Phosphoribosylamidotransferase: Regulation of Activity in Virus-Induced Murine Leukemia by Purine Nucleotides

Abstract. *Infection with Friend leukemia virus causes a marked increase in the activity of splenic phosphoribosylamidotransferase in mice. Intraperitoneal injection of purine nucleotides and their free bases inhibits this enzyme. This is the first example of the control of phosphoribosylamidostransferase in vivo in the mammalian system as well as in virus-induced leukemia. Experiments in vitro support the findings in vivo.*

Cellular metabolism and regulation of cellular growth are profoundly altered by viral infection. In the early changes that occur after viral infection, there is a marked increase in the enzymes associated with the synthesis of RNA and DNA within the cell. During experimental leukemia due to infection with FLV (*1*), in the spleens of infected animals there is an increase in the activity of a number of enzymes contributing to the synthesis of RNA (*2*). Because the increase in both RNA and DNA depends upon the availability of purines and pyrimidines, it seemed important to investigate the activity and the factors which control the first enzyme in purine synthesis in the spleens of FLV-infected mice. This enzyme, ribosylamine-5′-phosphate : pyrophosphate phosphoribosyltransferase (glutamate-amidating) (E.C. 2.4.2.14) (PRPP amidotransferase), is considered the key enzyme in purine synthesis because it catalyzes the first and irreversible step of the biosynthetic pathway. Studies in vitro of crude cell-free extracts derived from pigeon livers have shown the activity of this enzyme to be subject to feedback control by purine ribonucleotides (*3*). Decreased sensitivity to inhibitors occurs to various extents during purification. Purified enzyme preparations obtained from chicken liver are not subject to feedback inhibition (*4*). Studies on the mammalian system have been limited to the observation that partially purified enzyme from rat liver is subject to feedback control in vitro by end products of the pathway (*3*).

Studies performed in vivo are compatible with the concept of feedback inhibition of the biosynthetic pathway. In that such studies measure the incorporation of either glycine or formate into end product, they bypass the first step in synthesis which is catalyzed by PRPP amidotransferase, and therefore they fail to provide direct evidence for altered activity of this key enzyme.

We report on the relation between the development of FLV infection and changes in enzyme activity, and, in addition, on the mode of operation of the enzyme.

Swiss mice were infected with FLV by intraperitoneal injection of 0.2 ml of cell-free supernatant or filtrate of homogenate from leukemic spleens. Mice were approximately 6 to 8 weeks old at the time of infection. Infection with FLV caused splenic enlargement (*5*). The spleens of these animals were removed, homogenized in chilled 0.1*M* ammonium citrate buffer (*p*H 5.0), and centrifuged for 1 hour at 100,000*g* at 4°C. The PRPP amidotransferase activity was assayed as described by Nierlich and Magasanik (*6*). In this assay, the second enzyme of the pathway, PRG synthetase [5′-phosphoribosylamine : glycine ligase (ADP, E.C. 6.3.1.3)], is added in excess (*7*). With this assay, PRPP amidotransferase activity was not detectable in spleens of normal mice. In spleens of infected mice of the same strain, age, and sex, PRPP amidotransferase began to appear on the 4th day after infection and continued to rise during the following 6 to 9 days. Peak activity of the enzyme occurred approximately 1 week after infection, coinciding with the time at which there is appreciable infiltration of the spleens by neoplastic cells characteristic of FLV. Enzyme activity gradually decreased over the next 2 weeks and became quite low during the 3rd and 4th weeks of the disease. During the first 10 days after infection, spleen weight increased from 0.1 to 2.0 g, and some spleens weighed as much as 3 g by the end of 3 weeks (Fig. 1).

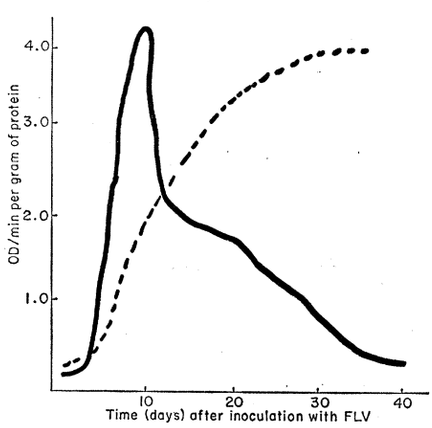

Fig. 1. The PRPP amidotransferase activity during development of Friend leukemia. Solid line, specific activity (O.D./min per gram of protein); dashed line, spleen weight (grams). Six assays were made.

During the time of peak enzyme activity, usually between the 5th and 8th day after infection, the response of PRPP amidotransferase to inhibition in vivo by end products of the pathway was studied. When 70 μmole of adenine, AMP, ADP, ATP, guanine, GMP, or GTP was injected intraperitoneally into leukemic mice (20 g), the residual specific activity in the spleen was 26, 55, 40, 57, 35, 47, and 50 percent, respectively. These determinations were made 1 hour later, at the time when the animals were killed. The response to feedback inhibition occurs within 20 minutes after the injection of adenine and lasts for at least 2 hours. This is the first demonstration that the administration of purines in vivo could influence the activity of the first enzyme in the purine biosynthetic pathway.

Incubation in vitro of these compounds with cell-free extracts from the spleens of leukemic mice support the findings in vivo. All the aminopurines tested—adenine, AMP, ADP, ATP, as well as guanine, GMP, and GTP—caused inhibition of the activity of PRPP amidotransferase. Guanine and GMP proved to be less inhibitory than adenine and AMP (Table 1). In view of the fact that the crude extract presumably contains adenine and guanine phosphoribosyltransferases which catalyze the conversion of adenine as well as guanine to the corresponding ribotides, it is reasonable to assume that free bases exert feedback control by means of the ribotides (*3*).

The PRPP amidotransferase activity which appears as a result of infection with FLV is thus subject to feedback control. This regulation of enzyme activity can be demonstrated after the injection of purines and purine ribonucleotides into the intact animal, as well as by incubation of the inhibitors with cell-free enzyme preparations. The correlation of increased enzyme activity with progressive infiltration of the spleen with neoplastic cells suggests that the increase in purine synthesis

Table 1. Inhibition in vitro of PRPP amidotransferase by adenine, guanine, AMP, and GMP. The incubation mixture contained 0.10 ml of crude enzyme extract, 20 μmole of glutamine, 0.35 μmole of $MgCl_2$, 0.28 μmole of ATP, and 30 μmole of tris buffer (*p*H 8.9). The incubations were carried out for 5 minutes at 37°C, and the reaction was stopped with 15 μmole of ethylenediaminetetraacetate. Residual activity expressed as percentage of control without inhibitor.

Substance	Concentration (mmole/liter)	Residual activity (%)
None	0.0	100
Adenine	0.5	70
Adenine	1.0	30
Adenine	2.0	20
Adenine	4.0	0
Guanine	0.5	70
Guanine	1.0	63
Guanine	4.0	66
AMP	1.0	50
AMP	4.0	30
AMP	8.0	15
GMP	1.0	90
GMP	4.0	60
GMP	8.0	60

de novo takes place in the cells that populate this organ during the disease. However, the reason for decrease in enzyme activity during the last phase of the disease is not entirely clear, since terminally the splenic architecture is almost completely disrupted by neoplastic cells. Several of the other enzymes studied (thymidylate synthetase, dihydrofolic reductase, and the formate-activating enzymes) have also shown a similar pattern of activity after viral infection (*2*).

GABRIELLE H. REEM
Department of Pharmacology, New York University Medical Center, New York

CHARLOTTE FRIEND
Department of Experimental Cellular Biology, Mount Sinai School of Medicine, New York

References and Notes

1. Abbreviations: FLV, Friend leukemia virus; AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; GTP, guanosine triphosphate; and GMP, guanosine monophosphate.
2. R. Silber, R. P. Cox, J. R. Haddad, C. Friend, *Cancer Res.* **24**, 1892 (1964).
3. C. T. Caskey, D. M. Ashton, J. B. Wyngaarden, *J. Biol. Chem.* **239**, 2570 (1964).
4. S. C. Hartman, *ibid.* **238**, 3024 (1963).
5. C. Friend, *J. Exp. Med.* **105**, 307 (1957).
6. D. P. Nierlich and B. Magasanik, *J. Biol. Chem.* **240**, 358 (1965).
7. J. E. Seegmiller, F. M. Rosenbloom, W. N. Kelly, *Science* **155**, 1682 (1967).
8. We thank Dr. S. Hartman for providing PRG synthetase, and Mrs. Myrna Zaret and Mrs. Hedva Baromi for technical assistance. Partially supported by the American Cancer Society (grant IN-14H), NCI (grant CA-10000), and the Leukemia Society.

22 June 1967

Absorption of Intact Protein Molecules across the Pulmonary Air-Tissue Barrier

Abstract. *The majority of heterologous serum albumin and globulin molecules introduced into the pulmonary alveoli of dogs are absorbed into the circulatory system antigenically intact. This function of the alveoli has both physiologic and pathologic importance.*

The aim of the experiments reported here was to determine to what extent protein molecules introduced into the alveoli can be absorbed intact. Heterologous serum protein was labeled with iodine-131, and the quantity in the blood was measured after precipitation with specific antibodies. Complete balance studies, however, proved impractical.

The experiments were carried out on 15 mongrel dogs of either sex weighing between 9 and 26 kg. Four of these animals were used to determine the specificity of the antiserums as well as the zone of equivalence. Data obtained from these animals are not included in this report. In ten dogs, 5 ml of human serum containing a tracer quantity of I^{131}-labeled human serum albumin or human γ-globulin (*1*) was instilled into the lungs (*2*). Blood samples were obtained at regular intervals from 15 minutes to 7 days after instillation or when the animal was killed. At death, the amount of the instilled material remaining in the lungs was also determined. A small quantity (2 to 4 ml) of each blood sample was heparinized and set aside for determination of the total radioactivity in the blood. The remainder was allowed to clot, and the serum was collected for the antibody studies.

Quantitative immune precipitation of the labeled protein in these serums was carried out with specific antiserums, usually in duplicate, after determination of the zone of equivalence. Goat antiserum to human serum albumin, as well as specific antiserums against human serum γ-globulin, was used (*3*). The formation of the antigen-antibody complexes was permitted to proceed at 4°C over a period of 4 days. After centrifugation of the samples at 10,000*g* for 30 minutes, the radioactivity of the supernatant and precipitate was measured with a Picker crystal-scintillation counter (counting efficiency approximately 45 percent).

One animal (K) was injected intravenously with 5 ml of human serum containing I^{131}-labeled albumin, and percentages of antibody-precipitate radioactivity were determined at intervals thereafter.

The stock solutions of the I^{131}-labeled albumin and γ-globulin, as well as the material instilled (mixture of serum with isotope-labeled albumin or globulin) into the lung, were subjected to precipitation with antibody. Over 96 percent of the radioactivity of these stock solutions and of the instilled material precipitated with the specific antiserums. Specificity of the antiserums was demonstrated by the absence of precipitins when serums containing I^{131}-labeled albumin were mixed under standard conditions with antiserum to human globulin, and vice versa.

To study absorption of human serum proteins from the canine lung, human serum containing I^{131}-labeled albumin was instilled into the lungs of six dogs. Three of these (A to C) were killed at 24, 48, and 72 hours, respectively. The percentages of radioactivity in the blood precipitable at these times with antibody to human serum albumin are recorded in Table 1 on the basis of triplicate determinations. Blood samples were obtained at regular intervals from the three other dogs (animals H to J), and the radioactivity precipitable with antibody was determined (Table 2). As much as 97 percent of the isotope in the blood remained attached to a protein molecule that could be precipitated with antibody to human serum albumin. Variation in the percentage of precipitable isotope was not great. A slight drop from the average of more than 90 percent was found 12 to 48 hours after the experiment was begun. Over 80 percent of the isotope which was not precipitated by the specific antibody was precipitable with 3.5 percent trichloroacetic acid. There was no radioactivity in the erythrocytes. Over 98 percent of the isotope remained in solution when serums obtained at various intervals after instillation were mixed with antiserums to human γ-globulin.

The results of the experiments with absorbed I^{131}-labeled γ-globulin (animals D to G) were more difficult to interpret in that there was a consistent