procedures (SIR, 84.4; 95% CI, 55.6–121.8). After the first year of follow-up, a deficit in cancers was observed in those who underwent cholecystectomy and combined procedures, although the difference was not statistically significant; 23 cancers were observed compared with 26.6 expected (SIR, 0.88; 95% CI, 0.56–1.31). A greater deficit in cancers, which was statistically significant, was observed 10 or more years after operation (SIR, 0.27; 95% CI, 0.06–0.80) among men and women undergoing cholecystectomy and combined procedures. Two potential sources of bias were examined: differences in the intensity of surveillance (or closeness of follow-up) and the accuracy of a bile duct cancer diagnosis between the study cohort and the general population. No evidence for either was detected.

The investigators concluded that gallstones probably play a causal role in the pathogenesis of bile duct cancer and that cholecystectomy markedly reduces the risk of extrahepatic bile duct cancer 10 years or more after the operation.

Comment. In patients from Western Europe and North America, virtually all gallstones are formed in the gallbladder. Because stones may pass from the gallbladder to the duodenum, a proportion of patients with gallbladder stones have common bile duct stones. Reports from patients undergoing common bile duct exploration either at the same time as cholecystectomy or following a previous cholecystectomy indicate that this proportion is about 10%–15% (Acta Chir Scand 1981;147:147–150).

Gallstones play a causal role in gallbladder cancer (Cancer epidemiology and prevention. Philadelphia: Saunders, 1982;683–691). Although the causes of extrahepatic bile duct cancer are unknown, gallstones have also been proposed to play a role (Surgery 1958;43:563–571). What information supports this hypothesis? Previous case-control research suggests an association between gallstones and bile duct cancer (Cancer 1987;59:2112–2116, Jpn J Cancer Res 1989;80:932–938). If gallstones are causally related to bile duct cancer, can a biologically plausible mechanism be offered to explain the association? Chronic bile duct inflammation seems to be the common feature among the diverse conditions associated with an increased risk of bile duct cancer, including liver flukes (Cancer 1973:31:468–473), primary sclerosing cholangitis (Ann Surg 1990;213:21–25), and choledochal cysts (Cancer 1977;40:880–883, Cancer 1979;44:1134–1141).

This study is the first to examine the incidence of extrahepatic bile duct cancer after cholecystectomy in a population-based cohort, which is a more rigorous research design than that of previous case-control studies (Cancer 1987;59:2112–2116, Jpn J Cancer Res 1989;80:932–938). The significant decrease in the incidence of extrahepatic bile duct cancer observed 10 or more years after the procedure suggests a long-term protective effect of cholecystectomy. The hypothesis that cholecystectomy may confer a long-term protective effect for cancer is not contradicted by the 59 cases that were identified within 1 year of surgery or the 20 cases that occurred from 1 to 10 years after the operation. The former were likely to have been present at the time of surgery, and the latter were likely to have been initiated before the surgery. If gallstones promote cancer, the process would be a slow one, and any protective effect of cholecystectomy would not be manifest in the early years after surgery.

The evidence from this cohort research is reasonably strong. If gallstones are causally related to bile duct cancer, what are the implications for clinical practice? Here, one must be cautious. The research was intended to address a question of biology (the relationship between gallstones and bile duct cancer), not one of patient management. We should not use the evidence to advocate cholecystectomy in patients with gallstones in an effort to reduce the risk of bile duct cancer. Further research specifically aimed at comparing the outcomes, costs, and cost-effectiveness of alternative strategies is needed to address this question.

LINDA RABENECK, M.D.

PARACRINE REGULATION OF INTESTINAL SECRETION

Madara JL, Patapoff TW, Gilles-Castro B, Colgan SP, Parkho CA, Delp C, Merney RJ (Division of Gastrointestinal Pathology, Department of Pathology, Brigham and Women’s Hospital, and Harvard Medical School, Boston, Massachusetts; and Genentech, Inc., South San Francisco, California). 5'-Adenosine monophosphate is the neutrophil-derived paracrine factor that elicits chloride secretion from T84 intestinal epithelial cell monolayers. J Clin Invest 1995;91:2320–2325 (May).

Secretion of fluid and electrolytes across the epithelial lining of intestinal crypts contributes to a broad range of intestinal functions. At the cellular level, efflux of Cl− through channels in the apical membrane is thought to be the rate-limiting step for secretion. These channels are regulated by changes in intracellular 5'-cyclic adenosine monophosphate, Ca2+, and other signaling factors that modulate the probability of opening and, accordingly, modulate transepithelial secretion (Proc Natl Acad Sci USA 1990;87:4956–4960). Traditionally, intestinal secretion has been thought to be regulated primarily by circulating hormones. The corresponding receptors are localized to the basolateral region, which is an anatomic orientation well suited to respond to varying hormone concentrations in the circulation. However, secretion is also influenced by factors within the intestinal lumen. The studies in this report identify 5'-adenosine monophosphate (5'-AMP) as a factor released by neutrophils that, after breakdown to adenosine, stimulates secretion from the luminal surface, presumably through activation of receptors in the apical membrane of epithelial cells.

Previous work by these investigators has shown that exposure of T84 monolayers to activated neutrophils stimulates vectorial Cl− transport, which is the electrophysiological equivalent of intestinal secretion. This represents an important component of the response to inflammation of many different causes. Interestingly, secretion is stimulated by application of neutrophils to the apical surface but does not require direct interactions between neutrophils and the cell surface because conditioned media is capable of eliciting the same effect. This led to the conclusion that luminal neutrophils release soluble factor(s) referred to as neutrophil-derived secretagogues (NDS) that interact with intestinal cells to stimulate secretion. The principal purpose of these studies was to identify NDS and determine how it modulates intestinal transport.

NDS-containing media was obtained by activating human neutrophils by exposure to phorbol myristic acid or lysing HL-
were size-filtered through a 1000-mol wt membrane, providing an enriched source for subsequent characterization. Isolation was guided by measurement of NDS bioactivity in an short-circuit current when applied to the apical membrane across T84 cell monolayers.

Application of NDS-enriched media elicited an increase in short-circuit current when applied to the apical membrane as previously described. When the NDS-enriched media was passed over a high-performance liquid chromatography anion exchange column, several absorbance peaks emerged. Separate testing showed that only the late fractions were capable of eliciting the short-circuit current response. Importantly, the biologically active peaks were clearly separated from adenosine. However, preincubation with either adenosine deaminase or 5′-adenylic acid deaminase eliminated NDS activity, suggesting that NDS activity may be related to the presence of an adenine nucleotide. Subsequent chemical evaluation showed that phosphorus was present in roughly equimolar amounts with adenosine, and mass spectral analyses showed that purified NDS shares a spectrum identical to 5′-AMP. In aggregate, these and other observations provide compelling evidence that 5′-AMP is the secretagogue released from activated neutrophils.

Extracellular 5′-AMP could activate P2 receptors directly. Alternatively, it could be degraded to the P1 receptor agonist adenosine by ectonucleotidases present on the cell surface. Consequently, structural analogues of 5′-AMP were also evaluated to determine their relative potency in stimulation of short-circuit current. An intact adenosine moiety appeared to be necessary for full activity because 5′-AMP, 3′-adenosine monophosphate, 5′-o-thio-adenosine monophosphate, adenosine diphosphate, adenosine triphosphate (ATP), and adenosine each caused comparable stimulation. However, the P2, preferring agonist uridine triphosphate had no effect, and nonhydrolyzable analogues of ATP failed to elicit any secretory response. This eliminates the possibility that 5′-AMP is serving as a partial agonist for P1 receptors. In contrast, the adenosine receptor antagonist 8-phenyltheophylline effectively blocked the response to both 5′-AMP and adenosine; the response to 5′-AMP was also blocked by inhibition of ectonucleotidase activity. These findings indicate that 5′-AMP does not stimulate secretion directly. Instead, breakdown by ectonucleotidases results in local generation of adenosine.

The secretory potency of 5′-AMP was an order of magnitude greater when applied to the apical vs. basolateral membrane. The 50% effective concentration for apical 5′-AMP was ~2 μmol/L, the same concentration had little or no effect when applied to the basolateral membrane. This concentration compares favorably with the 50% effective concentration estimated for apical NDS and to the concentrations of 5′-AMP found in supernatant solutions from isolated neutrophils. Moreover, the observed polarity of the response provides the best evidence to date for an apical orientation of the responsible receptors. Whereas adenosine was also released from neutrophils, the molar ratio of 5′-AMP exceeded adenosine 10- to 100-fold.

In summary, these studies identify 5′-AMP as the neutrophil-derived secretagogue that modulates intestinal Cl− transport. The biological effects require degradation of 5′-AMP to adenosine, which in turn activates receptors in the apical membrane. These observations support a novel working model for 5′-AMP as a paracrine factor mediating interactions between luminal neutrophils and intestinal epithelia, which results in stimulation of transepithelial secretion.

Comment. ATP is maintained inside most mammalian cells at concentrations of 2–5 mmol/L. Its central role as an intracellular source of high-energy phosphates, powering many transport and regulatory processes, is well established. Recently, ATP metabolites have also been identified outside of the cell, and considerable evidence has emerged to show that ATP and its metabolites can serve as signaling molecules, modulating a wide range of biological activities (Ann NY Acad Sci 1990;603:1-17). The present studies represent an important extension of this concept to gastrointestinal tissues by identifying 5′-AMP as the neutrophil-derived secretagogue that appears to work in a paracrine manner to modulate intestinal ion transport.

Extracellular nucleotides exert their effects on target tissues through activation of purinergic receptors. In general, these can be categorized as P1 receptors, which are preferentially activated by adenosine and are positively or negatively coupled to adenylyl cyclase, or as P2 receptors, which are preferentially activated by ATP (or uridine triphosphate) and coupled to phospholipase C and changes in [Ca2+]i.

This is an oversimplification of a rapidly growing field that has identified at least three types of P1 and five types of P2 receptors with coupling to diverse signaling pathways. Detailed study has been hindered by the lack of selective agonists and antagonists and by the frequent coexistence of more than one type of receptor. However, application of molecular techniques has resulted in cloning of several P1 receptors and, more recently, cloning of mouse brain P2x (Proc Natl Acad Sci USA 1993;90:5113–5117) and chick brain P2y (FEBS Lett 1993;324:219–225) receptors. Similar approaches promise to further clarify the classification and function of the purinergic receptors involved in epithelial secretion.

The identification of 5′-AMP as the neutrophil-derived secretagogue is well supported by complementary high-performance liquid chromatography and spectroscopic and functional techniques. Based on the relative potency of different nucleotides in the stimulation of short-circuit current, it seems equally clear that it is not 5′-AMP but adenosine that stimulates secretion. This is based on observations that inhibition of ectonucleotidase activity or inhibition of adenosine receptors blocks the increase in short-circuit current. Consequently, it is attractive to speculate that the apical receptor involved is in the P1 family and is positively coupled to adenylyl cyclase and that CI− efflux is mediated by Cl− channels previously localized to the apical membrane of T84 cells (J Clin Invest 1992;89:339–349). Interestingly, basolateral ATP has been shown to stimulate Cl− efflux from T84 cells through a Ca2+-dependent mechanism that presumably involves separate Cl− channels (Am J Physiol 1992;262:C67–C74). Assuming that observations in T84 cells are applicable to intestinal crypt epithelia, then an entirely separate purinergic network localized to the basolateral region and involving different receptors, intracellular signals, and channels may operate in parallel with the apical pathway.

The model of paracrine regulation developed in these studies compares favorably with emerging models of purinergic regulation in other settings. For example, vessel-mediated release of adenosine diphosphate from activated platelets induces local vasconstriction...
through activation of P2 receptors on the apical membrane of vascular endothelial cells (Ann NY Acad Sci 1990;603:1–17). Similarly, receptors for ATP stimulate ion transport pathways in human airway epithelia (Mol Pharmacol 1991;40:648–635) and amphibian gallbladder (J Gen Physiol 1991;97:949–971). In each case, the responsible receptors are localized to the apical membrane, but the origin of ATP in tracheal secretions and bile is not established. The presence of inflammatory cells could result in local release of nucleotides, as observed in the intestine. Alternatively, recent studies suggest that the multidrug-resistance (mdr1) gene product may serve as an ATP channel (Proc Natl Acad Sci USA 1993;90:312–316). This raises the possibility that regulated nucleotide release can occur locally in the absence of inflammation.

Like all good studies, this one invites additional investigation on several fronts. First, T84 cells provide an excellent model for investigation of intestinal secretion, but the applicability of these observations to tissue in vivo remains to be established. Second, it seems likely that apical adenosine receptors might also contribute to regulation of intestinal transport in the absence of inflammation. If so, then an alternative source for luminal nucleotides would be anticipated. This could be the epithelial cells themselves, representing an autocrine regulatory loop. Finally, the working model suggests several steps for regulation of neutrophil and epithelial interactions. Evaluation of the mechanisms responsible for nucleotide generation and release in neutrophils, nucleotide degradation by ectonucleotidases, and adenosine receptor signaling in the target tissues represent attractive opportunities for pharmacological manipulation of the biological response to inflammation.

Greg Fitz, M.D.

WHAT KIND OF FOOD AM I?


Chlebowski et al. noted that malnutrition is a common problem late in the course of acquired immunodeficiency syndrome (AIDS) and that such malnutrition is associated with a poor prognosis. The patients in the study were enrolled because they were judged to be at risk for the development of nutritional complications due to advanced disease. All patients were able to consume enteral feedings, and the result was that the study compared two diets: a standard diet and a peptide-based diet. The study measured a variety of outcomes, including weight change, muscle mass, infection rates, and hospitalization rates.

The standard diet was a standard enteral formula (Ensure; Ross Laboratories, Columbus, OH) with a standard supplement (Advera; Ross Laboratories, Columbus, OH). The experimental diet differed from the standard diet in a variety of ways; it contained a "patented" protein hydrolysate, a higher carbohydrate content (including soy polysaccharide), a lower fat concentration (with different fat sources, namely, canola oil, medium chain triglycerides, and deodorized sardine oil), and fiber. Eighty patients with early-stage HIV infection were randomized to receiving one of the supplements (2–3 eight-ounce cans per day for 6 months). They were assessed at 3 and 6 months for a variety of nutritional and biochemical parameters as well as for the development of gastrointestinal symptoms, their performance status (including workdays missed), and intercurrent illness (including hospitalization). At the conclusion of the study, 56 of the 80 initially enrolled patients (31 experimental and 25 standard) were considered for evaluation.

Each group consumed comparable daily volumes of the supplement (1.3–1.5 cans per day). No increase in any gastrointestinal symptoms was found in either group. Only the members of the experimental group gained weight (+4 lb vs. −1.5 lb; P = 0.04). The average triceps skinfold thickness decreased during the 6 months in the members of the standard group but did not change in those consuming the experimental supplement. No dramatic biochemical changes were found between the two groups; the recipients of the standard formulation had a slightly higher blood urea nitrogen and aspartate aminotransferase levels.

Perhaps the most important difference observed was in the subsequent hospitalization rates. In the latter 3 months, one in the experimental group was hospitalized. In contrast, 5 of the 25 standard supplement recipients (20%) were hospitalized (P < 0.05). It should be noted that during the entire course of the study, there was no statistically significant difference in these rates because a few more in the experimental group (7 of 31 [23%] vs. 4 of 25 [16%]; P > 0.05) were hospitalized in the first 3 months. The number of workdays missed in the two 3-month periods paralleled the hospitalization rates. The total number of days missed (if the data were reported as the average per patient) ranged between 45 and 60 days per 13 weeks, suggesting that most of these patients were not at work very often.

Comment. In virtually any disease, the presence of malnutrition predicts a poor clinical outcome, this association was observed more than 50 years ago (JAMA 1936;106:458–460). Since the 1960s, clinicians, apparently assuming that the association is a causative one, have been providing various regimens of nutritional support to their patients. Although the nutritional formulations have been marketed under the Food and Drug Administration regulations governing foods, they have been used in a manner that is philosophically more akin to the way medications are used, namely as adjunctive therapy to reduce the morbidity and/or mortality of an underlying disease. It has been distressing, at least to me, that a large number of PRCTs have been unable to show that nutritional support favorably affects the clinical outcome of those underlying diseases (Gastroenterology 1980;78:393–410, Dig Dis Sci 1984;29:577–588), even though such interventions usually do improve a variety of nutritional parameters (e.g., body weight or nitrogen balance) (Gastroenterology 1989;96:A269). From these observations, one could conclude that the association between malnutrition and poor outcome is not causative. On the other hand, it may be that generic nutritional support simply does not correct the specific nutrient deficiencies that account for the adverse clinical outcomes.

More recently, research efforts have begun to focus on a different approach to food formulation. These second-generation "diets" con-