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Nucleosides and Lymphocytes—An Overview (42116)

PHYLLIS R. STRAUSS,*[,1] J. FRANK HENDERSON,† AND MICHAEL G. GOODMAN‡

Department of Biology, Northeastern University, Boston, Massachusetts; †McEachern Laboratory and Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada; ‡Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Overview. In recent years nucleoside biochemistry and lymphocyte physiology have been brought together in a number of ways. Lymphocytes are particularly sensitive to the cytotoxic effects of low concentrations of nucleosides. Deficiencies in certain enzymes of nucleoside metabolism result in immune dysfunction. In addition, lymphocyte proliferation and differentiation can be stimulated by other nucleosides and numerous observations have been made of variations in the activities of the enzymes involved in nucleoside metabolism as they relate to different stages of lymphocyte activation or function. Naturally occurring purine and pyrimidine nucleosides participate in intermediary metabolism. Furthermore, many nucleoside analogs and derivatives have been synthesized, some of which have significant biological effects and serve as anticancer agents for lymphoid malignancies. The interface between different nucleosides and their effects on lymphocyte function is the topic of this issue.

Nucleosides and their phosphorylated derivatives participate in the general metabolism of lymphocytes as they do in all cells in a wide variety of biochemical reactions. ATP is the universal energy currency and adenine nucleotides are components of the major coenzymes FAD, NAD, and coenzyme A. Also, nucleotides are the building blocks for nucleic acids, both RNA and DNA. Nucleotides serve as activated intermediates in many biosynthetic reactions. Finally, nucleotides are regulator molecules for enzymes involved in their own metabolism: ribonucleotide reductase is activated or inactivated depending on the concentrations of several different nucleotides; adenylate cyclase is regulated by GTP which binds to modulatory proteins [for effects on lymphoid cells see Ref (1) and P. Insel, this issue].

Besides their overall importance in metabolism, nucleosides are highly cytotoxic, particularly for lymphocytes. Indeed, various diseases of the immune system have been correlated with a lack or reduced amounts of enzymes involved in nucleoside metabolism. The best studied of these are certain forms of inherited immunodeficiency disease. Cells from patients lacking adenosine deaminase or purine nucleoside phosphorylase tend to accumulate dATP or dGTP, respectively. The patients usually present with the clinical picture of acute immunodeficiency. However, a simple mechanism is unlikely, since deoxyadenosine also induces strand breaks in the DNA of resting cells and depletes NAD and ATP. Furthermore, in at least one B lymphoblastoid line increased levels of cytosolic 5′-nucleotidase protect cells from conversion of deoxynucleosides to deoxynucleotides (T. Iizasa and D. Carson, unpublished results). Interestingly, a different group of nucleosides, the C_8-substituted guanosine analogs, is able to stimulate cellular proliferation and differentiation in B lymphocytes. These exert both polyclonal and antigen-specific effects, the latter consisting of T cell-dependent and T cell-independent components (M. Goodman, this issue). The role of phosphorylation products of the C_8-substituted guanosine analogs is under investigation (G. Yeh, unpublished results).

The physiological importance of nucleosides is reflected in the careful regulation of both intra- and extracellular levels. Clearly, not only concentrations but also the locations of the various nucleosides and nucleotides are crucial to metabolic regulation. For example, normal plasma levels of adenosine

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are <1 μM and the intracellular levels of ATP are tightly maintained in a variety of cells. It follows that the concentrations and locations of the enzymes involved in nucleoside metabolism are the determining factors in metabolic control.

The meeting held at Henderson House, Northeastern University in October 1984 was designed to explore the interrelationships among the various nucleosides and nucleotides in lymphocytes, interrelationships involving metabolism, cytotoxicity, and cellular function. Practically speaking, the interlocking of different metabolic pathways has been downplayed in the past, probably due to the difficulties of assessing multiple parameters simultaneously. Rather than summarize the proceedings, this introduction is meant to assist the reader in putting the complexities of such a formidable topic into perspective. The next section is an overview of known nucleoside pathways. It is followed by a long range view of the need to evaluate nucleoside metabolism and function in the context of subcellular compartmentation and processing by multienzyme complexes. We hope that the meeting will serve a useful purpose in focusing future research on both broad issues and specific questions. We are grateful to the

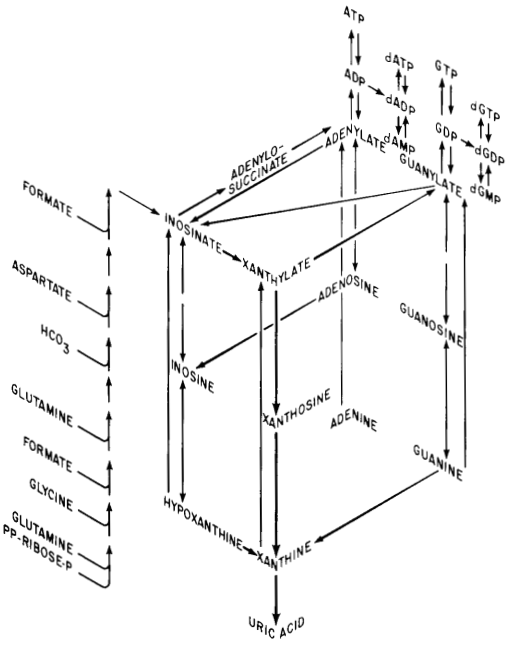

FIG. 1. Pathways of purine metabolism.

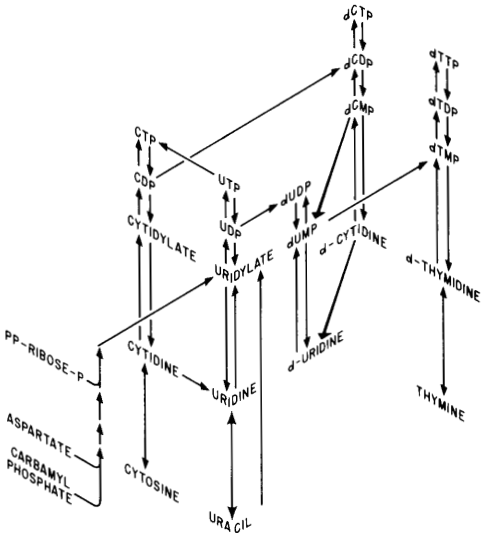

FIG. 2. Pathways of pyrimidine metabolism.

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Outline of Purine and Pyrimidine Metabolism. In order to synthesize, interconvert, and catabolize the various purine and pyrimidine nucleotides that are required for cellular metabolism, cells have evolved a series of alternative metabolic pathways (2–4); these are summarized in schematic fashion in Figs. 1 and 2. It should be noted that different cell types or cells in different stages of development favor some reactions over others. Consequently, it is critical to evaluate a given pathway in the particular cell of interest (J. F. Henderson, this issue; A. Cohen, this issue). This fact permits the selective use of nucleoside analogs in inhibiting reactions in one cell type and not another (A. Fridland, this issue). Inhibition of certain pathways may then promote differentiation along other pathways. One example involves differentiation of lymphoid and myeloid cells promoted by inhibitors of adenosine deaminase (M. Hershfield, unpublished results).

(1) *Synthesis of ribonucleotides.* The synthesis of purine and pyrimidine ribonucleotides may at least potentially be accomplished by three different kinds of processes.

(a) The pathway of pyrimidine biosynthesis *de novo* uses the nonpyrimidine precursors PP-ribose-P, aspartate, and carbamyl phosphate; the latter is formed from ATP,

bicarbonate, and glutamine. Though orotidylate is the first pyrimidine nucleotide formed, it is readily decarboxylated to uridylate.

(b) Purine bases may react with PP-ribose-P to form purine nucleoside monophosphates in one-step processes. The enzyme adenine phosphoribosyltransferase converts adenine to adenylate, whereas hypoxanthine–guanine phosphoribosyltransferase converts hypoxanthine to inosinate and guanine to guanylate. Xanthine is a weak substrate of the latter enzyme in some systems.

Uracil may be converted to uridylate by analogous phosphoribosyltransferase reactions using PP-ribose-P.

(c) Purine ribonucleosides may be phosphorylated to purine nucleoside monophosphates in single-step reactions utilizing ATP. The phosphorylation of adenosine to adenylate by adenosine kinase has been well documented and has been demonstrated in many biological systems. There is some evidence for the direct phosphorylation of inosine and guanosine as well, but these processes so far have not been well characterized.

The pyrimidine ribonucleosides uridine and cytidine may be phosphorylated to uridylate and cytidylate, respectively, by a specific kinase.

(2) *Interconversion of ribonucleotides.* Ribonucleotides may be metabolized by phosphorylation and dephosphorylation and by reactions that interconvert their purine or pyrimidine base moieties.

(a) The purine nucleoside monophosphates adenylate and guanylate, and the pyrimidine nucleoside monophosphates uridylate and cytidylate are readily phosphorylated to the corresponding di- and triphosphates. The tri- and diphosphates are also readily dephosphorylated to di- and monophosphates.

(b) The base moieties of purine ribonucleotides are interconverted as nucleoside monophosphates. Inosinate may be converted via adenylosuccinate to adenylate, and via xanthylate to guanylate. In turn, both adenylate and guanylate may be deaminated back to inosinate.

The interconversion of base moieties of pyrimidine ribonucleotides is a simpler matter; uridine triphosphate may be aminated to form cytidine triphosphate.

(3) *Synthesis of deoxyribonucleotides.* Purine and pyrimidine deoxyribonucleotides may be synthesized by two different processes.

(a) The enzyme ribonucleotide reductase converts the diphosphates of adenosine, guanosine, uridine, and cytidine to the corresponding deoxyribonucleoside diphosphates; these are readily phosphorylated to the corresponding triphosphates and dephosphorylated to the corresponding monophosphates.

(b) Pyrimidine and purine (not shown) deoxyribonucleosides may be phosphorylated to the corresponding monophosphates; deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine, and deoxythymidine may be substrates for the appropriate kinases.

(c) The only reactions of base moiety interconversion that occur in deoxyribonucleotide metabolism are involved in the synthesis of deoxythymidylate; this nucleotide is formed from deoxyuridylate and 5,10-methylene tetrahydrofolate. Two sources of deoxyuridylate are deoxyuridine and deoxyuridine diphosphate produced by ribonucleotide reductase. A third source is deoxycytidylate, which in turn may be produced from deoxycytidine or from deoxycytidine diphosphate.

(4) *Catabolism of ribonucleotides and deoxyribonucleotides.* Nucleotides are catabolized by (a) removal of the phosphates, (b) removal of amino groups, (c) cleavage of the glycosidic bond and loss of the ribose or deoxyribose moiety, and (d) oxidation or reduction of the purine or pyrimidine rings. Adenosine deaminase removes amino groups from both adenosine and deoxyadenosine (not shown), and purine nucleoside phosphorylase catalyzes the removal of both ribose and deoxyribose (not shown) moieties from purine nucleosides. Adenosine deaminase is distinct from AMP deaminase (J. Dorand, this issue) and it is markedly higher in T lymphoblasts than in B lymphoblasts. The synthesis and turnover of human lymphocyte purine nucleoside phosphorylase is discussed by F. Snyder (this issue).

Compartmentation. *Nucleoside receptors and the transport of nucleosides.* As mentioned earlier, not only concentrations but also locations of the various nucleosides and nucleotides are crucial to metabolic activity and regulation. Consequently compartmentation becomes an important issue in any

overview of present findings and for evaluating future directions for the field.

The first issue in maintaining compartmentation of purine and pyrimidine pools involves the translocation process across a membrane from one compartment to another. Translocation can occur from inside the cell to outside and vice versa or from one intracellular compartment to the other. It is specific, saturable, rapid, reversible, and temperature dependent. The transport step itself may not involve subsequent metabolic steps such as phosphorylation, as in the case of flux from inside to outside the cell, or it may involve phosphate exchange as in the case of the mitochondrial ATP transporter.

At the plasma membrane nucleosides are transported by a multicomponent system (or systems) which contain at least a nucleoside binding moiety which is distinct from the transporter [Refs. (5) and (6); B. Ullman, this issue]. Continued dissection is likely to demonstrate more components. Since the extracellular concentration of nucleosides is so low, it is to be expected that the affinities of physiologically important transporter(s) will be high. Furthermore, the role of ecto-5′-nucleotidase in supplying the cell with nucleosides from extracellular fluids is currently under investigation (L. Thompson, this issue).

Nucleoside receptors also function in regulation of the inflammatory and immune response. In particular binding of exogenous adenosine to its receptors inhibits superoxide anion production without affecting lysosomal release generated by neutrophils stimulated with *N*-formyl-methionyl-leucyl-phenylalanine, concanavalin A, ionophore A23187, and zymosan-treated serum but not phorbol myristic acetate. Cellular uptake of adenosine is not required, since inhibition persists despite addition of dipyridamole, which blocks transport. Metabolism of adenosine is also not required, since inhibitors of adenosine deaminase do not prevent inhibition (R. Hirschhorn, unpublished results).

Intracellular pools. The intracellular compartmentation of purine and pyrimidine nucleotide pools has been well reviewed recently by Moyer and Henderson (4). Both historically and at the practical level of laboratory investigation it is usually assumed that measured nucleotide pools are homogeneous. In cases where this assumption has been questioned it has often been shown that nucleotide pools are not homogeneous in various organelles. The most striking examples are mitochondrial nucleotide pools; both ATP and GTP are present in higher concentrations in mitochondria than in the rest of the cell. The problems attendant on rapid isolation of the subcellular organelle in question, the presence of nucleotide transporters as an integral part of the organelle's membrane system, and the danger of inadvertantly mixing enzymes from other compartments, especially those whose job it is to metabolize the various nucleotides, make the problem highly complex.

A second area of interest lies with the pools from which precursors for nucleic acids are drawn, both mitochondrial and nuclear. It is clear that the dTTP pool used for synthesis of mitochondrial DNA is distinct from total cellular dTTP of mouse L cells and HeLa cells. Furthermore, the possibility that the source of nucleotides for nuclear nucleic acid synthesis is not the same as the general pool has long been the object of investigation. Still another pool may be involved in direct insertion of the base hypoxanthine into transfer RNA (R. Trewyn, this issue). The presence of Q nucleosides in transfer RNA may imply that purine salvage pathways are directly involved in protein synthesis (J. Katze, this issue).

Intracellular compartmentation of nucleotide metabolizing enzymes. It is a tenet of modern cell biology that if the concentration of substrates is closely controlled in individual subcellular compartments then the locations of the enzymes involved in biosynthetic and salvage pathways are also compartmentalized. Many of the biosynthetic and regulatory enzymes are associated with membrane systems in a particular subcellular space. For example adenylate cyclase is a plasma membrane enzyme with an external receptor for polypeptide hormones and internal binding proteins for various nucleotides which act as modulators. Also, the biosynthesis of ATP occurs on the F1–ATPase of mitochondrial cristae, closely apposed to the electron transport chain from which the energy to generate ATP is derived.

On the other hand, very little is known about the intracellular locations of most of the nucleoside and nucleotide salvage en-

zymes. Adenosine deaminase is entirely soluble as shown by lysis of cells with nonionic detergent and complete recovery of the enzyme in the supernate (P. Strauss, unpublished data). The locations of other purine salvage enzymes including purine nucleoside phosphorylase and hypoxanthine guanine phosphoribosyltransferase are also unknown. The presence of extracellular purine nucleoside phosphorylase (B. Mitchell, this issue) poses the possibility of its secretion by viable cells and/or release upon cell death. Even less is known about the pyrimidine pathway enzymes. Furthermore, the physiological role of thymidine kinase or its subcellular location is yet to be understood, since this enzyme is not involved in pyrimidine biosynthesis or metabolism under normal conditions.

Macromolecular Assemblies. The roles played by multienzyme complexes in metabolic processing of a variety of substrates have proven to be interesting and may be highly significant in nucleoside metabolism. A multienzyme complex can be defined as an aggregate of distinct but functionally related enzymes bound together by noncovalent forces into a highly organized structure [for review, see Refs. (7–9)]. Potential advantages of such multienzyme complexes include (a) decreased substrate diffusion time; (b) minimal competition for an intermediate product by competing enzyme systems; (c) a high relative concentration provided by a small number of substrate or product molecules for the ensuing complexed enzymes; (d) transmission of the effects of a specific metabolite with one protein to other regulatory mechanisms of the system via protein–protein interactions; (e) protection of unstable intermediates; and (f) enhancement of specific reactions by microenvironments of particular natures (e.g., hydrophobicity). Conduction of a metabolite from one enzyme to the second to the third, and so on, without free access of the metabolite to the intracellular pool of that metabolite is spoken of as "channeling." The existence of multienzyme complexes, presumably for channeling of relevant substrates, has been demonstrated in many systems, such as the *de novo* synthesis of pyrimidines from carbamyl aspartate and carbamyl phosphate (pyr 1–3), or from orotidylate (pyr 5–6); production of deoxynucleoside triphosphates in CHEF cells, Novikoff Hepatoma cells, and human lymphoblastoid cells; synthesis of DNA by DNA polymerase, thymidine kinase, dihydrofolate reductase, nucleoside diphosphate kinase, and ribonucleotide reductase (called "replitase") present in S phase (but not G_0) CHEF cells; synthesis of uridylic acid in Ehrlich ascites cells; action of several aminoacyl-tRNA synthetases; fatty acid synthesis; and the urea cycle, among others.

An increasing awareness and interest in the importance of multienzyme complexes for processing of purine and pyrimidine substrates is developing. The incorporation of various ^{3}H-nucleosides into both chromosomal and detergent soluble DNA of lymphocytes may involve a similar multienzyme complex since the incorporation of ^{3}H-nucleosides into detergent soluble DNA is sensitive to inhibitors of polymerase α and ribonucleotide reductase (P. Strauss, this issue). Future years may provide crucial information concerning the existence and composition of such complexes in nucleoside metabolism and utilization, where characteristic enzymatic composition may serve to channel metabolites predominantly into one or another of the pathways illustrated in Figs. 1 and 2. If such complexes exist, it is possible that an allosteric regulator can regulate the function of an entire group of nucleoside pathways rather than of a single enzyme, through a series of protein–protein interactions. Binding of several different regulators to different enzymes could conceivably activate or inactivate distinct *subcomplexes* of enzymes within the complex, leading to alternative modes of channeling as a function of the regulators bound. Moreover, consideration of the functional capabilities of such complexes within the microenvironments of their own subcellular compartments will provide still more information relating to the biological relevance of different pathways. Finally, study of such complexes may ultimately lead to discovery and characterization of new enzymatic activities and pathways.

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