DECREASED GASTRIN SECRETION IN PATIENTS WITH LATE-ONSET HYPOGAMMAGLOBULINEMIA

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Abstract We undertook this study to determine whether patients with late-onset hypogammaglobulinemia, who are at very high risk for gastric cancer, have a reduced secretion of gastrin after stimulation with food or bombesin, a potent gastrin-releasing stimulus. We compared the plasma gastrin responses to bombesin and to a standard test meal in 18 patients with late-onset hypogammaglobulinemia with those in patients with X-linked agammaglobulinemia, early-onset hypogammaglobulinemia, or hypogammaglobulinemia due to lymphoproliferative cancer, and in 30 normal control subjects.

Thirteen of 18 patients with late-onset hypogammaglobulinemia (72 percent) had an abnormally low gastrin response to bombesin, as compared with none of 21 patients with other forms of hypogammaglobulinemia (P<0.05). After a test meal, abnormally low gastrin secretion was found in 6 of 14 patients with late-onset hypogammaglobulinemia (43 percent) and in 1 of 18 patients with other forms of the disease (6 percent) (P not significant).

The plasma gastrin responses to stimulation with bombesin or food distinguished late-onset hypogammaglobulinemia from other forms, with sensitivities of 72 and 43 percent and specificities of 100 and 94 percent, respectively. Stimulated gastrin response can therefore be used as a marker for this type of immunodeficiency. The test responses also showed heterogeneity among patients with late-onset hypogammaglobulinemia and may help to identify patients with an increased risk for gastric cancer.

HYPOGAMMAGLOBULINEMIA comprises a heterogeneous group of immunodeficiency disorders. Of the congenital forms, X-linked agammaglobulinemia has been studied the most thoroughly and is characterized by an absence of B lymphocytes in the bone marrow, peripheral blood, and lymphoid tissues. There are other forms of early-onset hypogammaglobulinemia, but differentiating them from isolated forms of X-linked agammaglobulinemia or from early manifestations of late-onset hypogammaglobulinemia is difficult. When primary hypogammaglobulinemia develops later in life, the diagnosis of late-onset hypogammaglobulinemia (also called common variable immunodeficiency) is clear. In patients with late-onset hypogammaglobulinemia, B lymphocytes are usually present, but they do not differentiate into plasma cells. This humoral immunodeficiency is often accompanied by abnormalities in the T lymphocytes. The disease has been classified as a combined immunodeficiency disorder by the World Health Organization. The cellular immune defects accompanying late-onset hypogammaglobulinemia are by no means constant or homogeneous, and it remains uncertain whether late-onset hypogammaglobulinemia represents a single entity. Low serum concentrations of gammaglobulin can occur not only in primary forms of hypogammaglobulinemia but also in hematologic cancer or intestinal or renal disease (as a result of loss of protein). In addition to an increased susceptibility to infection, patients with primary hypogammaglobulinemia often have abnormalities of the gastrointestinal tract, including malabsorption, lymphonodular small-intestinal hyperplasia, pernicious anemia, and gastric cancer. These gastrointestinal disorders have been...
reported mainly in patients with late-onset hypogammaglobulinemia,\textsuperscript{5,14,24,25} but recent studies indicate that patients with other types of hypogammaglobulinemia may also have such abnormalities.\textsuperscript{15,26} Patients with late-onset hypogammaglobulinemia have a 50-fold increase in the incidence of stomach cancer.\textsuperscript{27} Patients with the intestinal type of gastric cancer have a reduced plasma gastrin response to the gastrin-releasing peptide bombesin.\textsuperscript{28} We therefore studied plasma gastrin secretion during bombesin infusion in patients with various forms of hypogammaglobulinemia to determine whether such patients have abnormalities in gastrin secretion after stimulation and whether those abnormalities are specific to subgroups of patients with hypogammaglobulinemia. Since bombesin is not readily available, we also measured postprandial plasma gastrin levels to determine whether bombesin can be replaced by a meal as a stimulant of gastrin secretion.

**METHODS**

**Patients**

The study was performed in a group of 33 patients with primary hypogammaglobulinemia, consisting of 10 male patients with X-linked hypogammaglobulinemia (mean age, 23 years; range, 14 to 35), 5 patients (4 male and 1 female patient) with early-onset hypogammaglobulinemia (mean age, 22 years; range, 17 to 25), and 18 patients (13 men and 5 women) with late-onset hypogammaglobulinemia (mean age, 42 years; range, 21 to 67). We also studied six patients (five men and one woman; 40 to 70 years of age) with hypogammaglobulinemia due to lymphoproliferative cancer—two with chronic lymphocytic leukemia, one with non-Hodgkin’s lymphoma, and three with multiple myeloma. Thirty normal subjects (24 men and 6 women; mean age, 43 years; range, 24 to 72) with neither immunodeficiency nor recurrent infection served as controls. Before treatment, all patients with hypogammaglobulinemia except one had markedly reduced serum gammaglobulin concentrations (range, 0 to 4.5 g per liter). One patient with a gammaglobulin concentration of 6.0 g per liter, a member of a family with X-linked hypogammaglobulinemia (mean age, 39 years; range, 24 to 72) with neither immunodeficiency nor recurrent infection served as controls. Before treatment, all patients with hypogammaglobulinemia except one had markedly reduced serum gammaglobulin concentrations (range, 0 to 4.5 g per liter). One patient with a gammaglobulin concentration of 6.0 g per liter, a member of a family with X-linked hypogammaglobulinemia, had no IgA, IgM, IgG2, or IgG4, but had nearly normal concentrations of IgG1 and IgG3—a finding that has been reported elsewhere.\textsuperscript{29}

Informed consent was obtained from all subjects, and the protocol was approved by the ethics committee of the University Hospital of Leiden.

**Criteria**

The criteria for the diagnosis of X-linked agammaglobulinemia were the onset of clinical signs during the first two years of life, a family history compatible with an X-linked pattern of inheritance, and an absence of or profound decrease in B lymphocytes in the peripheral blood or bone marrow. In patients in whom clinical symptoms began before the age of two years but no evidence for X-linked inheritance was found, the diagnosis of early-onset hypogammaglobulinemia was made. In these patients, B lymphocytes were either absent, scarce, or present in normal numbers. Patients were considered to have late-onset hypogammaglobulinemia if clinical manifestations began after the age of 10 years and B lymphocytes, but not plasma cells, were found in the bone marrow.

**Bombesin-Infusion and Food Stimulation of Gastrin Secretion**

Patients were studied after they had fasted overnight. Plasma gastrin levels after stimulation with bombesin were measured in all subjects, whereas gastrin levels after stimulation with food were measured in all 10 patients with X-linked agammaglobulinemia, in 14 of the 18 patients with late-onset hypogammaglobulinemia, in 4 of the 5 patients with the early-onset form, in 4 of the 6 patients with hypogammaglobulinemia due to lymphoproliferative cancer, and in all 30 control subjects. Synthetic bombesin-14 (UCB, Brussels) was infused intravenously (60 pmol per kilogram of body weight for 20 minutes).\textsuperscript{28,30,31} Blood samples for measurements of gastrin were obtained at −5, 0, 5, 10, 15, and 20 minutes. The standard test meal consisted of one slice of bread, 50 g of cheese, 15 g of margarine, one boiled egg, and 200 ml of skim milk. Serum gastrin was measured at 15-minute intervals for 120 minutes. Plasma gastrin levels were assessed by radioimmunoassay.\textsuperscript{30,31}

**Statistical Analysis**

A mean basal plasma concentration of gastrin was calculated. The integrated plasma gastrin secretion was determined by calculating the area under the time curve for plasma concentration, after basal values had been subtracted. To calculate the incremental value, the mean basal level was subtracted from the peak plasma gastrin concentration during stimulation. Statistical analysis was performed with use of the chi-square test with Yates’ correction and Spearman’s correlation test.

**RESULTS**

In the normal subjects, plasma concentrations of gastrin ranged from 10 to 40 pmol per liter. A reduced basal plasma concentration of gastrin after bombesin stimulation was observed in 6 of the 18 patients (33 percent) with late-onset hypogammaglobulinemia as compared with none of the 21 patients with the other forms of hypogammaglobulinemia (P<0.05). Both bombesin and food increased plasma gastrin levels in all groups of subjects studied (Fig. 1 and 2). A reduction in integrated plasma gastrin secretion in response to bombesin was observed in 13 of the 18 patients (72 percent) with late-onset hypogammaglobulinemia, as compared with none of the 21 patients with other forms of hypogammaglobulinemia (P<0.05; Fig. 1), whereas a reduced postprandial level of plasma gastrin secretion was found in 6 of the 14 (43 percent) and 1 of the 18 patients (6 percent) with late-onset and other forms of hypogammaglobulinemia, respectively (P not significant; Fig. 2). The incremental plasma gastrin secretion in the patients with late-onset hypogammaglobulinemia was reduced in 13 of the 18 patients who received bombesin (72 percent) and in 4 of the 14 who were given food (29 percent), as compared with none of the 21 and 18 patients with other forms of hypogammaglobulinemia who received bombesin (P<0.05) and food (P not significant), respectively.

The low levels of plasma gastrin secretion in the patients with late-onset hypogammaglobulinemia were not correlated with age (r = 0.19), sex, or duration of illness (r = 0.12).

**DISCUSSION**

We found a reduced gastrin response to the infusion of bombesin or the ingestion of food in 33 and 22 percent, respectively, of patients with hypogammaglobulinemia. Interestingly, these abnormalities were restricted to patients with late-onset hypogammaglobulinemia.
integrated plasma gastrin (pM - 20min)

Figure 1. Integrated Plasma Gastrin Secretion during the Infusion of Bombesin.
Bombesin was given to 30 normal subjects, 18 patients with late-onset hypogammaglobulinemia, and 21 patients with other forms of hypogammaglobulinemia: 10 with X-linked agammaglobulinemia (crosses), 5 with early-onset hypogammaglobulinemia (solid circles), and 6 with secondary hypogammaglobulinemia (triangles). pM-20min denotes picomoles per liter per 20 minutes.

ulineria; none of the 21 patients with other forms of hypogammaglobulinemia had reduced plasma gastrin secretion. A reduced integrated plasma gastrin response but a normal incremental response to stimulation with food was observed in only 1 of the 18 patients with the other forms of hypogammaglobulinemia. Among the patients with late-onset hypogammaglobulinemia, 72 percent had an abnormally low gastrin response to bombesin, and 43 percent had a reduced response to food. Thus, the plasma gastrin response to bombesin distinguished late-onset hypogammaglobulinemia from the other forms, with a sensitivity of 72 percent and a specificity of 100 percent. This discrimination was better than that obtained with the food-stimulation test, which yielded a sensitivity of 43 percent and a specificity of 94 percent. Since there was no correlation between a low plasma gastrin response and the age of the patient, or the duration of clinical symptoms, it is unlikely that such a response is merely a late manifestation of any type of hypogammaglobulinemia. Furthermore, gastrin responses were normal in the 10 patients with X-linked agammaglobulinemia, whose ages overlapped with those of patients with the late-onset form.

Our findings may have clinical importance. The stimulation test may help in the classification of cases of primary hypogammaglobulinemia. Although various immunologic markers are available, such classification is difficult in certain patients.1,2,6 Also, the test may aid in the differentiation of primary hypogammaglobulinemia from hypogammaglobulinemia due to chronic lymphocytic leukemia and other lymphoproliferative cancers.11 We do not know whether, among patients with late-onset hypogammaglobulinemia, those with and without an abnormal gastrin response represent separate disease entities; this must be determined in large comparative studies.
We observed no obvious clinical or biochemical differences between the two groups.

The test may also provide a noninvasive way to identify patients with hypogammaglobulinemia who are at risk for gastric cancer. It has been shown that among patients with late-onset hypogammaglobulinemia, there is a 50-fold increase in the incidence of gastric cancer; such an elevation has not been observed among patients with other types of hypogammaglobulinemia. In fact, patients with late-onset hypogammaglobulinemia have the highest known risk of gastric cancer. Therefore, a low plasma gastrin response to bombesin or food may serve as a marker in the selection of patients for prospective gastroscope evaluation. Since a low plasma gastrin response to bombesin has been found in patients with gastric cancer but normal serum gammaglobulin levels, it is tempting to hypothesize that the development of gastric cancer is restricted to patients with abnormal gastrin secretion. Prospective studies are needed to support or reject this hypothesis, however.

The mechanism of the low plasma gastrin response to bombesin and food is unknown. Bombesin, a neuropeptide, is involved in the vagal stimulation of gastrin release from the gastric antrum. The infusion of bombesin is a potent stimulus of gastrin secretion in several species of animals and in humans. Unlike food, which stimulates gastrin release from both the antrum and the duodenum, bombesin mainly stimulates the antral G cells. The normal postprandial secretion of plasma gastrin in some patients with late-onset hypogammaglobulinemia, despite a reduced response to bombesin, may be caused by release of gastrin from the duodenum after stimulation with food. It is not known whether the abnormally low gastrin response is due to a reduced sensitivity of antral G cells to stimulation with bombesin or food, or a reduction in the number of G cells. It should be noted that none of our patients had abnormal gastric symptoms. Low basal plasma concentrations of gastrin have previously been reported in patients with pernicious anemia associated with hypogammaglobulinemia, whereas elevated gastrin levels are usually observed in patients with pernicious anemia who have normal concentrations of serum gammaglobulin. In the present study, abnormally low plasma gastrin secretion was not restricted to patients with pernicious anemia (3 of the 18 patients studied).

We have shown that, in contrast to patients with other types of hypogammaglobulinemia, the majority of patients with late-onset hypogammaglobulinemia have a reduced plasma gastrin response to food or bombesin. Plasma gastrin secretion can therefore be used as a marker of this type of immunodeficiency. Since bombesin is not readily available, stimulation with food may be the preferred initial diagnostic procedure, although the sensitivity of the test of postprandial gastrin secretion is somewhat lower than that of the test using bombesin. Both tests indicate heterogeneity among patients with late-onset hypogammaglobulinemia and may help to identify those with an increased risk of gastric cancer.

References

CLINICAL IMPORTANCE OF NEAR-DIPLOID TUMOR STEM LINES IN PATIENTS WITH OSTEOSARCOMA OF AN EXTREMITY

A. Thomas Look, M.D., Edwin C. Douglass, M.D., and William H. Meyer, M.D.

Abstract We determined the clinical value of flow-cytometric measurement of tumor-cell DNA content, which reflects the chromosome number (ploidy), in patients with osteosarcoma of an extremity. Hyperdiploid stem lines were identified in 25 of 26 tumor samples obtained at diagnosis from patients who did not have clinically overt metastases. Near-diploid tumor stem lines coexisted with hyperdiploid lines in 15 of these 25 cases; an isolated near-diploid line was present in the 26th case. All 26 patients underwent definitive surgery and then were treated uniformly with intensive adjuvant combination chemotherapy.

Kaplan–Meier analysis of both relapse-free and overall survival times showed that the presence of a near-diploid tumor stem line was associated with improved outcome (P = 0.003 for each comparison). After a median follow-up time of three years, pulmonary metastases developed in only 2 patients in the group with near-diploid lines, in contrast to 7 of the 10 with hyperdiploid lines exclusively. Near diploidy remained significantly associated with improved relapse-free survival after adjustment for the influence of age, the only clinical variable that showed prognostic strength in this analysis (P < 0.01; relative risk, 0.08; 95 percent confidence interval, 0.02 to 0.48).

Our findings demonstrate the usefulness of flow-cytometric determination of tumor-cell ploidy for predicting the sensitivity of histologically high-grade osteosarcoma to chemotherapeutic agents. Patients with a near-diploid tumor stem line can be expected to respond favorably to adjuvant chemotherapy as used in this study, whereas those with only hyperdiploid lines should be considered as candidates for alternative therapy. (N Engl J Med 1988; 318:1567-72.)

OSTEOSARCOMA is a rapidly metastasizing bone tumor that poses difficult challenges to the therapist. Early development of pulmonary metastases in most patients who have undergone amputation but not adjuvant chemotherapy indicates that clinically inapparent tumor cells are usually present in the lungs at the time of diagnosis. Recently, two groups of investigators established in controlled clinical trials that multiagent chemotherapy administered after definitive surgery improves the relapse-free survival of patients with high-grade osteosarcoma. However, in those studies and in others, metastases eventually appeared in approximately one third to one half of all patients who received adjuvant treatment.

A major obstacle to the development of uniformly effective adjuvant chemotherapy for osteosarcoma has been the lack of understanding of the biologic factors that determine the sensitivity of a particular tumor to drugs. As a first step toward identifying clinically useful prognostic markers in this disease, we measured the DNA content of tumor cells collected before treatment in patients who had been enrolled in the multi-institutional study of Link et al. This approach was attractive because measurement of cellular DNA content according to flow-cytometric techniques provides reliable estimates of the chromosome number (ploidy) in malignant stem lines and correlates well with the response to chemotherapy in childhood acute lymphoblastic leukemia, neuroblastoma of infants, and Wilms’ tumor. Hiddemann et al. have shown that measurement of DNA in patients with osteosarcoma can distinguish between hyperdiploid tumors with highly malignant features and tumors classified as parosteal variants, which have diploid DNA stem lines, well-differentiated histologic features, and a relatively favorable prognosis.

The results of our investigation indicate that histologically high-grade osteosarcomas with near-diploid stem lines respond significantly better to adjuvant combination chemotherapy than do tumors with only hyperdiploid lines. Patients whose tumors do not have...