Gastrin, Secretin, GIP and VIP Alter Levels of IL-2 and IFN-γ in Human Peripheral Blood Mononuclear Cells under Various Culture Conditions

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Abstract

Background: Gastrointestinal hormones have traditionally been viewed as mere regulators of gut movement and secretions, but, it is becoming increasingly apparent that other body systems may be affected by these hormones. Secretion of gut hormones is influenced by the type of food we take. Therefore, the more we know about the effects of gut hormones on the various body tissues, the more we know about the different mechanisms by which our diets affect our health. **Objectives:** This in vitro study aimed to explore the effects of physiologically-relevant concentrations of four gut hormones on the production of IL-2 and IFN-γ by human peripheral blood mononuclear cells and how culture conditions may modify those effects. **Methods:** Peripheral blood mononuclear cells were separated by density gradient centrifugation from the blood of 15 adults. Cells were cultured with/without PHA and treated with four concentrations of gastrin, secretin, GIP and VIP. IL-2 and IFN-γ in culture supernatants were assayed by ELISA. **Results:** Gastrin, secretin, GIP and VIP increased IL-2 and IFN-γ levels under some culture conditions and depressed IL-2 under other conditions. An increase was often observed under culture conditions in which the cytokine production was not initially high. Repeated administration of the hormone was also more likely to result in a stimulatory effect. **Conclusions:** Physiologically-relevant concentrations of gastrin, secretin, GIP and VIP are potential immunomodulators as they have shown their ability to alter the production of IL-2 and/or IFN-γ under various culture conditions.

Keywords: Gastrin, Secretin, GIP, VIP, IL-2, IFN-γ

INTRODUCTION

The numerous peptides, of mucosal and neural origin, collectively known as the gastrointestinal hormones, are not really exclusive products and regulators of the gastrointestinal tract (GIT). Many of these peptides are expressed in extra-intestinal tissues, notably, the neuroendocrine tissues, the central nervous system and the urogentital tract (1).

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However, production of these peptides by extra-intestinal tissues seems to serve the local needs of communication and growth regulation rather than systemic hormonal effects (1). On the other hand, when the same peptides are secreted by the gastrointestinal cells, they behave, not only in paracrine and autocrine fashions, but as standard hormones in the sense that they enter the circulation to reach the various body tissues (1, 2). It is, therefore, important to explore the possibility that these hormones are not mere regulators of the secretions and the motility of the gastrointestinal tract, but could have effects on other body tissues. The secretion of these hormones by GIT cells is influenced by the type of our diet (3). Hence, the more we know about the effects of GIT hormones on the various body tissues, the more we know about possible mechanisms by which food may affect our health

The gastrointestinal tract has large mucosal collections of immune cells; the gut associated lymphoid tissues (GALT). Are these immune cells not targeted by GIT hormones? It has been noticed that the numbers of lymphocytes in these collections are reduced in patients and animals on parenteral nutrition but not those on enteral nutrition (4). Some researchers have concluded that exogenous administration of neuropeptide GIT hormones may preserve the integrity of mucosal immune mechanisms in patients on parenteral nutrition (5). It seems that the immune system is an important potential target for GIT hormones.

Cytokines control the proliferation and activation of the cells of the immune system. Researchers have reported conflicting stimulatory and inhibitory effects of GIT hormones on cytokine production, migration and proliferation of immune cells (6, 7). Furthermore, there is no comprehensive data on which GIT hormone affects the production of which cytokine. This *in vitro* study of human peripheral blood mononuclear cells (PBMC) was designed to investigate the ability of physiologically-relevant concentrations of gastrin, secretin, gastric inhibitory peptide (GIP) and vasoactive intestinal peptide (VIP) to influence the production of two cytokines that are central in adaptive and natural immunity; IL-2 and IFN-γ. We also aimed to determine whether culture conditions could be a factor influencing the response of immune cells to these hormones.

MATERIALS AND METHODS

RPMI-1640 culture medium (with L-glutamine and sodium bicarbonate), heat inactivated fetal calf serum, antibiotic solution, phosphate buffered saline, phytohaemagglutinin (PHA), human recombinant Gastrin, human recombinant Secretin, human recombinant GIP, human recombinant VIP, and Histopaque density gradient solution were all supplied by Sigma Aldrich company. The kits used for measurements of the cytokines IL-2 (minimum detectable concentration less than 4pg/ml) and IFN-γ (minimum detectable concentration less than 15pg/ml) were supplied by RayBiotech, Incorporation.

Preparation of the Culture Medium. Complete RPMI medium was prepared by adding antibiotics (penicillin 100u/ml and streptomycin 100μg/ml final concentration) and heat-inactivated calf serum (5% final concentration).

Preparation of Peripheral Blood Mononuclear Cells. Fifteen healthy male volunteers from the staff of AlQassim University donated the blood samples. 25 ml of venous blood were collected aseptically in heparinized 50 ml tubes (final concentration of heparin was 20 units/ml). The blood was diluted 1:1 with phosphate buffered saline (PBS). The peripheral blood mononuclear cells (PBMC) were prepared using density gradient

centrifugation according to the method developed by Boyum (8) and previously described by us (9). Cells were counted in a haemocytometer.

Concentrations of GIT Hormones and PHA. Four final concentrations were used in our cultures for each of the GIT hormones gastrin, secretin, GIP and VIP. The concentrations were: 50, 200, 500, and 1000 pg/ml. The hormones were added at the beginning of the cultures. The final concentration of PHA was 5µg/ml. The stock solutions of the hormones and PHA were diluted to the working concentrations in complete RPMI.

Cultures of Peripheral Blood Mononuclear Cells. 100,000 to 150,000 cells per well (depending on yield) were cultured in the wells of 48-well flat bottom plates. Control cultures of PHA-stimulated and non-PHA-stimulated PBMC were done in triplicates for 24, 48 and 72 hours to determine baseline cytokine production. Four concentrations of gastrin, secretin, GIP and VIP were added to duplicate cultures of non-PHA-stimulated PBMC and PHA-stimulated PBMC that were harvested after 24, 48 or 72 hours. The GIT hormone was added at the start of the culture. Some of the 72 hour cultures had the hormone added to them repeatedly every 24 hours. After a preliminary study, we decided to have five experimental groups as follows: **1**- No PHA, for 48 hours, **2**- No PHA, for 72 hours with the hormone repeated every 24 hours, **3**- PHA, for 24 hours, **4**- PHA, for 72 hours, **5**- PHA, for 72 hours with the hormone repeated every 24 hours. The final volume per well was 500 µl. For cultures that had the GIT hormone added every 24 hours, the same volume of RPMI was added to controls. Plates were incubated at 37^0 C in 5% CO_2. At the end of the predetermined incubation period, supernatants were harvested, centrifuged and immediately stored in a freezer at -86^0 C.

Measurement of Cytokines. Cytokine levels were measured using ELISA kits and the plates were then read at 450 nm in an ELISA plate reader. Log-log Figures were used to determine concentrations.

Statistical Methods. Volunteers have been studied one at a time, and for each an equal number of cells have been used in control and test cultures. The hormone induced change for that volunteer was then calculated as a percentage of his own control. The percentage changes of all volunteers have been pooled to calculate a mean percent change for the whole sample.

For each volunteer, changes induced by a GIT hormone were calculated as percentages of the control according to the following formula:

$$\text{GIT hormone induced change} = \frac{\text{Mean of Test Wells - Mean of Control wells}}{\text{Mean of Control wells}} \times 100$$

Where; GIT hormone refers to one of the concentrations of the GIT hormone, test wells are the cultures treated with that concentration of the GIT hormone, and the control wells are those not treated with the GIT hormone.

The mean percent change and standard deviation induced by each hormone concentration were then calculated for each of the experimental groups. The significance of change was assessed by the two-tailed paired t-test with the significance level set at 5%. The SPSS statistical package was used.

RESULTS

Cytokine Production by PHA-Stimulated and Non-PHA-Stimulated PBMC. Table 1 shows that un-stimulated peripheral blood mononuclear cells do not produce detectable

amounts of IL-2 in the culture supernatants. IL-2 production by PHA-stimulated PBMC was high in the first 48 hours and had declined by the third day. IFN-γ was detectable in the cultures of both un-stimulated and PHA-stimulated PBMC and its level was maintained throughout the three days period.

Table 1. Pattern of cytokine levels in the culture supernatants of human peripheral blood mononuclear cells over a 3-day period

Cytokine	After 24 hours		After 48 hours		After 72 hours	
	spontaneous	PHA	spontaneous	PHA	spontaneous	PHA
IL-2	Not detectable	470±130 pg/ml	Not detectable	400 ±80 pg/ml	Not detectable	18±10 pg/ml
IFN-γ	17±4 pg/ml	3500 ± 1300 pg/ml	15± 4 pg/ml	5400 ± 1800 pg/ml	22 ±7 pg/ml	6500 ± 2100 pg/ml

The values given are mean ±SD of eight experiments using cells from different volunteers. Each experiment was started by culturing 150,000 PBMC. Spontaneous = PBMC not treated with a mitogen. PHA = PBMC treated with 5µg/ml PHA.

Effect of Gastrin on IL-2 and IFN-γ. Figure 1 demonstrates that gastrin increases IL-2 levels in PHA-stimulated 72-hour cultures that had received the hormone daily for three days. However, statistically significant increases were only seen with the two highest concentrations. The stimulatory effect seen in the PHA-stimulated 24-hour cultures was not statistically significant. Gastrin did not induce IL-2 production in non-PHA-stimulated cultures.

Figure 2 shows a considerable increase in the level of IFN-γ in the non-PHA-stimulated PBMC cultures that had received the three highest concentrations of gastrin. There was no remarkable change in PHA-stimulated cultures

Effect of Secretin on IL-2 and IFN-γ. Figure 3 demonstrates that secretin had an inhibitory effect on IL-2 level in the 72-hour cultures that received the hormone once, but it only reached statistical significance with the 200pg/ml concentration. Adding the hormone repeatedly for 72 hours resulted in a rise of IL-2 level, but only with the highest two hormone concentrations. However, this effect did not reach statistical significance. Secretin did not induce IL-2 in non-PHA-stimulated cultures.

Figure 4 demonstrates that secretin induced a rise in IFN-γ level in non-PHA-stimulated PBMC cultures. The effect of secretin on IFN-γ level in PHA-stimulated cultures was not remarkable.

Effect of GIP on IL-2 and IFN-γ. Figure 5 shows that GIP has increased IL-2 level in PHA-stimulated PBMC cultures. The highest two concentrations appeared most active and caused statistically significant changes. GIP did not induce production of IL-2 in non-PHA-stimulated cultures.

GIP had an enhancing effect on IFN-γ level in non-PHA-stimulated, 48-hour PBMC cultures but this effect did not reach statistical significance.

Effect of VIP on IL-2 and IFN-γ. Figure 6 shows that the effect of VIP on PHA-stimulated, 72- hour cultures was inhibitory. Although the overall trend is inhibitory, only two of the hormone concentrations showed statistically significant effect. VIP did not induce IL-2 production in non-PHA-stimulated cultures. VIP did not have a clear effect on IFN-γ production.

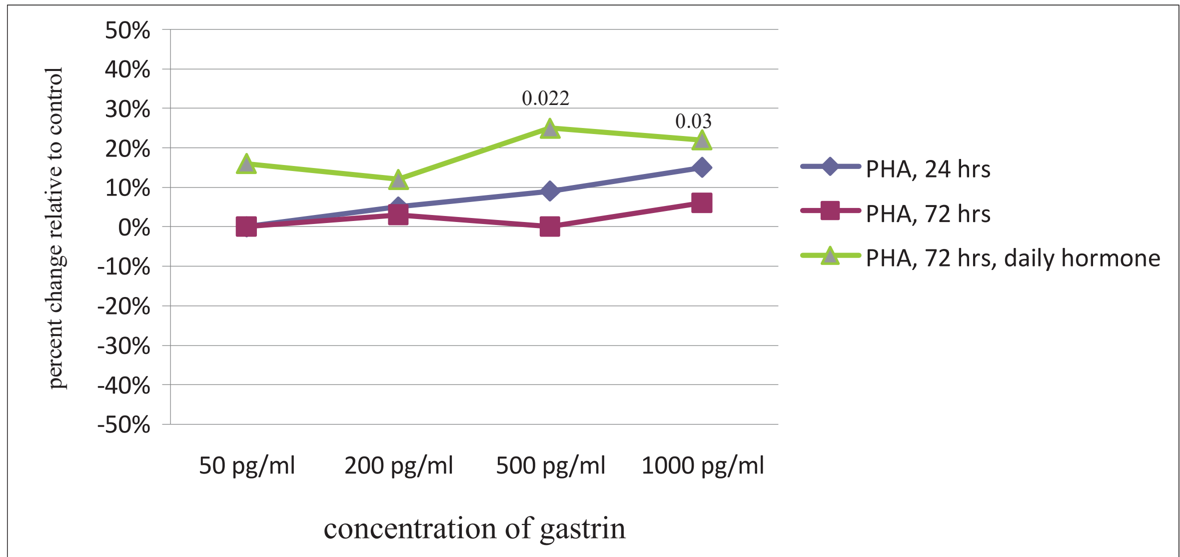

Figure 1. Effect of gastrin on IL-2

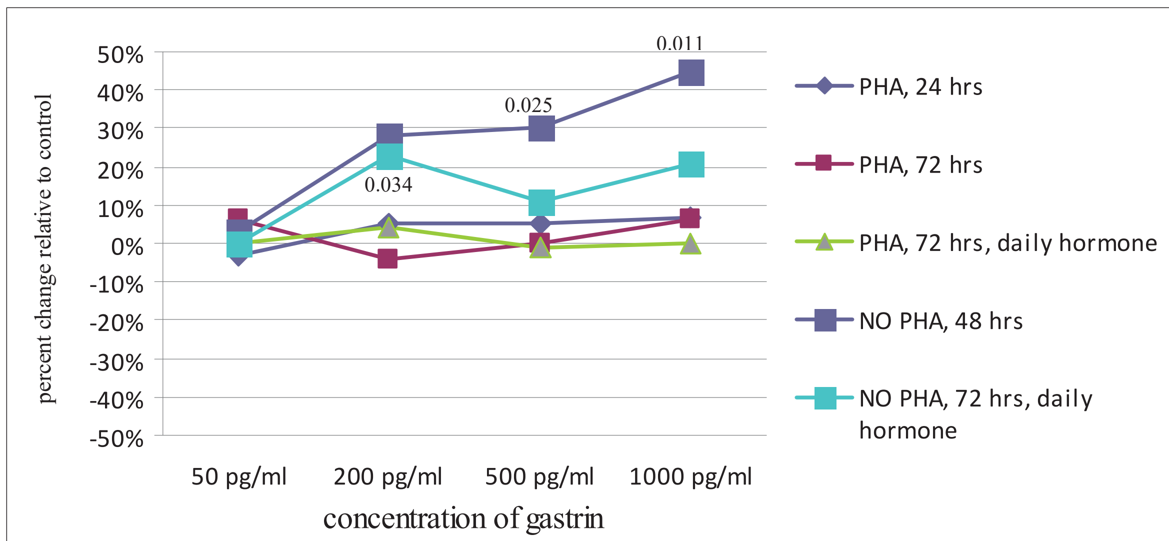

Figure 2. Effect of gastrin on IFN-γ

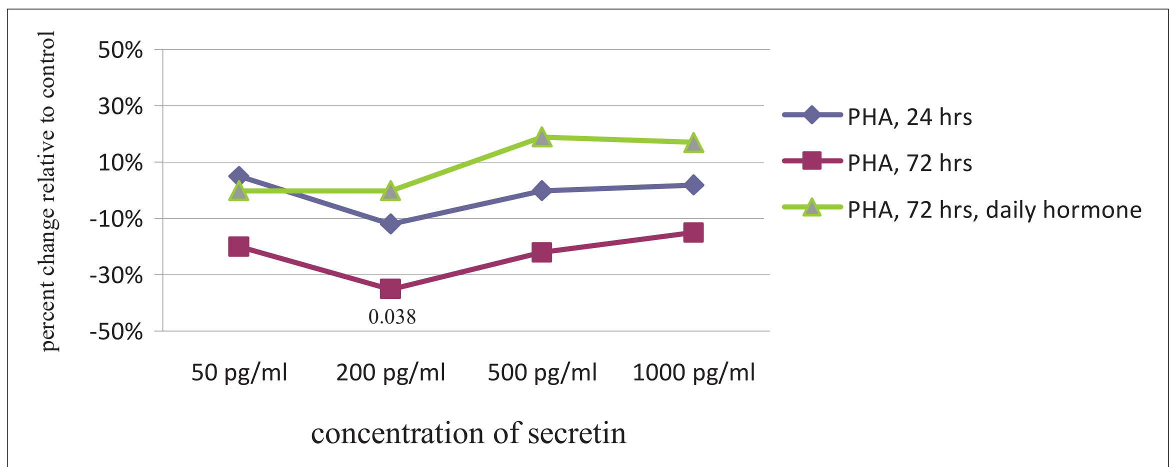

Figure 3. Effect of secretin on IL-2

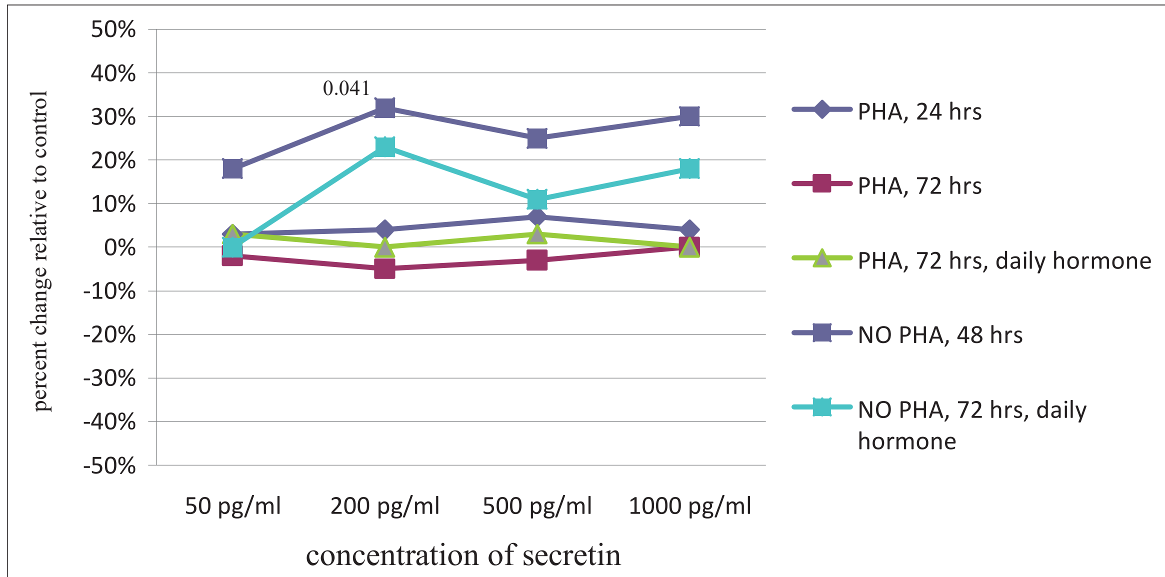

Figure 4. Effect of secretin on IFN-γ

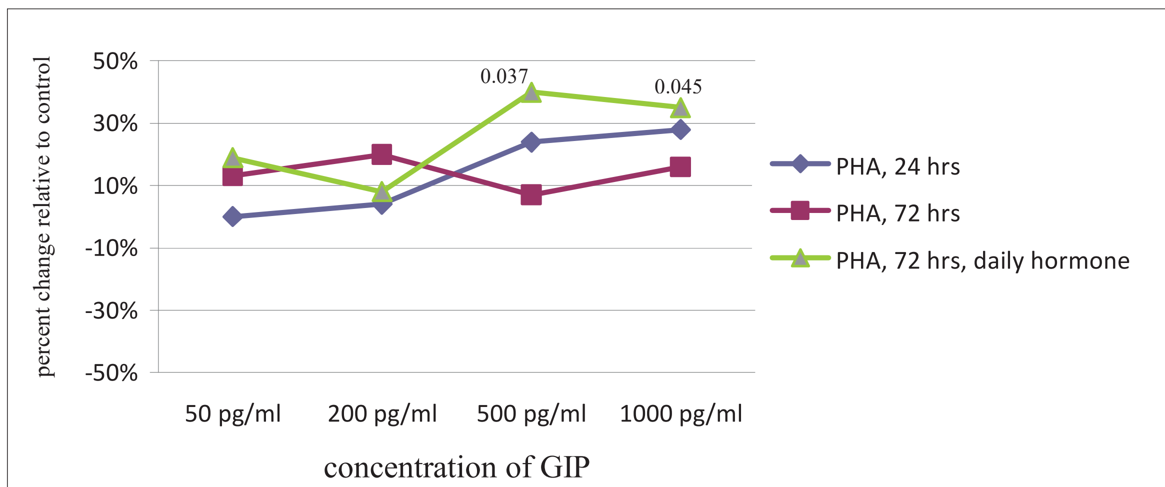

Figure 5. Effect of GIP on IL-2

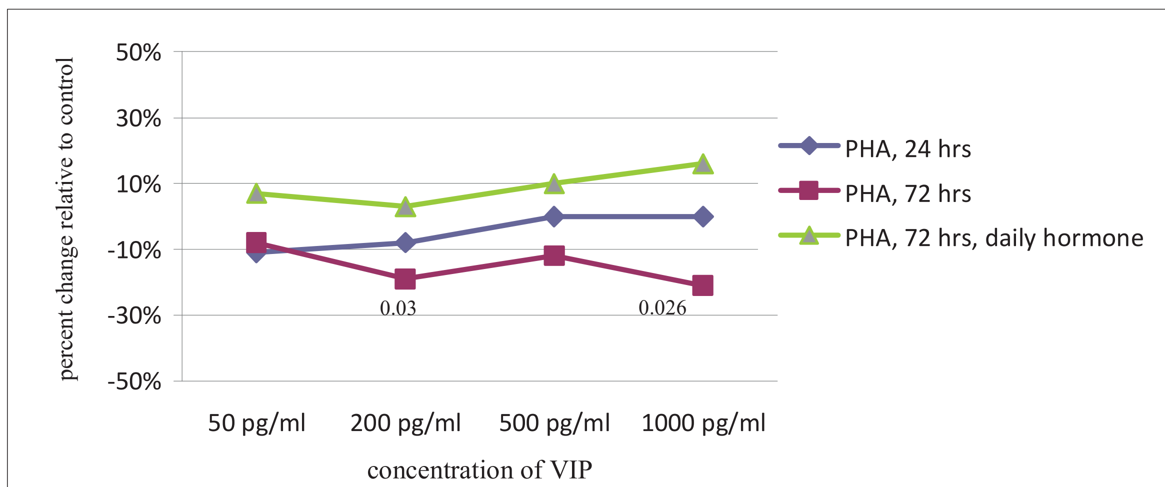

Figure 6. Effect of VIP on IL-2

Figure 1-6. The percent change relative to control is the mean of the hormone-induced percentage changes of volunteers (*see statistics section for calculation*). Stimulation is above the line of 0% change (the control) and inhibition is below. The time in hours is the duration of the culture. PHA means cells were stimulated with PHA. Cultures were treated with the corresponding concentration of the hormone at initiation of the culture. Daily hormone means the same concentration of the hormone was added every 24 hours. The P values of statistically significant changes are indicated.

DISCUSSION

All four GIT hormones that have been used in this study had some effect on the cells of the immune system; the lymphocytes and monocytes which constitute the bulk of PBMC (8). These effects are evidenced by changes in cytokine production. The changes in the levels of the cytokines IL-2 and/or IFN-γ produced by gastrin, secretin, GIP or VIP were not consistently uniform or large. Also, none of the four hormones could induce the production of IL-2 in non-PHA-stimulated cultures. Yet, from their effects on IL-2 in PHA-stimulated cultures and their effects on IFN-γ in non-PHA-stimulated cultures, it can be argued that these GIT hormones have shown their potential as immunomodulators. Gastrin and GIP were primarily stimulators of IL-2 and IFN-γ production whereas secretin and VIP showed inhibitory effects on IL-2.

The culture conditions in terms of duration, concentration and repetition of hormonal treatment and mitogen stimulation, appeared to affect whether a hormone increased or decreased cytokine production. Hormone-induced stimulation of IL-2 secretion was seen more often in the PHA-stimulated, 72-hour cultures that had received the hormone daily. These are cultures in which IL-2 level was expected to be low (Table1). GIP and gastrin tended to stimulate IL-2 production in PHA-stimulated 24- and 72-hour cultures that were treated with the hormone once, whereas secretin and VIP tended to inhibit IL-2 productions in those cultures. However, not all changes were statistically significant.

Gastrin and secretin induced marked increases of IFN-γ levels in non-PHA-stimulated cultures but not in PHA-stimulated cultures. The small enhancing effect of GIP on IFN-γ levels was also primarily seen in non-PHA-stimulated cultures. It may be hypothesized that PHA-stimulated cells are producing IFN-γ at maximum rates which precludes the observation of the stimulatory effects of the GIT hormones.

It seems that these hormones were more likely to have a stimulatory effect on the production of IL-2 when the production of the cytokine was initially low and the hormones were added daily as seen in PHA-stimulated 72-hours cultures with daily hormone addition. A stimulatory effect on IFN-γ was more probable in non-PHA-stimulated cultures that were treated with gastrin or secretin. We do not have any information about the half life of these hormones under our culture conditions. It is therefore not possible to say whether the effect of repeated administration of the hormones was due to a constant effective concentration or to a rising concentration.

The work of several researchers has indicated that GIT hormones have the potential to influence the various cells of both the innate and adaptive immunity. Similar to what we have noticed, both stimulatory and inhibitory effects have been reported. Stimulatory and inhibitory effects of gastrin and cholecystokinin (CCK) on lymphocyte proliferation and IL-2 production have been reported by Carrasco et al (7). Inconsistent effects of VIP on cytokine production have also been reported (10). In this study we pointed out some of the culture conditions that may explain some of the inconsistencies. Our results show that there is not always a clear

linear correlation between the hormone concentrations and the effect on cytokine production, as low concentrations can be more effective than higher ones (e.g. effect of secretin in Figure 3 and effect of VIP in Figure 6). The reason is not clear to us, but a larger sample size may show more consistent results. Possible causes of the inconsistencies could be inter-individual variations in the effect of these hormones on human cells or heterogeneity of the cells used.
Our in vitro observations would be more relevant than the in vivo situation if they were produced by physiological concentrations of these hormones. In peripheral blood, the normal plasma level of gastrin is up to 75 pg/ml (11), of secretin is 12 - 75 pg/ml (12), of GIP is 75 - 325 pg/ml (13) and of VIP is 12.9 - 98.5 pg/ml (14). With regard to the portal circulation, the concentration of a GIT hormone is expected to be about double its concentration in the peripheral blood (15). We can, therefore, state that the lowest two concentrations (50 pg/ml and 200 pg/ml) used in this study are well within the range to which circulating immune cells are exposed. Although the higher pharmacological concentrations of the hormones in this study were more consistent in their effects, the physiological concentrations did show their activity.
Cytokines occupy the centre position in the regulation of both the adaptive specific immune mechanisms and the natural innate mechanisms. It is worth noting that the gastrointestinal tract houses vast numbers of lymphocytes and macrophages. It is conceivable then that GIT hormones not only affect secretions, motility and growth of gut cells, but also its defensive capabilities. This could well mean that our diets affect our immunity not just by providing nutrients, but also through the GIT hormones.

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REFERENCES

1 Rehfeld JF. The new biology of gastrointestinal hormones. Physiol Rev. 1998; 78: 1087-108.
2 Montuenga LM, Guembe L, Burrell MA, Bodegas ME, Calvo A, Sola JJ et al. The diffuse endocrine system: from embryogenesis to carcinogenesis. Progr Histochem Cytochem. 2003; 38: 155-272.
3 Ganong WF (editor): Regulation of gastrointestinal function. In: Review of Medical Physiology. 22nd edition. Lange/McGraw-Hill. 2005: 479-512.
4 Okamoto K, Fukatsu K, Ueno C, Shinto E, Hashiguchi Y, Nagayoshiet H et al. T-lymphocyte numbers in human gut associated lymphoid tissue are reduced without enteral nutrition. J Parenter Enteral Nutr. 2005; 29: 56-8.
5 Genton L, Kudsk K. Interactions between the enteric nervous system and the immune system: role of neuropeptides and nutrition. Am J Sur. 2003; 186: 253-8.
6 Goetzl EJ, Pankhaniya RR, Gaufo GO, Mu Y, Mxia M, Sreedharan SP. Selectivity of effects of vasoactive intestinal peptide on macrophages and lymphocytes in compartmental immune responses. Ann NY Acad Sci. 1998; 840: 540-50.
7 Carrasco M, Hernandz A, De La Fuente M. Effect of cholecystokinin and gastrin on human peripheral blood lymphocyte functions, implication of cyclic AMP and interleukin 2. Regul pept. 1997; 70: 135-142.
8 Boyum A. Separation of leukocytes from blood and bone marrow. Scan J Clin Lab Invest. 1968; 97: 7.
9 Abdalla EE, Adam I, Blair GE, Boylston A, Sue-Ling HM, Finan P et al. The immunomodulatory effect of levamisole is influenced by postoperative changes and type of lymphocyte stimulant. Cancer Immunol Immunother 1995; 41: 193-8.
10 Delgado M, Pozo D, Ganea D. The significance of vasoactive intestinal peptide in immunomodulation. Pharmacol Rev. 2004; 56: 249-90.
11 Bostwick DG, Bensch KG. Gastrin releasing peptide in human neuroendocrine tumors. J Pathol. 1986; 146: 237-44.
12 Christ A, B Werth B, Hildebrand P. Human secretin: Biologic effects and plasma kinetics in humans. Gastroenterology. 1988; 94: 311-6.
13 Krarup T. Immunoreactive Gastric Inhibitory Polypeptide. Endocr Rev. 1988; 9: 122-134.
14 Hejna M, Hamilton G, Brodowicz T. Serum levels of vasoactive intestinal peptide (VIP) in patients with adenocarcinomas of the gastrointestinal tract. Anticancer Res. 2001; 21:1183-7.
15 Boden G, Essa N, Owen OE, Reichle FA. Effects of intraduodenal administration of HCl and glucose on circulating immunoreactive secretin and insulin concentrations. J Clin Invest. 1974; 53: 1185-93.