- (Fig. 6A) and time-dependent (Fig. 6B). After a pretreatment of the animals with bestatin at a dosage higher than 0.5 mg/kg an activation of the enzyme is observed after 15 hr (Fig. 6A). Using a dose of 5 mg bestatin/kg the DNA polymerase α activation begins around 5 hr after bestatin treatment; after 15 hr the cells are fully activated (Fig. 6B).

Distribution of terminal deoxynucleotidyl transferase activity in lymphoid tissue. The determination of terminal deoxynucleotidyl transferase, especially in bone marrow cells, can be performed only in crude extract for practical reasons (low amount of cell material). Therefore it is crucial to use a specific assay system for the determination of the activity of this enzyme. As shown by Coleman [30] only oligo $d(pA)_{12-18}$ is an absolute specific initiator for this non-template directed DNA polymerase and initiator concentrations of approx 10 μM are appropriate. These prerequisites are fulfilled in the present study.

The experiments revealed that in fractionated spleen as well as thymus lymphocytes no terminal deoxynucleotidyl transferase activity could be determined (data not shown). Only in bone marrow cells could this enzyme be detected. As first described by Bollum's group [31], this enzyme is present in a low amount in bone marrow cells from healthy adults and is found in a

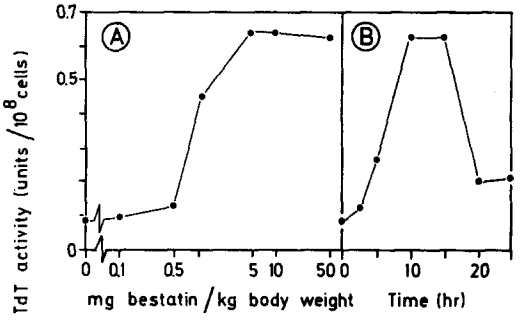

Fig. 7. Activation of terminal deoxyribonucleotidyl transferase *in vivo* in bone marrow cells by bestatin as a function of bestatin concentration (A) and time (B). (A) Mice were pretreated with different doses of bestatin; 15 hr later bone marrow cells were isolated and determined for enzyme activity. (B) Animals were pretreated with 5 mg bestatin/kg; at the time indicated the enzyme activity was determined. TdT, terminal deoxyribonucleotidyl transferase. Values represent means of 5 parallel experiments. The S.D. does not exceed 10%.

higher concentration in bone marrow cells from patients with acute myelomonocytic leukemia. Our experiment, using bone marrow from untreated mice, revealed a base level of terminal deoxynucleotidyl transferase activity of 0.09 units/10^8 cells. After treatment of mice with bestatin, at a dosage higher than 0.5 mg/kg, this enzyme is activated in bone marrow cells (Fig. 7A); the cells reach the highest level (0.64 units/10^8 cells) after administration of 5 mg/kg. In this series of experiments the enzyme activity was determined 15 hr after bestatin injection.

The activation of terminal deoxyribonucleotidyl transferase in bone marrow cells in response to bestatin treatment *in vivo* is is time-dependent (Fig. 7B). After administration of 5 mg bestatin/kg the period until the onset of the detectable increase of enzyme activity is around 2.5 hr. The cells are fully activated 10 hr after the administration of bestatin; at this time the extent of induction is 7.8-fold. 15 hr after the bestatin administration the enzyme activity decreases and reaches a level which is only slightly higher than the base level.

DISCUSSION

One aim of future studies in developing new anti tumor agents must be the search for immune enhancers. In the present study it is shown that the microbial product bestatin causes *in vivo* an activation of T-cells in spleen and thymus and of lymphocytes in bone marrow. As parameters for the activation the increase of dThd incorporation into DNA as well as the induction of DNA polymerase α and terminal deoxynucleotidyl transferase have been chosen.

Although dThd incorporation studies probably reveal no absolute values for the extent of DNA synthesis, because possible alterations of the dThd pool in response to bestatin treatment are not known, they still provide us with at least relative values for the rate of DNA synthesis. The second parameter used, the determination of the activity of the two DNA polymerizing enzymes, directly reflects the physiological state of the cellular DNA metabolism: the level of DNA polymerase α activity is closely correlated with the extent of DNA synthesis *in vivo* and *in vitro* [32] and the amount of terminal deoxynucleotidyl transferase obviously reflects the maturation state of T-cells in bone marrow [5].

Our *in vivo* experiments clearly indicate that bestatin selectively stimulates the DNA-synthesizing systems in lympoid tissue, in particular in the T-cells from spleen and thymus, as well as in lymphocytes from bone marrow. In these cells both the incorporation rate of dThd into DNA and the level of DNA polymerase α increased dramatically. This result can, at the present stage of knowledge, only be interpreted by the assumption that bestatin stimulates the T-cell proliferation in the mentioned organs. Preliminary animal studies revealed an increase in the percentage of T-cells in the circulating blood after bestatin administration (B. Fukushima, Teikyo University, Tokyo, unpublished results). This could mean that bestatin stimulates proliferation of T-cells in the lymphoid tissue, which are subsequently released into the blood.

The fact that bestatin causes not only a strong increase in the dThd incorporation rate and a stimulation of the DNA polymerase α after *in vivo* application, but also an activation of the T-cell specific terminal deoxynucleotidyl transferase seems to be important. In the light of the results that, first, this T-cell specific enzyme can be detected in only 1.8% of adult rat bone marrow cells [33] and second, that 13% of the total bone marrow cells are of the T-cell type [29], the conclusion has been drawn that only the T-precursor cells contain the transferase (A.L. Goldstein, George Washington University School of Medicine, Washington, personal communication). From our findings demonstrating that after *in vivo* administration of bestatin the terminal deoxynucleotidyl transferase is activated up to 8-fold, it seems probable that in the bone marrow of the treated animals the number of T-precursor cells is enlarged several-fold. In addition, our data show that bestatin does not cause a terminal deoxynucleotidyl transferase expression in T-cells from thymus. In this organ the enzyme is present in large amounts only during embryonic development, and its level decreases rapidly in young animals [34].

It is too early to formulate the mode of action of bestatin in T-cells. However, it is already established that: (a) the microbial product binds to cells with a density of 2.2×10^7 molecules/cell; (b) it inhibits a specific cell surface protease [10]; (c) it stimulates DNA and RNA synthesis in T-cells both *in vivo* and *in vitro* [13, 14, this paper]; and (d) it causes the expression of terminal deoxynucleotidyl transferase in bone marrow cells (this paper).

Because bestatin enhances cell-mediated immunity in a cell culture system [12] and it is a non-toxic substance [35], its antitumor effect was tested both in animal systems [35] and in humans [36]. The outcome of these trials revealed that the compound causes a significant regression of some animal tumors and a favorable response in human cancer patients.

In contrast to other immunoadjuvants belonging to the class of lectins which bind specific cell surface oligosaccharides and thus cause an alteration of the stereochemical structure of these non-enzymic membrane macromolecules, bestatin specifically inhibits the activity of a cell surface bound enzyme. While bestatin causes an activation of terminal deoxynucleotidyl transferase no lectin provided with such a function is known.

Acknowledgements—The authors would like to thank Prof. H. Wagner, Prof. M. Röllinghoff and Prof. E. M. Lemmel (Institut für Medizinische Mikrobiologie, Universität Mainz) for their advice. R. Steffen provided excellent technical assistance. W. E. G. M. wishes to thank Prof. F. J. Bollum (University of Health Services, Bethesda, MD) for introducing him to the preparation procedures for the three DNA-synthesizing enzymes and oligodeoxynucleotides during the tenure of a Yamagiwa-Yoshida Memorial International Cancer Study Grant awarded to him.

REFERENCES

1. J. Harris and D. Copeland, *Ann. N.Y. Acad. Sci.* **230**, 56 (1974).
2. C. M. Haskell, *A. Rev. Pharmac. Tox.* **17**, 179 (1977).
3. A. L. Goldstein, A. Guha, M. M. Zatz, M. A. Hardy and A. White, *Proc. Natn. Acad. Sci. U.S.A.* **69**, 1800 (1972).
4. M. A. Hardy, A. M. Dattner, D. K. Sarker, J. A. Stoffer and N. Friedman, *J. med. Chem.* **37**, 98 (1976).

5. N. H. Pazmino, J. N. Ihle and A. L. Goldstein, *J. exp. Med.* **147**, 708 (1978).
6. T. Aoyagi, H. Suda, M. Nagai, K. Ogawa, J. Suzuki, T. Takeuchi and H. Umezawa, *Biochim. biophys. Acta* **452**, 131 (1976).
7. W. E. G. Müller, J. Arendes, B. Kurelec, R. K. Zahn and I. Müller, *J. biol. Chem.* **252**, 3836 (1977).
8. W. E. G. Müller, R. K. Zahn, B. Kurelec, I. Müller, G. Uhlenbruck and P. Vaith, *J. biol. Chem.* **254**, 1280 (1979).
9. H. Umezawa, *Enzyme Inhibitors of Microbial Origin*, University of Tokyo Press, Tokyo (1972).
10. R. Nishizawa, T. Saino, T. Takita, H. Suda, T. Aoyagi and H. Umezawa, *J. med. Chem.* **20**, 510 (1977).
11. H. Suda, T. Aoyagi, T. Takeuchi and H. Umezawa, *Archs biochem. Biophys.* **177**, 196 (1976).
12. M. Saito, T. Aoyagi, H. Umezawa and Y. Nagai, *Biochem. biophys. Res. Commun.* **76**, 526 (1977).
13. W. E. G. Müller, R. K. Zahn, A. Maidhof and H. Umezawa, *J. Antibiotics*, in press.
14. W. E. G. Müller, in *Effects of Drugs on the Cell Nucleus* (Ed. H. Busch), Academic Press, London, in press.
15. H. Umezawa, T. Aoyagi, H. Suda, M. Hamada and T. Takeuchi, *J. Antibiotics* **29**, 97 (1976).
16. R. K. Zahn, E. Tiesler, A. R. Kleinschmidt and D. Lang, *Biochem. Z.* **336**, 281 (1962).
17. H. N. Munro and A. Fleck, *Analyst* **91**, 78 (1966).
18. W. Plunkett and S. S. Cohen, *Cancer Res.* **35**, 415 (1975).
19. M. H. Julius, E. Simpson and L. A. Herzenberg, *Eur. J. Immun.* **3**, 645 (1973).
20. M. S. Coleman, J. J. Hutton and F. Bollum, *Blood* **44**, 19 (1974).
21. W. E. G. Müller, A. Maidhof, R. K. Zahn and W. M. Shannon, *Cancer Res.* **37**, 2282 (1977).
22. W. E. G. Müller, R. K. Zahn and D. Falke, *Virology* **84**, 320 (1978).
23. W. E. G. Müller, R. K. Zahn and J. Arendes, *FEBS Lett.* **94**, 47 (1978).
24. F. J. Bollum, in *Procedures in Nucleic Acid Research* (Eds G. L. Cantoni and D. R. Davies), Vol. 1, p. 296. Harper & Row (1967).
25. W. E. G. Müller, H. C. Schröder, J. Arendes, R. Steffen, R. K. Zahn and K. Dose, *Eur. J. Biochem.* **76**, 531 (1977).
26. H. W. Aposhian and A. Kornberg, *J. biol. Chem.* **237**, 519 (1962).
27. F. J. Bollum, in *Procedures in Nucleic Acid Research* (Eds G. L. Cantoni and D. R. Davies), Vol. 1, p. 577. Harper & Row (1967).
28. F. J. Bollum, *Fedn. Proc. Fedn Am. Socs. exp. Biol.* **34**, 494 (1975).
29. I. Goldschneider and D. D. McGregor, *J. exp. Med.* **138**, 1443 (1973).
30. M. S. Coleman, *Nucleic Acids Res.* **4**, 4305 (1977).
31. M. S. Coleman, J. J. Hutton, P. de Simone and F. J. Bollum, *Proc. natm. Acad. Sci. U.S.A.* **71**, 4404 (1974).
32. F. J. Bollum, *Prog. Nucleic Acid. Res. molec. Biol.* **15**, 109 (1975).
33. I. Goldschneider, K. E. Gregoire, R. W. Barton and F. J. Bollum, *Proc. natn. Acad. Sci., U.S.A.* **74**, 734 (1977).
34. L. M. S. Chang, *Biochem. biophys. Res. Commun.* **44**, 124 (1971).
35. H. Umezawa, *Acta biol. med. germ.* **36**, 1899 (1977).
36. T. Ichikawa, I. Hirokawa and K. Isurugi, *Effect of a New Immunomodulator, Bestatin, in the Treatment of Urogenital Malignancies.* XII*th International Congress of Cancer, Buenos Aires* (1978).