progresses extrinsic acidic degradation of the eggshell exposes large areas of the honeycomb zone at the bases of the erosion craters, so increasing the shell porosity, which facilitates the exchange of respiratory gases and water vapor from the ever enlarging alligator embryo. The erosion craters also weaken the eggshell causing it to crack and fall off the eggshell membrane about 60 days, so facilitating hatching at 65 days. This is the first time that normal extrinsic degradation of the eggshell of any animal has been described and the first report of the ability of an eggshell to change its porosity as incubation progresses. It would be interesting to learn if the eggs of other crocodilians, which are often laid in a sandy hole as opposed to a nest of vegetation (*7*), also show this extrinsic degradation. Preliminary observations (*8*) of eggshells of *Crocodylus niloticus* (which lay their eggs in sandy holes) have shown that they exhibit erosion craters similar to those described here for *Alligator mississippiensis*. Perhaps these are formed by the action of mineral acids in the moist sand or else by acidic microbial metabolites. Further study of the microbial flora of crocodilian nests (both vegetative and sandy) and of the structure of other archosaurian eggshells is indicated. It is very important when alligator eggs are artificially incubated that they are completely surrounded by nesting medium at 28° to 30°C and 100 percent humidity. Otherwise, if nesting medium is excluded, hatching success is reduced (*9*) because the young cannot break out of the abnormally tough shell and quickly die from asphyxiation.

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Inhibition of Purine Nucleoside Phosphorylase by 8-Aminoguanosine: Selective Toxicity for T Lymphoblasts

Abstract. *The guanosine analog 8-aminoguanosine is an effective inhibitor of the purine degradative enzyme purine nucleoside phosphorylase, both in vitro and in intact lymphoid cells. In a human lymphoblast tissue culture system, 8-aminoguanosine, in combination with low concentrations of 2′-deoxyguanosine, causes toxicity toward T cells but not B cells. The selective T cell toxicity correlates with increased accumulation of deoxyguanosine triphosphate in the treated T lymphoblasts.*

An inhibitor of the purine degradative enzyme, purine nucleoside phosphorylase (PNP) (E.C. 2.4.2.1), has been sought because of its potential usefulness as a selective immunosuppressive agent for the treatment of certain autoimmune diseases, for the prevention of tissue rejection after organ transplantation, and for the treatment of malignant lymphoproliferative diseases. Interest in PNP as a target for pharmacologic inhibitors stems from the discovery that the hereditary deficiency of PNP is associated with severe, selective T lymphocyte dysfunction and resultant cellular immunodeficiency, without compromise of humoral immunity (*1*). The conversion of guanosine and inosine and their 2′-deoxy derivatives is catalyzed by PNP to their respective purine bases. 2′-Deoxyguanosine accumulates in the absence of PNP activity and results in a striking increase in levels of deoxyguanosine triphosphate (dGTP) in the erythrocytes of affected individuals (*2*). Similar elevations of dGTP occur in human T lymphoblasts, but not in B lymphoblasts, cultured in the presence of 2′-deoxyguanosine (*3*). The accumulation of dGTP in the T cell inhibits ribonucleotide reductase, the enzyme that catalyzes the reduction of nucleoside diphosphates to their corresponding 2′-deoxy derivatives (*4*); depletion of other deoxynucleoside triphosphates by this mechanism then results in inhibition of DNA synthesis and cell death.

Previous approaches to the selective biochemical control of immune function have focused on the use of inhibitors of adenosine deaminase (ADA) (E.C. 3.5.4.4), another enzyme in the purine catabolic pathway, which is responsible for the conversion of adenosine to inosine and deoxyadenosine to deoxyinosine. Several observations would suggest, however, that the inhibition of ADA would not be as effective or as selective in its lymphocytotoxic effect as the inhibition of PNP. While the hereditary deficiency of ADA results in T lymphocyte dysfunction and depressed cellular immunity, as does PNP deficiency, ADA-deficient patients also exhibit impairment of B lymphocyte function and associated abnormalities of humoral immunity not found with PNP deficiency (*5*). In addition, the accumulation of the ADA substrate 2′-deoxyadenosine causes not only the accumulation of deoxyadenosine triphosphate (dATP) in erythrocytes of affected individuals (*6*) as well as in immature T lymphocytes in vitro (*3*, *7*) but also inactivation of the enzyme *S*-adenosylhomocysteine hydrolase (E.C. 3.3.1.1) (*8*). The latter effect may adversely alter the function of both lymphoid and nonlymphoid cells as a consequence of *S*-adenosylhomocysteine accumulation and inhibition of *S*-adenosylmethionine–dependent methylation reactions. Indeed, the administration of deoxycoformycin, a potent inhibitor of ADA, to patients with acute lymphoblastic leukemia as well as to patients with nonhematologic malignancies produced not only a marked reduction in the numbers of both neoplastic and normal lymphoid cells but also serious toxicity involving the kidneys, lungs, and central nervous system (*9*).

These experiences with the clinical use of an ADA inhibitor have stimulated interest in the development of an inhibitor of PNP. 8-Aminoguanosine, an analog of guanosine (*10*), is the most potent PNP inhibitor discovered to date. With a K_i (inhibition constant) of 17 μM for purified erythrocyte PNP (*11*), it is six times more potent on a molar basis than formycin B, the strongest previously recognized PNP inhibitor (*12*). Thus, 8-aminoguanosine has the potential of serving as a lymphocytotoxic agent with considerable selectivity for T lymphocytes.

We have described (*3*) a cell culture system using human T and B lymphoblast cell lines with which we can reproduce the biochemical changes and selective T cell lymphocytotoxicity observed in PNP deficiency disease. In this system, a high concentration of 2′-deoxyguanosine, 50 μM, added to culture me-

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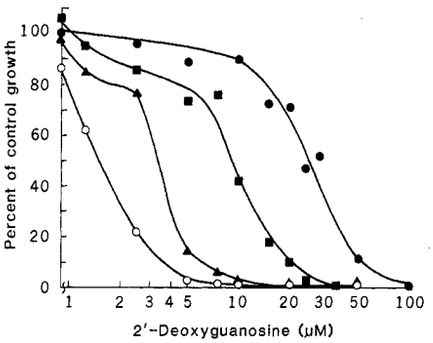

Fig. 1. Effects of varying concentrations of 8-aminoguanosine and 2′-deoxyguanosine on the growth of MOLT-4 T lymphoblasts. Results are expressed as the percent of control growth defined as the percent of viable control cell number at 72 hours. Cells were cultured with a starting concentration of 2.5×10^5 cells per milliliter in RPMI 1640 medium plus 10 percent heat-inactivated horse serum. Control cultures contained no additives. Values represent the mean of determinations from two or more separate experiments. 8-Aminoguanosine concentrations: (●) none; (■) 10 μ*M*; (▲) 50 μ*M*; and (○) 100 μ*M*.

dium, leads to the phosphorylation of some of this deoxynucleoside to produce an increased level of dGTP and cytotoxicity in T cells, but not in B cells (*3*). B cells may be protected from dGTP accumulation by their increased ability to degrade deoxynucleotides (*13*). We have used this lymphoblast culture system to study the potentiating effect of the PNP inhibitor 8-aminoguanosine on 2′-deoxyguanosine toxicity for T and B lymphoblasts in vitro.

The T lymphoblast line MOLT-4, derived from a patient with acute lymphoblastic leukemia, and the human B lymphoblast line MGL-8, derived from a normal individual, were maintained in exponential phase growth in RPMI 1640 tissue culture medium containing 10 percent heat-inactivated horse serum at 37°C. Cell growth studies were performed during a 72-hour period on cells cultured in medium without additives and in medium containing various concentrations of 8-aminoguanosine and 2′-deoxyguanosine, both alone and in combination. Cell growth was determined at 24-hour intervals by counting viable cells on a hemacytometer. Trypan blue dye exclusion was used as an index of viability.

8-Aminoguanosine alone had no effect on the growth of MOLT-4 cells (Fig. 1) or MGL-8 cells (data not shown) at concentrations of up to 100 μ*M*. As shown in Fig. 1, 2′-deoxyguanosine alone had no effect on the growth of MOLT-4 cells at concentrations of up to 10 μ*M* and resulted in 50 percent inhibition of growth (ID_{50}) at a concentration of 26 μ*M*. In the presence of 10, 50, or 100 μ*M* 8-aminoguanosine, the ID_{50} for 2′-deoxyguanosine was markedly reduced to 9.0, 3.5, and 1.5 μ*M*, respectively, in the MOLT-4 cell line. In contrast, the ID_{50} for 2′-deoxyguanosine in MGL-8 cells was 85 μ*M* in the absence of 8-aminoguanosine and 45 μ*M* in the presence of 100 μ*M* inhibitor (data not shown). These results reflect the selective toxicity of this combination for T lymphoblasts at low deoxyguanosine concentrations.

A corresponding selective accumulation of dGTP was observed in T cells, but not in B cells, incubated with 2′-deoxyguanosine alone, or in combination with 8-aminoguanosine (Table 1). The addition of 100 μ*M* 8-aminoguanosine increased dGTP levels by as much as fourfold in the presence of 2′-deoxyguanosine concentrations of up to 10 μ*M* in the T lymphoblasts. In the B cell line, however, 2′-deoxyguanosine concentrations of up to 20 μ*M* in the presence or absence of 8-aminoguanosine (100 μ*M*) did not significantly enhance dGTP accumulation.

The selective toxicity of 8-aminoguanosine for T lymphoblasts in the presence of 2′-deoxyguanosine could not be attributed to differences between T and B cell lines in either the levels of PNP or the sensitivity of PNP to the inhibitor. The MOLT-4 and MGL-8 lymphoblasts were harvested during exponential growth and lysed by freezing and thawing. The extracts were dialyzed overnight against 1000 volumes of 10 m*M* tris-HCl, *p*H 7.4, and centrifuged at 20,000*g* for 20 minutes. The specific activities of PNP in the T and B lymphoblast extracts, as determined by a radiochemical enzyme assay (*14*), were 36 and 54 nmole/min-mg, respectively. The K_m (Michaelis constant) of PNP for inosine was 40 μ*M* in the T cells and 58 μ*M* in the B cells. Finally, 8-aminoguanosine competitively inhibited PNP activity with a K_i of 8 μ*M* in both the T and B cell extracts.

Furthermore, the differential sensitivity of T cells was not related to differences in either the rate of metabolism or the transport of 8-aminoguanosine into the cell. Intact MOLT-4 and MGL-8 lymphoblasts were incubated in RPMI 1640 medium for up to 60 minutes at 37°C in the presence of [^{14}C]inosine (200 μ*M*; specific activity, 22 μCi/μmole) with and without 8-aminoguanosine (100 μ*M*). The hypoxanthine, inosine, and inosine monophosphate present in boiled extracts of cells plus medium were separated by high-voltage paper electrophoresis, and the ultraviolet-absorbing spots corresponding to the standards were counted, as described in (*14*). The PNP activity, as measured by the conversion of radioactive inosine to hypoxanthine and inosine monophosphate, was inhibited by more than 98 percent in both the T and B cells at time periods from 5 minutes to 1 hour. The concentration of 8-aminoguanosine in the medium, as determined by high-performance liquid chromatographic analysis on a C-18 μBondapak column (*15*), did not decline during incubation with either cell type. Finally, we further established that the selective T cell cytotoxicity associated with 8-aminoguanosine was mediated by PNP inhibition by demonstrating that 8-aminoguanosine (250 μ*M*) did not inhibit the enzymatic activity of either ADA or *S*-adenosylhomocysteine hydrolase in T cell extracts using K_m concentrations of substrates and previously described assay methods (*8*, *16*).

We conclude that 8-aminoguanosine is a potent competitive inhibitor of PNP with a K_i of 8 μ*M* for the enzyme from either T or B lymphoblasts. The synergistic combination of 8-aminoguanosine and low, nonlymphocytotoxic concentrations of 2′-deoxyguanosine causes profound selective T cell toxicity in a tissue culture system that is a biochemical model of PNP deficiency disease. This cytotoxicity correlates with a marked accumulation of dGTP in T cells exposed to the combination, suggesting a specific mechanism of action, probably involving feedback inhibition of ribonucleotide reductase by dGTP. The potential usefulness of 8-aminoguanosine or

Table 1. Effect of 8-aminoguanosine on dGTP levels in T and B lymphoblasts incubated with 2′-deoxyguanosine. Duplicate cultures containing 10^7 lymphoblasts were incubated in RPMI 1640 medium with 10 percent heat-inactivated horse serum for 4 hours in the presence of the additives indicated. Cells were then centrifuged at 400*g* for 5 minutes, the supernatant was removed, and the cells were extracted in 60 percent methanol at −20°C overnight. Assays of dGTP were performed by the DNA polymerase method, as described in (*3*). Values represent the mean of duplicate determinations.

2′-Deoxyguanosine (μ*M*)	dGTP (pmole/10^6 cells)	
	Without 8-aminoguanosine	With 100 μ*M* 8-aminoguanosine
MOLT-4: T lymphoblasts		
0	16.6	13.7
2.5	18.6	64.7
5.0	23.8	105.1
10.0	48.7	229.4
20.0	173.2	366.4
MGL-8: B lymphoblasts		
0	6.3	5.0
20.0	4.2	8.4

similar analogs in the treatment of T cell lymphoproliferative diseases or as immunosuppressive agents in the treatment of autoimmune disorders has yet to be explored. However, the results of this study suggest that 8-aminoguanosine could be both extremely effective as a lympholytic agent and more specific for T lymphocytes than are other agents that are currently available.

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Vibrio damsela, a Marine Bacterium, Causes Skin Ulcers on the Damselfish *Chromis punctipinnis*

Abstract. *A previously undescribed marine bacterium,* Vibrio damsela, *was isolated from naturally occurring skin ulcers on a species of temperate-water damselfish, the blacksmith* (Chromis punctipinnis). *Laboratory infection of the blacksmith with* Vibrio damsela *produced similar ulcers.* Vibrio damsela *was pathogenic for four other species of damselfish but not for members of other families of fish. The bacterium has also been isolated from water and from two human wounds and may be a cause of human disease.*

During the summer and fall spawning season in southern California, the blacksmith, a temperate-water damselfish (*Chromis punctipinnis*), may have irregular skin ulcers along its flanks (Fig. 1). There has been much speculation on the cause of these lesions, which are rare among naturally occurring marine fish. We now report that these skin lesions are caused by a newly described marine bacterium, *Vibrio damsela* sp. nov. (*1*), and we discuss the possible role of *V. damsela* in human wound infections.

The blacksmith is a small (total length, up to 35 cm), schooling, mid-water planktivore that lives along inshore reefs, to depths of 33 m, from Baja California to Monterey, California (*2*). Ulcerated *C. punctipinnis* have been observed from August through October in King Harbor, Redondo Beach, California, and from June through August off Ship Rock, Catalina Island (*3*). Although ulceration rates are often around 10 percent, in some aggregations more than 70 percent of the individuals had ulcers.

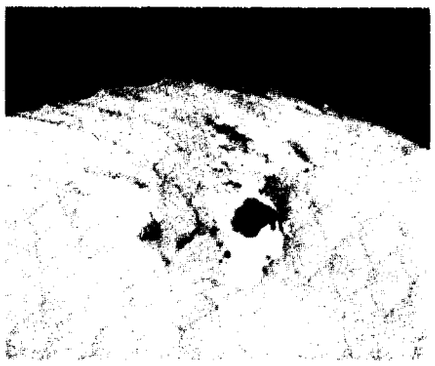

Fig. 1. Ulceration on skin of garibaldi (*Hypsypops rubicunda*) caused by laboratory infection with *Vibrio damsela*.

The ulcers are 0.5 to 2.0 cm in diameter and are usually near the pectoral fin and on the caudal peduncle. Histopathological examination of the lesions indicates a granulomatous ulcerative dermatitis. Lesions are characterized by muscle lysis and by histiocytes present in the dermis and skeletal muscles (*4*).

Bacterial isolations were made by swabbing the ulcerated lesions with a sterile Dacron swab and then streaking a brain-heart infusion agar culture medium containing 5 percent sheep blood. All bacterial incubations were carried out at 25°C unless otherwise stated. Cultures from the lesions yielded a number of different bacterial species and protozoan parasites, so that it was initially difficult to determine which, if any, was causing the lesion. Thirteen different bacteria were obtained in pure culture and tested for ability to produce ulcers. Healthy specimens of *C. punctipinnis* were collected from King Harbor and maintained in the laboratory (*5*). Eight fish were anesthetized with tricaine methane sulfonate (MS-222; 1:5000 in seawater). Four to six scales were removed from the flanks of each fish and the dermis scarified. The resulting lesions of four fish were swabbed with 10^7 to 10^8 viable cells. Four control fish were swabbed with sterile medium. Only *V. damsela* proved pathogenic.

Vibrio damsela produced large ulcers within 3 days and caused death in all animals within 4 days. Experimentally induced wounds in control animals had healed completely by this time. At the time of death, the ulcers in the fish inoculated with *V. damsela* were larger than the area originally scarified. Cultures from each ulcer yielded *V. damsela*. One of these cultures was reintro-

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