Serum immunoreactive gastrin in rheumatoid arthritis

Further observations on its identity, clinical specificity, and relationship to gastric function

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Rooney, P. J., Kennedy, A. C., Gray, G. H., Sturrock, R. D., Buchanan, K. D., and Dick, W. C. (1976). *Annals of the Rheumatic Diseases*, 35, 246–250. **Serum immunoreactive gastrin in rheumatoid arthritis. Further observations on its identity, clinical specificity, and relationship to gastric function.** Serum immunoreactive gastrin is raised in some patients with rheumatoid arthritis (RA). In this paper paired samples taken within 30 minutes of each other show that this phenomenon is reproducible. Dilution curves show identity between gastrin from RA patients and from patients with no RA, and the raised immunoreactive gastrin concentration is demonstrable in patients who do not have chronic atrophic gastritis and in whom both basal and stimulated acid output concentrations are normal. Samples of gastric mucous membrane obtained from the body of the stomach and gastroscopy under direct vision in 18 patients with raised serum immunoreactive gastrin concentrations were normal.

The demonstration of conspicuously raised concentrations of serum immunoreactive gastrin (IRG) in a proportion of patients suffering from 'classical' or 'definite' rheumatoid arthritis (RA) (Rooney and others, 1973) raised a series of provocative questions. Did changes in IRG concentration merely represent another in a long list of nonspecific acute phase reactants? Did some component of the deranged immunological response in RA produce this effect? Was the observation simply a laboratory artefact?

These suggestions can now be refuted or at least rendered unlikely by direct and indirect evidence. Firstly, there is evidence against this being a pharmacological artefact: (i) IRG levels in adjuvant arthritis in rats rise during induction of the disease (Rooney and others, 1973), an experiment in which no drugs are used; (ii) administration of single anti-inflammatory drugs to patients with RA does not alter either normal or raised serum IRG levels; (iii) IRG levels are not raised in patients with chronic inflammatory nonrheumatoid joint diseases who were receiving broadly similar anti-inflammatory drugs (Rooney

and others, 1976). Secondly, the failure of serum IRG concentrations to rise and fall in parallel with validated clinical and laboratory indices of the severity of the degree of inflammatory involvement, together with the fact that serum IRG levels are normal in patients with other articular and nonarticular inflammatory and tissue destructive diseases suggests that IRG is not an acute phase reactant. Thirdly, the fact that IRG concentration does not correlate with the titre of rheumatoid factor or the serum immunoglobulin concentration makes it unlikely that this is a nonspecific immunological epiphenomenon. The possibility of a laboratory artefact is rendered less likely by the fact that IRG levels reported from the same laboratory in other disease situations are meaningful.

We report similar results in repeat samples taken within 30 minutes from patients with both raised and normal IRG concentrations. We also show that the dilution curve for IRG taken from patients with RA and raised serum IRG concentrations behave identically in the assay to that obtained with IRG

taken from nonrheumatoid control subjects and to synthetic human gastrin 1. The likelihood of a laboratory artefact is remote therefore.

An important possibility remains. Did the patients with raised serum IRG levels suffer from chronic atrophic gastritis? The results of gastric parietal cell autoantibody testing, the results of maximally stimulated gastric acid outputs using both histamine and pentagastrin, and gastric biopsy histology all show clearly that the high IRG levels cannot be explained by chronic atrophic gastritis in all cases.

Patients and methods

Fifty-one patients with 'classical' or 'definite' RA (Ropes and others, 1959), of whom 13 were male, volunteered to participate in these studies with full knowledge of their content. The mean age was 57.2 years (range 22–67 years) and the mean duration of their arthritis was 15.6 years (range 3–24 years). None had at any time received corticosteroid or cytotoxic drugs or chrysotherapy, and only 13 had evidence of Sjögren's syndrome. In none was there evidence of disease of any other major system and, in particular, renal function was normal in all. The patients were assessed clinically, noting particularly disease duration, presence of nodules, articular index of joint tenderness, full blood count, and erythrocyte sedimentation rate. Immunological studies included rheumatoid factor, antinuclear factor, and gastric parietal cell autoantibody. All the patients had a full joint radiological examination, 22 had a full barium examination of the upper gastrointestinal tract, and 25 underwent gastroscopy (Olympus GFBK side-view fibre optic gastroscope) which was carried out on patients basally sedated by intravenous diazepam 4–47 mg.

Serum immunoreactive gastrin was assayed using a

sensitive and specific radioimmunoassay. The antibody is raised in rabbits to synthetic human gastrin and radio-labelling of the hormone is accomplished by a modification of the chloramine T method (Hunter and Greenwood, 1962). Separation of free from antibody-bound hormone was achieved using dextran-coated charcoal (Buchanan and McCarroll, 1971). The sensitivity of this method is greatest below 600 pg/ml, and the lower limit of reliable detection is of the order of 10 pg/ml. The antibody used detects both the heptadecapeptide and 'big' gastrin (Yalow and Berson, 1970), and there is minimal cross reactivity with cholecystokinin pancreozymin.

Plasma from RA patients was pooled in two groups. (1) From subjects with normal levels of IRG; (2) from subjects with high levels of IRG. These pooled plasmas were then run under standard immunoassay conditions at different dilutions and compared against pooled plasma from nonrheumatoid subjects.

Maximal gastric acid output was determined using the classical technique of Kay (1953). Nasogastric tube positioning was achieved by direct x-ray screening in 5 patients and in the others by the water recovery technique (Findlay, Prescott and Sircus 1972); stimulation was by histamine acid phosphate 40 µg/kg body weight or pentagastrin 6 µg/kg body weight.

Results

The results are shown in Figs. 1 and 2 and in Tables I and II. Plasma IRG from the two groups of RA patients diluted identically with the nonrheumatoid subjects, indicating that the radioimmunoassay did not detect immunological differences between the IRG of rheumatoid plasma and nonrheumatoid plasma. This is plotted in linear fashion in Fig. 1. The paired samples for IRG in repeat samples withdrawn

FIG. 1 Gastrin dilution curves for labelled human synthetic gastrin and sera from patients with rheumatoid arthritis with both high and normal immunoreactive gastrin content

within 30 minutes are shown in Table I. The standard error of the difference between these samples was ± 12.6 pg/ml. The reproducibility of our assay is such that in 10 consecutive assays on the same control sample the assays did not differ by more than 15 pg/ml and the standard error of the difference of 10 samples assayed on two occasions was ± 1.02 pg/ml.

The results of gastric function analysis of 16 patients with markedly raised IRG are shown in Table II and Fig. 2. Basal gastric acid concentrations were within the normal range (0–4.6 mEq/h), and in all but one patient the response to pentagastrin and to histamine

Table I Gastrin concentrations (pg/ml) in 2 samples taken within 30 minutes in the fasting state from one subject

Sample 1	Sample 2 (gastrin pg/ml)
20	10
60	50
70	15
265	300
900	1065
1125	1145
55	50
155	130
60	45
55	70
90	105
70	50
200	190
10	10
50	70

Standard error of difference ± 12.6 pg/ml.

FIG. 2 Acid outputs during basal and post-stimulated hour with both pentagastrin and histamine in 14 subjects with markedly raised serum immunoreactive gastrin concentrations

was also within the normal range (7–41 mEq/l) (Avery-Jones, Gummer and Lennard-Jones, 1968). This one patient (Case 6) has subsequently been shown to have had early pernicious anaemia. Table II also shows that in the 10 patients in whom both

Table II Acid output in 16 patients with raised immunoreactive gastrin

Case no.	Basal acid			Pentagastrin stimulated output			Histamine stimulated output		
	Volume (ml)	Concentration (mEq/l)	Output (mEq)	Volume (ml)	Concentration (mEq/l)	Output (mEq)	Volume (ml)	Concentration (mEq/l)	Output (mEq)
1	20	35.8	0.72	181.5	109.4	19.8			
2	85	20.2	1.71	187	112	20.9	160	120	19.2
3	55	0	0	48	147	7.05			
4	30	14.6	0.44	139	78.8	10.95			
5	14	69.5	0.97	151.5	88	13.3	200	60	12.0
6*	4	0	0	15	0	0	20	0	0
7	35	49.5	1.73	138	92	12.69	150	93	13.95
8	8.4	10	0.08	279	84	23.4	206	80	16.48
9	69	142	9.8	230	220	50.6			
10	15	100	1.5	56	75	4.20			
11	17.5	175	3.06	245	161	39.4	193	173	33.4
12	88	60	5.28	181	97	17.6	130	93	12.09
13	72	23	1.65	118	86	10.15			
14	23	138	3.17	129	132	17.0	150	93	13.95
15	29	153	4.43	179	154	27.56	110	162	17.8
16	18	52	0.94	203	154	31.2	150	163	24.5
Mean			2.217			19.11			16.33
SD			2.55			13.17			8.71
SEM			0.64			3.29			2.75

Standard error of difference between histamine- and pentagastrin-stimulated acid output. 1.1 mEq.

* This patient was subsequently shown to have pernicious anaemia.

stimuli were used the response to both pentagastrin and histamine was similar (standard error of difference 1.1 mEq/l).

In 18 patients a sample of gastric mucous membrane was obtained from the body of the stomach at gastroscopy under direct vision. Histology of these samples was normal.

Discussion

The results of this study are pertinent to two questions. Is the gastrin produced in RA patients with raised serum IRG levels the same gastrin that is produced normally? What is the functional state of the end organ—the gastric mucosa? Although identity can only be conclusively established by amino acid sequence analyses, the results do support the contention that IRG produced in RA patients is similar immunologically and in its dilution curve characteristics to gastrin produced in normal subjects. Studies are currently being done in bioassay models to determine whether this gastrin is biologically as well as immunologically active. The fact that almost all of the bioassays are orders of magnitude less sensitive than radioimmunoassay presents a major difficulty. Gastrin measured previously in the same laboratory by the same method has been shown to be biologically active.

If the IRG in patients with RA is biologically active then the results of the present study leave us with an enigma. In the presence of such markedly raised levels of gastrin conspicuous gastric hypersecretion of acid and pepsin would be expected. In fact the levels of IRG we have reported in some patients are of the same order of magnitude as those seen in patients suffering from the Zollinger–Ellison syndrome (Rooney and others, 1973). The results of the gastric function studies reported have shown not only that the high IRG levels do not reflect chronic atrophic gastritis but also that the resting and stimulated acid output of these subjects is entirely normal. Since the subject of these studies was gastrin it was absolutely necessary to show the response to stimulation by histamine as well as pentagastrin.

Recent observations in the heterogeneity of

circulating gastrin (Walsh, Debas and Grossman, 1974) have suggested that the larger molecules in the circulation have a lesser biological activity. The possibility must therefore remain that until full gel filtration and bioassay studies are completed high IRG levels in RA are due to large biologically inert forms of the hormone.

Clearly in the presence of grossly raised hormone levels with an apparently normal end organ either the IRG measured is not biologically active or its activity is blocked in some way *in vivo*. Refutation of the former must await the results obtained in our bioassay systems. In considering the latter possibility it is conceivable that other substances are present which would interfere with the response to available gastrin. Prostaglandins, particularly of the E type are known to block pentagastrin-stimulated acid production (Karim and others, 1973) and this group of compounds has also been clearly shown to be involved in the inflammatory process (Greaves, Sondergaard, and McDonald-Gibson, 1971).

It is also conceivable that a specific low affinity antigastrin antibody is produced *in vivo* which blocks the N terminal biologically active site but that does not block the immunoreactive site and therefore raised IRG levels are measured in the radioimmunoassay. An alternative possibility is that gastrin degradation is abnormal, allowing accumulation of immunoreactive, biologically inactive, degradation products of gastrin (Levin and others, 1971).

The gastric histological studies in these patients are of interest. While no small sample biopsy can conclusively exclude the presence of chronic atrophic gastritis, the consistently normal mucosal appearance obtained, accompanied by the normal acid output, militate strongly against the presence of such a lesion.

Whatever the outcome of this fascinating problem we are left in practical terms with an observation that by at least one parameter two subgroups exist within those patients hitherto considered to be homogeneous. This does not seem to be due to a pharmacological or immunological epiphenomenon and does not appear to be a nonspecific acute phase reactant. Whether or not rheumatoid arthritis may prove to be different in these different subgroups is the subject of ongoing studies.

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