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Cell-mediated Immunity to Intrinsic Factor in Autoimmune Disorders

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British Medical Journal, 1974, 4, 494-496

Summary

Evidence of cell-mediated immunity to gastric intrinsic factor was present in 86% of patients with pernicious anaemia and in at least 13% of patients with hyperthyroidism, 21% of patients with atrophic gastritis, and four out of nine (46%) patients with hypogammaglobulinaemia. Controls gave negative results. The four patients with hypogammaglobulinaemia and cell-mediated immunity to intrinsic factor had evidence of impaired gastric function.

Introduction

Pernicious anaemia is common in some disorders in which autoantibodies appear. Thus some 10% of patients with primary hypothyroidism, 2% of patients with Graves's disease, and about 4% of insulin-dependent diabetics have pernicious anaemia. Sera from these groups of patients often contain gastric antibodies, particularly parietal-cell antibodies, which are generally demonstrable in about 30% of such samples. There is also a high incidence of pernicious anaemia among patients with acquired hypogammaglobulinaemia. Intrinsic-factor antibodies are less common in diseases other than pernicious anaemia, but they occur in 2% of patients with Graves's disease and less frequently in the other groups as well as in relatives of patients with pernicious anaemia.

The association of pernicious anaemia with hypogammaglobulinaemia is particularly interesting because patients with hypogammaglobulinaemia may lack normal B-lymphocyte function responsible for producing humoral antibodies while they often retain T lymphocytes responsible for cell-mediated immunity. This suggests that at least in these patients, and

probably in other patients, cell-mediated immunity may be more important in producing pernicious anaemia than humoral antibodies. This view is reinforced by the failure to show humoral intrinsic-factor antibody in either the serum or gastric juice of almost one-third of patients with pernicious anaemia.

This study was undertaken to determine the frequency of cell-mediated immunity to intrinsic factor in these susceptible groups and, in the longer term, whether cell-mediated immunity to intrinsic factor would prove a better indication of the risk of the patient developing pernicious anaemia than tests for humoral antibodies.

Patients and Methods

Observations were made on 51 patients with proven pernicious anaemia, 14 with histologically-proven atrophic gastritis without pernicious anaemia, eight with acquired hypogammaglobulinaemia and one with an isolated IgA deficiency, 24 with insulin-dependent diabetes, 14 with primary hypothyroidism, 22 with Graves's disease, and eight patients with toxic nodular goitre. Observations were made also on 105 controls, 51 of whom corresponded in sex and age to the pernicious-anaemia group and 54 of whom were matched to patients in the other groups.

Crude hog intrinsic factor (Armour Pharmaceutical Company) was concentrated by the method of Ellenbogen and Highley (1967) so that 1 mg dried powder bound 45 ng vitamin B₁₂.

Human intrinsic factor was contained in gastric juice collected after pentagastrin stimulation. Bile-stained samples were discarded. The gastric juice was concentrated by ultrafiltration through an Amicon filter so that the intrinsic factor content was 600 U/ml. It was used at an intrinsic factor concentration of 150 intrinsic factor U/ml.

The *leucocyte migration inhibition (M.I.F.) test* was set up as described by Rose et al. (1969) using disposable migration chambers (Sterilin Ltd.). Leucocytes were harvested from 25 ml of heparinized blood. Tubes were set up in quintuplicate, with control tubes from which antigen was omitted. The fluid in the chamber contained either hog intrinsic factor (5 mg) diluted in 1 ml TC-199 (Burroughs Wellcome) followed by the addition of 10% fetal calf serum (Burroughs Wellcome) or human gastric juice (600 intrinsic factor U/ml) diluted with three parts TC-199 followed by the addition of 10% fetal calf serum. Intrinsic factor was replaced by additional TC-199 in controls. Migration zones were traced on paper through a photographic enlarger ($\times 15$) and areas read with a planimeter. Migration zones from the control group were within a range of 80%-120% of the mean value (see fig. 1). Inhibition of migration

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TABLE I—Frequency of Gastric Antibodies in Patients and Controls

Diagnosis	No. of Patients	Sex		No. (%) with Parietal-cell Antibodies	No. (%) with Intrinsic-factor Antibodies
		F.	M.		
Controls	105	75	30	6 (6)	0
Pernicious anaemia	51	37	14	34 (67)	26 (51)
Hyperthyroidism	30	24	6	3 (10)	0
Primary hypothyroidism	14	13	1	5 (36)	0
Diabetes	24	15	9	4 (17)	0
Atrophic gastritis	14	8	6	7 (50)	0
Hypogammaglobulinaemia	9	3	6	0 (0)	0

TABLE II—Cell-mediated Immunity to Intrinsic Factor in Patients and Controls

Group	No. Tested	Positive for Migration Inhibition	No. Tested	Positive for Transformation	Both Tests Performed	
					No. Tested	No. Positive
Controls	59	0	54	0	35	0
Pernicious anaemia	39	26	52	10	35	30
Hyperthyroidism	28	2	11	2	10	4
Primary hypothyroidism	13	0	6	0	0	5
Diabetes	16	0	13	0	5	0
Atrophic gastritis	14	3	14	0	14	3
Hypogammaglobulinaemia	9	2	9	2	9	4

was considered significant when the leucocyte migration in the presence of intrinsic factor was less than 80% of that of the mean control.

Lymphocyte transformation tests using ¹⁴C-thymidine were set up as described by Janossy and Greaves (1972). All tests and controls were set up in triplicate. Two million lymphocytes were used in each culture, which each contained 1 ml of a mixture of TC-199 with 5% fetal calf serum and 0.1 ml of human gastric juice (60 intrinsic factor units). Intrinsic factor was replaced by additional TC-199 in the controls. In this test the controls gave results of up to 3 on the transformation index, and hence only values above 3 were regarded as significant (see fig. 2).

Parietal cell antibodies were measured by a fluorescent method (Taylor *et al.*, 1962), and intrinsic-factor antibodies were measured by the method of Ardeman and Chanarin (1963).

Results

The presence of parietal-cell and intrinsic-factor antibodies in the various groups is shown in table I.

The results indicating cell-mediated immunity to intrinsic-factor are shown in table II. M.I.F. tests were carried out on 59 controls and lymphocyte transformation tests with human gastric juice in 54 controls. All these gave negative results.

Positive M.I.F. test results were obtained in 26 out of 39 pernicious anaemia and positive transformation test results in 10 out of 52 patients (figs. 1 and 2). All three tests (M.I.F. test with human and hog intrinsic factor and transformation test with human intrinsic factor) were carried out in 35 patients and 30 of these gave a positive result with one or more methods.

Lymphocytes from 28 patients with hyperthyroidism were tested by M.I.F. assay, and two were weakly positive. One of these two patients had a toxic nodular goitre and the other had Graves's disease. Two of the 11 patients tested by the lymphocyte transformation test gave positive results. Altogether four out of 30 patients had possible evidence of cell-mediated intrinsic factor immunity.

Three out of 14 patients with atrophic gastritis gave weakly positive results on M.I.F. testing, but transformations were negative.

Four out of the nine patients with acquired hypogammaglobulinaemia gave positive results, two using M.I.F. technique and two with the transformation technique. These four patients all had pentagastrinfast achlorhydria with extremely low intrinsic factor output. Three out of the four had malabsorption of vitamin B₁₂ corrected by additional intrinsic factor. None were anaemic and the mean corpuscular volume was either normal or

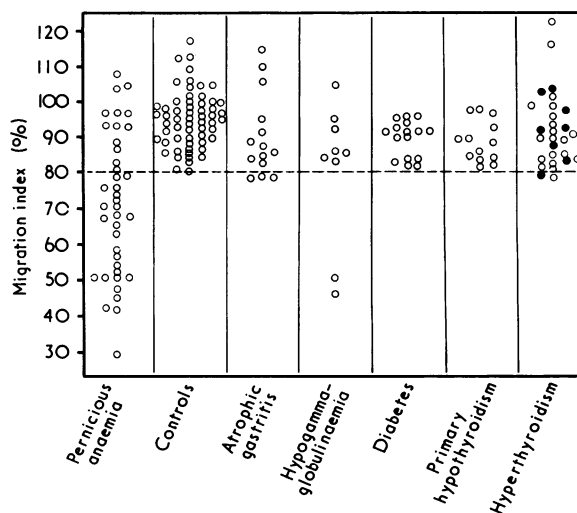


FIG. 1—Results of leucocyte migration test for cell-mediated immunity against intrinsic factor in patients and controls. Values below 80% were considered positive. In patients with hyperthyroidism O = Graves's disease, ● = toxic nodular goitre.

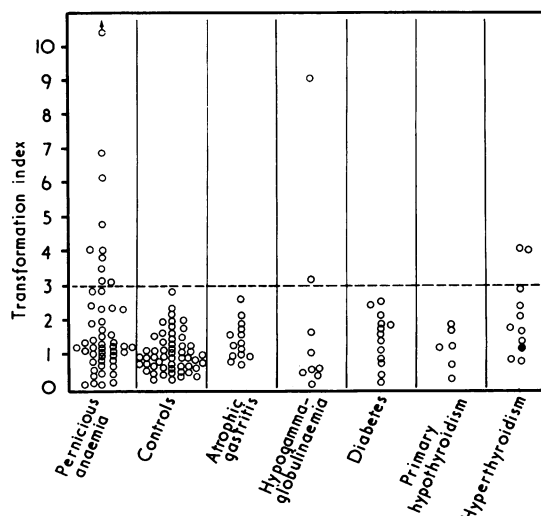


FIG. 2—Transformation of lymphocytes in presence of human gastric juice as test for cell-mediated immunity against intrinsic factor in patients and controls. Values above 3 were considered positive. In patients with hyperthyroidism O = Graves's disease, ● = toxic nodular goitre.

TABLE III—Details on Four Patients with Cell-mediated Immunity to Intrinsic Factor and Hypogammaglobulinaemia

Case No.	Serum		Lymphocytes		Hb (g/100 ml)	Mean Corpuscular Volume (fl)	Intrinsic-factor Secretion (U/hr)	Serum B ₁₂ (pg/ml)	% B ₁₂ Absorption*	
	Parietal-cell Antibodies	Intrinsic-factor Antibodies	Intrinsic Factor on M.I.F. Test	Intrinsic Factor on Transformation Test					B ₁₂ Alone	B ₁₂ + Intrinsic Factor
1	—	—	—	+	13.8	82	141	—	0.6	16.4
2	—	—	+	—	14.0	82	60	250	0.7	15.5
3	—	—	+	—	15.6	78	0	75	0.8	18.9
4	—	—	—	+	11.1	66	82	130	Normal plasma level	—
Normal Values	—	—	—	—	—	80–90	≥2000	≥170	≥10	≥10

*% of 1.0 µg oral dose of B₁₂ in 24-hour urine in Schilling test.

low, due to either iron deficiency or anaemia of infection (table III).

Discussion

Cell-mediated intrinsic-factor immunity was present in at least 85% of patients with pernicious anaemia and was absent in 105 controls. A few patients with thyrotoxicosis, atrophic gastritis, or hypogammaglobulinaemia also gave positive results.

The association between pernicious anaemia and hypogammaglobulinaemia was reviewed by Chanarin (1969). Twomey *et al.* (1970) found that four out of 10 immunoglobulin-deficient patients had pernicious anaemia, and Hughes *et al.* (1972) found five examples of pernicious anaemia among 12 such patients. Additional patients were described by Douglas *et al.* (1970), Cowling *et al.* (1974), and Ginsberg and Mullinax (1970). Gelfand *et al.* (1972) reported a mother and identical twins all with pernicious anaemia; the twins had immunoglobulin deficiency.

Serum intrinsic factor antibody was present in the case reported by Ginsberg and Mullinax (1970) and in one of the twins reported by Gelfand *et al.* (1972). This child also gave a positive result on M.I.F. and transformation tests using hog intrinsic factor.

Our results show that out of nine patients with hypogammaglobulinaemia four had cell-mediated immunity against intrinsic factor by conventional tests and in this respect resembled patients with pernicious anaemia. None of these patients had serum antibodies against intrinsic factor or parietal cells. These data support the view that the cell-mediated immunity is the

prime factor in bringing about atrophic gastritis in this group. But the low serum gastrin levels found in these patients, together with atrophy of the antrum as well as body of the stomach, suggest that the pathological condition in these patients differs from that seen in cases of pernicious anaemia (Hughes *et al.*, 1972).

Atrophic gastritis of varying degree is a very common lesion of the stomach, which increases in frequency with increasing age. Only very few cases progress to pernicious anaemia and the role of intrinsic factor antibody in this transition is not known. It is of particular interest that evidence of cell-mediated immunity was present in some patients in this group. Only long-term follow up, however, can indicate whether these particular patients will progress eventually to pernicious anaemia. The same arguments apply to the positive results in the other groups.

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Double-blind Cross-over Trial of Flurbiprofen and Phenylbutazone in Ankylosing Spondylitis

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British Medical Journal, 1974, 4, 496-499

Summary

A double-blind cross-over study in 35 patients with ankylosing spondylitis was carried out comparing flurbiprofen (150 mg daily)—a new non-steroidal anti-inflammatory agent—with phenylbutazone (300 mg daily) over a four-week

period. Flurbiprofen was well tolerated and shown to have therapeutic efficacy approaching that of phenylbutazone. The results suggest that flurbiprofen may prove a valuable alternative in the treatment of ankylosing spondylitis, and long-term efficacy and tolerance studies are clearly indicated.

Introduction

Flurbiprofen (B.T.S. 18-322, 2-(fluorobiphenyl) propanoic acid) is a member of the phenylalkanoic acid series. It differs from its predecessors (ibuprofen and ibufenac) in having a biphenyl nucleus but like them possesses antipyretic, anti-inflammatory, and analgesic properties—a triad essential

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