Role of Purines in Lymphocyte Function*

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All cells require a balanced supply of purines for growth, proliferation and survival. The major roles of purines in cellular metabolism and function are given in Table 1. In Figure 1, a simplified outline is shown of the basic pathways by which purines are metabolised. The purpose of this article is to review those aspects of purines that appear unique to lymphocytes and thus their functions in immune processes. Basically, the rationale for this focus comes from the involvement of purines in immunodeficiency diseases and the use of purine analogues as immunosuppressive agents.

Purine catabolism and immunodeficiency diseases

The discovery that hereditary disturbances in purine metabolism interfere with lymphocyte function has produced an intense interest over the past 10 years in the biochemistry of the lymphoid immune system.[1-3] Three lymphocyte enzymes have been associated with immunodeficiency diseases: adenosine deaminase, purine nucleoside phosphorylase and 5'-nucleotidase.

Adenosine deaminase deficiency

The first case of immunodeficiency disease associated with an inherited purinogenic defect in lymphocytes was reported by Eloise Giblett and colleagues in 1972.[4] They described two children with severe combined immunodeficiency disease (SCID) whose RBC and other tissues lacked the enzyme adenosine deaminase (ADA). The next year ten more cases of SCID associated ADA deficiency were reported. The parents of affected children were found to have reduced RBC ADA activity indicating an autosomal recessive form of the disease. Thus it appeared that at least some forms of immunodeficiency disease were "inborn errors of metabolism."

Adenosine deaminase (adenine aminohydrolase) (E.C. 3.5.4.4) catalyses the irreversible deamination of adenosine to inosine or deoxyadenosine to deoxyinosine. ADA has a monomeric structure and a molecular weight of 38,000 daltons when extracted from RBC. RBC ADA is the smallest and simplest form of the enzyme. Several different tissue-specific isozymes exist consisting of two catalytically active subunits identical to RBC ADA – combined with a dimeric conversion factor or binding protein of 190,000 daltons. The properties of the human red cell ADA enzyme have been described in detail by Daddona and Kelley.[5] ADA occurs in most body tissues. Especially high levels of ADA enzyme activity are found in thymus, spleen and other lymphoid tissues.

ADA deficiency is characterised by both T and B lymphocyte defects. There is a profound lymphopenia, absence of delayed cutaneous hypersensitivity and reduced responsiveness of lymphocytes to mitogens, allogeneic cells, and other antigens.[1] There is severe hypogammagloblulinaemia or agammaglobulinaemia with no evidence of specific antibody synthesis. Such individuals are prone to increasingly severe infections with eventual fatal outcome in the absence of successful therapy. Successful immunological reconstitution has been achieved in ADA deficient SCID patients with histocompatible bone marrow transplantation.[6] Another therapy effective in some indivi-

Table 1 Purine functions.

1. Energy metabolism (ATP)
2. Monomeric precursors of DNA and and RNA
3. Structural components
   a) Coenzymes (FAD, NAD, NADP)
   b) Methyl donor (SAM)
4. Regulatory roles
   a) Metabolic signals (cAMP/cGMP)
   b) Allosteric effects
5. Physiological roles
   a) Smooth muscle
   b) Platelets
   c) Neurotransmission

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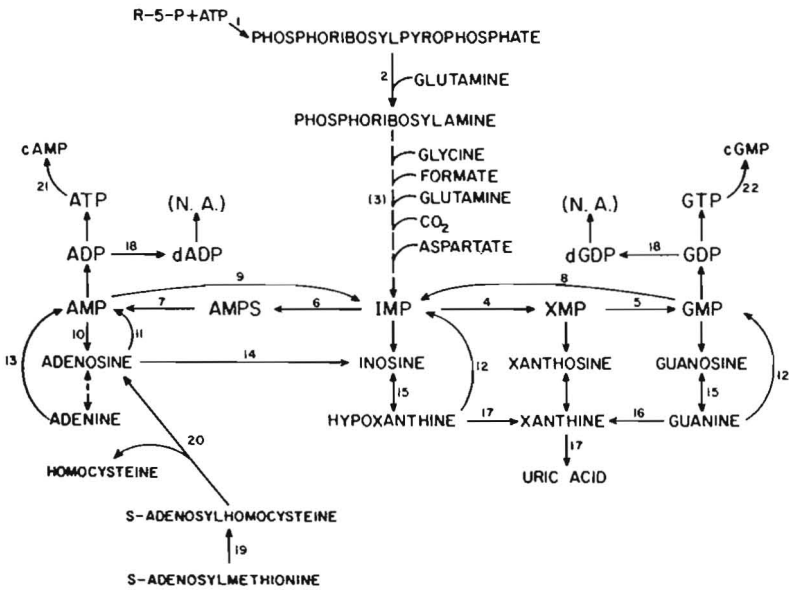

Fig. 1 Purine pathway enzymes
1) Phosphoribosyl pyrophosphate synthetase (EC 2.7-6.1),
2) Amidophosphoribosyl transferase (EC 2.4.2.14),
3) (De Novo pathway enzymes),
4) IMP dehydrogenase (EC 1.2.1.14),
5) GMP synthetase (EC 6.3.5.2),
6) Adenylosuccinate (AMPS) synthetase (EC 6.3.4.4),
7) Adenylosuccinate lyase (EC 4.3.2.2),
8) GMP reductase (EC 1.6.6.8),
9) AMP deaminase (EC 3.5.4.6),
10) 5'-nucleotidase (EC 3.1.3.5),
11) Adenosine kinase (EC 2.7.1.20),
12) Hypoxanthine – Guanine phosphoribosyl transferase (EC 2.4.2.8),
13) Adenine phosphoribosyl transferase (EC 2.4.2.7),
14) Adenosine deaminase (EC 3.5.4.4),
15) Purine nucleoside phosphorylase (EC 2.4.2.1),
16) Guanine deaminase (EC 3.5.4.3),
17) Xanthine oxidase (EC 1.2.3.2),
18) Ribonucleotide reductase,
19) Protein carboxymethylase (EC 2.1.1.24),
20) S-adenosylhomocysteine hydrolase (EC 3.3.1.1),
21) Adenylate cyclase (EC 4.6.1.1),
22) Guanylate cyclase (EC 4.6.1.2)

duals involves infusion of normal irradiated RBC as a source of encapsulated ADA which can result in restoration of immune responses.[7] This form of enzyme replacement therapy is particularly interesting in that the RBC enzyme acts to correct the biochemical problem in ADA defective lymphocytes. This observation suggests an important relationship between RBC and lymphocytes in terms of systemic purine homeostasis.

A number of biochemical mechanisms have been proposed to explain how ADA deficiency affects the function of lymphocytes. No single proposal has as yet been totally accepted. There are four basic biochemical changes observed in ADA-SCID patients. 1) elevated plasma adenosine and deoxyadenosine;[8] 2) accumulation of adenosine deoxynucleotides, especially dATP, in lymphocytes and RBC;[9] 3) increased cAMP levels in lymphocytes;[10] and, 4) decreased S-adenosylhomocyteine hydrolase activity resulting in accumulation of S-adenosylhomocyteine which can inhibit S-adenosylmethionine mediated methylation reactions.[11] This latter effect may be due to the high level of deoxyadenosine which acts as a "suicide" inhibitor of S-adenosylhomocyteine hydrolase.[12] A combination of these biochemical changes may provide a plausible basis for the lymphocyte dysfunction in ADA deficiency. The sequence of toxic events may be as follows. The absence of ADA activity leads to accumulation of adenosine (AR) and deoxyadenosine. Deoxyadenosine (dAR) is acted on by nucleoside kinase (dAR kinase occurs specifically in lymphoid tissues) producing increased ("trapped") dATP. The accumulated dATP acts to allosterically inhibit ribonucleotide reductase which leads to disruption of DNA synthesis and thus lymphocyte killing. An interesting alternative hypothesis has been recently advanced by Carson and colleagues[13] who proposed that the elevated dATP levels kill mature resting T lymphocytes by depleting ATP levels. This could well be a contributing biochemical factor that in combination with dATP mediated ribonucleotide reductase inhibition produces an overwhelming metabolic disruption in ADA deficient lymphocytes.

Purine nucleoside phosphorylase deficiency

Following the discovery of the association of ADA deficiency with SCID, a second purine defect was discovered during active screening of patients for ADA deficiency. Again in 1975, Eloise Giblett and colleagues described an association of purine nucleoside phosphorylase (PNP) deficiency with a selective T-lymphocyte dysfunction.[14] Like ADA deficiency PNP deficiency is a autosomal recessive disease.

Purine nucleoside phosphorlyase (purine riboside – orthophosphate

ribosyl transferase) (EC 2.4.2.1) catalyses the reversible coversion of guanosine and deoxyguanosine to guanine and of inosine and deoxyinosine to hypoxanthine. The enzyme occurs in most tissues with particularly high levels in RBC. Red cell PNP is a trimer composed of identical 28,000 dalton subunits with each subunit having one substrate-binding site.[15] The RBC isozyme shows a seven-banded pattern on starch gel electrophoresis with the slowest moving band representing the primary gene product; other bands represent postgenic modifications.[16]

Clinically, PNP deficient individuals have a severe lymphopenia with marked loss of T-lymphocyte function. There appears to be a total loss of T suppressor lymphocytes.[17] B-lymphocyte functions are intact with no impairment of specific antibody synthesis to immunising antigens. Most children show a hypergammaglobulinaemia characterised by monoclonal IgG spikes.[2] There is some evidence of autoimmune disease. Individuals tend to have infections that are predominantly viral in nature. Overall there is an age-dependent progressive organ failure particularly involving the thymus-dependent lymphoid system.[1]

The major biochemical features of PNP deficiency are: 1) increased levels of deoxyguanosine and deoxyinosine in urine and serum;[18] 2) marked decrease in serum uric acid levels with attendent hypouricosuria.[19] and 3) a dramatic increase in dGTP levels in lymphocytes and RBC.[20]

A biochemical mechanism to explain the selective T cell defect in PNP deficiency disease may be developed as follows. Absence of PNP activity leads to the accumulation of deoxyguanosine (dGR). The dGR is acted on by lymphocyte dGR kinase to produce dGTP which is trapped intracellularly. It is noteworthy that thymus contains the highest levels of dGR kinase in the body. dGTP then acts to inhibit ribonucleotide reductase which leads to disruption of DNA synthesis. dGR and dGTP appear to be extremely toxic to T-suppressor lymphocytes but less so to T-helper lymphocytes.[17] This differential toxicity may explain the sparing of antibody mediated immune function in PNP deficiency.

A basically unaddressed but fundamental concern in trying to mechanistically explain the immune defect in both PNP and ADA deficiency is how the observed biochemical changes relate to the ontogeny of T and B lymphocytes in these disease states. Research is currently underway on this issue and results from these studies may improve our understanding of the fundamental role of purines in immune cell function.

5'Nucleotidase deficiency

Deficiency of lymphocyte ecto-5' nucleotidase has been reported in certain patients with adult-onset hypogammaglobulinaemia, Wiskott-Aldrich syndrome and familial reticuloendotheliosis.[2]

Ecto-5'nucleotidase (5'-ribonucleotide phosphohydrolase) (EC 3.1.3.5) is a surface enzyme that catalyzes the conversion of 5'-nuceotide monophosphates to their respective purine bases (5'-MNTD --> Purine Base + Pi). At present it is uncertain whether there is a true etiological relationship between the enzyme deficiency and the immune dysfunction associated with such a diverse group of immunological disorders.[1,2,21]

Attempts to understand a possible role of ecto-5'-nucleotidase in immune cell function have forced a close look at the role of this enzyme in intermediary metabolism. As a result of such studies evidence has been obtained for the presence of cytoplasmic 5'-nucleotidase.[22] Particularly interesting is the observation that B lymphoblasts contain considerably more cytoplasmic 5'-nucleotidase activity than T lymphoblasts. Such a metabolic difference between T and B cells could explain in part the differential behavior of these cells in PNP-deficiency disease.

Another interesting observation has been the demonstration of a lymphocyte ecto-ADPase.[23] During platelet aggregation ADP is released into the extracellular space. Given ADP as a physiological substrate, lymphocytes thus have an ectoenzyme system for localised production of adenosine (viz: ADP $\xrightarrow{\text{ADP-ase}}$ AMP $\xrightarrow{\text{5'-NTD-ase}}$ AR).

Adenosine: role in normal lymphocyte function.

The biochemical consequences of ADA and PNP immunodeficiency diseases suggest that pathways of purine nucleoside catabolism are essential to normal lymphocyte function. Perhaps more importantly they suggest that the nucleosides, adenosine and guanosine, whose turnover is regulated by these catabolic pathways have critical roles in cellular function. In particular adenosine has been increasingly regarded as having the role of an immunoregulatory molecule. There is now considerable evidence that extracellular adenosine has an important physiological role as a regulator of adenosine 3', 5'-monophosphate (cAMP) metabolism.[24] The prospect that adenosine acts as an intercellular messenger capable of influencing second messenger effects mediated by intracellular cAMP is both novel and exciting. Implicit in this concept in which adenosine has the role of a first-messenger is the requirement that the adenosine molecule interact with a specific receptor on the lymphocyte surface.

Adenosine has been shown to inhibit a number of lymphocyte responses both *in vivo* and *in vitro*. The *in vivo* effects have already been discussed under the enzyme associated immunodeficiency diseases. *In vitro* effects of adenosine are as follows: 1) inhibition of PHA induced lymphocyte blastogenesis;[25] 2) inhibition of Con A induced lymphocyte blastogenesis;[26] 3) inhibition of lymphocyte-me-

diated cytolysis[27] – effects augmented by the presence of an ADA inhibitor; and 4) inhibition of pokeweed mitogen – stimulated synthesis of IgG by B lymphocytes.[28]

A number of mechanisms have been proposed to explain the inhibition by adenosine of lymphocyte responses: 1) uptake of adenosine leading to increased cAMP production;[29] 2) cAMP phosphodiesterase inhibition shuting off catabolism of cAMP;[30] and, 3) adenosine mediated activation of cAMP.[31] All three proposals have in common the final effect of increased cAMP. Adenosine binding studies[32] and work with competitive inhibitors of adenosine[24] support, however, the concept of a specific plasma membrane receptor to which adenosine binds at physiological concentrations. Interaction of the adenosine molecule with its receptor on the lymphocyte surface then leads to activation of adenylate cyclase and consequent increased production of intracellular cAMP. Figure 2 shows a generalised model for the adenosine receptor – adenylate cyclase system.

What this model attempts to do is bring together what little is known about adenosine receptors, adenylate cyclase and cAMP mediated cellular responses and to apply this in the context of lymphocyte function. Adenosine effects on adenylate cyclase appear to be mediated via two types of receptors termed R-sites and P-sites.[33] R-site adenosine receptors are located on the outer surface of the plasma membrane. R-receptors activate adenylate cyclase (AC), require an intact ribose moiety in the effector molecule and are antagonised by theophylline. Recently R-receptors have been divided into two subclasses – A1 and A2.[34] A1 adenosine receptors are inhibitory to AC and show high affinity with a Ki for adenosine and analoges of between 5 to 50 nM. A2 adenosine receptors stimulate AC and are characterised by low affinity with Ki in the range of 5-20 μM. The case for A2 receptors on lymphocytes is well established whereas A1 receptors have only been demonstrated on a few other cell types. P-site adenosine receptors are located on the inner surface of the cell membrane and always inhibit AC. These are low affinity receptors that require an intact purine ring and are not antagonised by theophylline. There is some evidence that the P site receptor may be regulated by nucleotide analogues of adenosine such as 5'-AMP.[34]

Given a stimulatory interaction of adenosine with a lymphocyte surface A2 receptor the molecular sequence of events would be basically as follows (Figure 2). Binding of adenosine to its membrane receptor (A2) triggers the GTP dependent activation of AC by a membrane bound coupling protein.[35] cAMP synthesised from ATP by AC diffuses through the cytoplasm and activates cAMP-dependent protein kinase, which in turn catalyses the phosphorylation of specific substrate proteins (S), usually enzymes (Figure 2). The phosphorylated substrates (S ∿ P) due to their altered activity produce the characteristic effects of cAMP at specific intracellular loci. cAMP phosphodiesterase regulates activation by degrading the newly produced cAMP. Further regulation is accomplished by cellular phosphatases which dephosphorylate the substrates of cAMP-dependent protein kinases. This model of adenosine mediate cAMP production provides a conceptual framework for consideration of adenosine and cAMP roles in lymphocyte function.

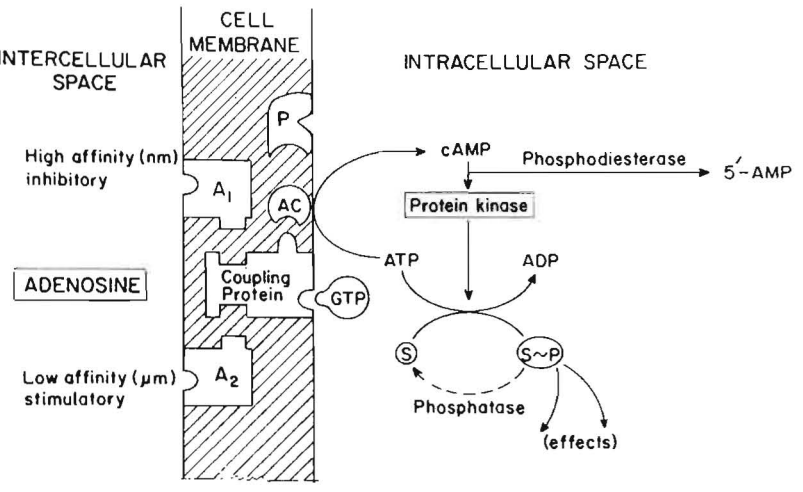

Fig. 2 A generalized model for the adenosine receptor – adenylate cyclase system for cAMP. A1 and A2 are R-site adenosine receptors.

Basically, as already noted, increased cellular cAMP blocks the proliferative response of lymphocytes to mitogens and inhibits antigen induced secretion of lymphokines and direct lymphocytotoxicity. It is important to emphasise, however, that these basically inhibitory effects of cAMP on lymphocyte function are occuring in initially resting peripheral blood lymphocytes. The absence of a physiological response may be simply due to a block in the cell surface triggering mechanism which produces a specific effector cell proliferative response. Indeed there is evidence for a positive cAMP influence over cellular differentiative processes.[36] Thus consideration must be given to the stage of lymphocyte development and the physiological compartment in which cAMP mediated responses occur.

A fascinating observation regarding cAMP and lymphocyte function has to do with the formation of rosettes between sheep RBC (E)

and human lymphocytes (RFC). The sheep cell receptor is a surface marker for T lymphocytes[37] (T cell E-rosettes are referred to as E-RFC). The formation *in vitro* of E-RFC is inhibited by agents which raise intracellular cAMP levels.[38] Using this observation it is possible to separate T lymphocytes into theophylline – resistant (Tr) E-RFC (E rosette formation in the presence of theophylline) and theophylline-sensitive (Ts) E-RFC (no E rosette formation in the presence of theophylline).[39] Based on an antigen-specific *in vitro* anti-sheep-RBC plaque-forming cell (PFC) assay Tr E-RFC are helper T cells and Ts E-RFC are suppressor T cells.[39] It has been demonstrated that almost all Ts E-RFC have Fc receptors (FcR) for IgG (RFcγ+) whereas essentially none of the Tr E-RFC have FcR for IgG (RFcγ−).

Recently it was shown that treatment of Tr lymphocytes with adenosine or impromidine (an H2 – histamine agonist) produced an increase in the percentage of RFcγ+ cells showing suppressor activity against B cell immunoglobulin synthesis.[28,40] This was the first direct evidence for an immunoregulatory role of adenosine on lymphocyte function.

In vitro adenosine clearly has a direct role in the modulation of lymphocyte function through its interaction with a specific receptor capable of mediating intracellular cAMP response. The situation *in vivo* is more difficult to assess. Although adenosine is being continually generated its turnover in the extracellular environment is very rapid. The magnitude of adenosine production and catabolism can be most appreciated by the consequences observed in ADA or PNP deficiency states where toxic levels of nucleotide and products rapidly accumulate. What probably occurs is a finely tuned steady-state which permits a constant but critical range of adenosine concentration to be in contact with cell surface receptors, enzyme activities and nucleoside transport loci. Perturbation in adenosine homeostasis would be expected to influence adenosine-receptor interactions in a manner that produces either compensatory or abnormal cellular responses.

Adenosine: role in abnormal lymphocyte function

Recent work by Steven Polmar and colleagues[41] provides strong evidence for what appears to be an immunoregulatory defect in systemic lupus erythematosus (SLE) due to an abnormality in adenosine receptor-mediated cAMP metabolism. SLE is an autoimmune disease characterised by impaired suppressor T-lymphocyte function during active disease. In particular SLE T-lymphocytes do not develop suppressor activity in response to concanavalin A.[42]

When normal human Tr lymphocytes are exposed to 10 μM adenosine (30 min, 37°C) there is an increase in the percentage of cells expressing RFcγ+ with a proportional increase in OKT8 and decrease in OKT4 antigens.[40] Tr lymphocytes are enriched for helper/inducer cells which have the OKT4 antigen.[41] The Tr (RFcγ−) lymphocytes act as helper cells in pokeweed mitogen-induced differentiation of B cells to cytoplasmic immunoglobulin-containing plasma cells. Adenosine treatment of Tr lymphocytes produces a loss of helper function with active suppression of B cell differentiation corresponding to an increase in RFcγ+ and OKT8 expression.[40] In comparison, adenosine treatment of Tr lymphocytes from patients with SLE does not produce an increase in the proportion of OKT8+ (RFcγ+) cells and there is no induction of immunosuppressor activity.[41] Incubation of normal Tr lymphocytes with adenosine produces a transient increase (160% of control, 5 min) in cAMP levels whereas SLE Tr lymphocytes show a fall (50%, 5 min) in cAMP levels. Treatment of both normal and SLE Tr lymphocytes with 8-bromoadenosine cyclic monophosphate (a cAMP analogue that traverses the cell membrane) produces an equivalent increase in the OKT8+ (RFcγ+) cell fraction. Polmar *et al*,[41] interpret these results as indicating that cAMP mediates the effects of adenosine on cell surface markers of T lymphocytes and suggest that the lack of an adenosine receptor – coupled adenylate cyclase activity in SLE Tr lymphocytes accounts partly for their lack of immunosuppressive activity.

Another example of how altered adenosine metabolism may influence lymphocyte function in a disease state comes from recent work in our laboratory. We have observed a marked increase in ADA activity in haemolysates from malaria infected humans *(P. falciparum)* and monkeys *(P. cynomolgi)*.[43] A distinct malaria parasite enzyme was identified using a noval biological isolation technique that involved growing the human parasite in ADA deficient host RBC.[44]

We were particularly interested in the implications of elevated ADA activity in the peripheral blood for overall purine homeostasis. One prediction would be a decrease in the steady-state concentration of adenosine in the extracellular compartment. This could influence the intercellular messenger role proposed for adenosine and alter adenosine-receptor interactions with a consequent perturbation in lymphocyte cAMP metabolism.

When peripheral blood lymphocytes were isolated from individuals with acute *P. falciparum* malaria a 6-fold decrease in endogenous cAMP level was observed for malaria lymphocytes compared to normal controls. The effect of adenosine on cAMP levels in malaria and normal lymphocytes is presented in Figure 3. In normal lymphocytes exposure to adenosine (10μM) produced an increase in cAMP level that appeared maximal at 20 minutes. Malaria lymphocytes, however, showed a significant depression in the cAMP response to exogeneous

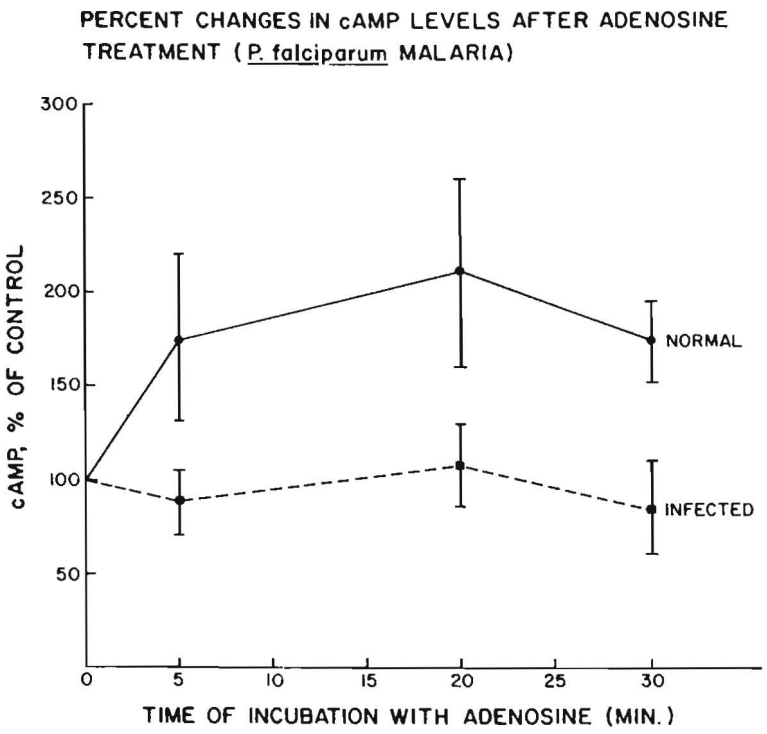

Fig. 3 Percent change in cAMP levels following adenosine treatment. Normal (●) and malaria (■) lymphocytes were incubated with 10 μM adenosine. Aliquots were removed at the indicated times, boiled for 3 min, and cAMP determined by radioimmunoassay. (values are mean ± SEM, n = 4)

adenosine. Similar observations were made on lymphocytes from *P. cynomolgi* infected rhesus monkeys.[45] In these studies a marked decrease in mitogen induced blastogenesis was found in parallel with the cAMP depression. Recovery of both the biochemical and functional defects were observed during convalescence. These data suggest that malaria lymphocytes acquire a reversible defect in cAMP production that may be related to an uncoupling of the adenosine receptor from adenylate cyclase. The mechanism for the cAMP defect is currently under study.

Summary

Purines have multiple roles in cellular function. In addition to their fundamental role in intermediary metabolism purines appear to have a special role for lymphocytes in immune processes. Studies of immune deficiency diseases have shown that enzyme defects in the purine nucleoside catabolic pathways can produce accumulation of toxic metabolites that interfer drastically with both cell-mediated and humoral immunity. Under normal conditions the purine nucleoside, adenosine, appears to have an immunoregulatory role functioning as an intercellular messenger molecule. Lymphocytes have surface receptors for adenosine that are coupled to plasma membrane adenylate cyclase. Exposure of helper/inducer T lymphocytes to adenosine produces a rapid change in the expression of cell surface markers (OKT antigens and Fc receptors for IgG) accompanied by the appearance of suppressor activity. These affects appear to be correlated with adenosine receptor mediated changes in cAMP. Alterations in the adenosine receptor/adenylate cyclase system have been identified in systemic lupus erythematosus and malaria infection which may, with further work, clarify the role of purines in the molecular control of lymphocyte function in immune regulation.

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