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Reassessment of the relative prevalences of antibodies to gastric parietal cell and to intrinsic factor in patients with pernicious anaemia: influence of patient age and race

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SUMMARY

Anti-parietal cell antibody has been reported in nearly all patients with pernicious anaemia in past studies, in contrast with anti-intrinsic factor (IF) antibody which occurs in only 50–70% of such patients. However, observations in the more diverse patient population at our hospital suggest that these prevalences, originally described in predominantly elderly, white patients of European origin, no longer apply. Anti-IF antibody was found in 70% of the 324 patients, with blacks (84%) and Latin Americans (69%) having a significantly higher prevalence than whites (55%). In contrast, only 55% of the 266 patients tested had anti-parietal cell antibody. It was noteworthy that this low rate was similar in all racial groups. However, patients lacking anti-parietal cell antibody were significantly younger than those who had the antibody ($54{\cdot}8 \pm 17{\cdot}8$ *vs* $59{\cdot}6 \pm 16{\cdot}2$ years, $P = 0{\cdot}022$). Overall, a striking 30% of all patients had anti-IF antibody but not anti-parietal cell antibody, while only 13% had the latter antibody without having anti-IF antibody. This pattern was particularly striking among black patients. An interesting incidental observation was that gastrin levels were not associated with antibody status, but were significantly higher not only among women than among men but also among white and black patients than among Latin-American patients. The findings show that anti-parietal cell antibody is not found nearly as often in pernicious anaemia as has been reported in the past, and thus has no value as a diagnostic tool in pernicious anaemia. They also suggest clues to different expressions of pernicious anaemia or of its immunologic response, particularly among younger patients with the disease.

Keywords parietal cell antibody intrinsic factor antibody pernicious anaemia gastrin age blacks

INTRODUCTION

Circulating antibodies to two gastric antigens are commonly found in pernicious anaemia. Anti-parietal cell antibody is associated with atrophic gastritis, and often occurs in subjects with gastritis who do not have pernicious anaemia [1–6]. Its prevalence is considerably higher in those in whom pernicious anaemia develops [2–6]. Antibody to intrinsic factor (IF), on the other hand, rarely occurs in atrophic gastritis that is not also accompanied by cobalamin (vitamin B_{12}) malabsorption, i.e. pernicious anaemia [3]. For that reason, it is considered a much more specific marker for pernicious anaemia than is anti-parietal cell antibody. However, its prevalence has been consistently described as much lower than that of anti-parietal cell antibody in this disease [2,3,6–12]. Thus, while anti-IF antibody is viewed as a less sensitive but more specific diagnostic marker of pernicious anaemia, anti-parietal cell antibody has been considered as a highly sensitive, if non-specific, marker.

The pathogenetic roles, if any, of these two antibodies remain a mystery. Indeed, considerable uncertainty persists about the nature of the antigen or antigens for the anti-parietal cell antibody. One of the antigens is thought to be the H^+, K^+-adenosine triphosphatase found in the tubulovesicular membranes of the parietal cell [13]. Animal experiments have produced suggestive but not entirely clear support for an *in vivo* role of the antibody in inducing gastritis [14,15].

Since anti-IF antibody usually only appears once pernicious anaemia develops [3], a pathogenetic role for it in that disease, rather than just atrophic gastritis, has been more attractive. However, pernicious anaemia also develops without this antibody. All of these issues await clarification.

Despite the uncertainty surrounding pathogenesis, the diagnostic roles of the two antibodies, along the lines outlined in the first paragraph, have become widely accepted. However, obser-

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vations at our medical centre, where younger patients and more black and Latin-American patients are seen with pernicious anaemia than the more classical preponderance of older white patients of European origin, have shown a higher prevalence of anti-IF antibody among them [16,17]. At the same time, the prevalence of the presumably more common anti-parietal cell antibody has seemed to be lower than expected [17]. This combination of preliminary observations led to the following more complete survey, which also attempts to establish what factors influence the antibody frequencies and to suggest possible different patterns or forms of the disease.

PATIENTS AND METHODS

Data were accumulated on all patients with documented pernicious anaemia over the past 15 years at our medical centre. The diagnosis of pernicious anaemia had been made by standard criteria. In brief, the criteria required present or past evidence of cobalamin deficiency and evidence of IF dysfunction. Nearly all the patients were tested for antibody at the time of diagnosis of their disease. Because of the high diagnostic specificity of anti-IF antibody, that test was done for clinical purposes in all 324 patients. Anti-parietal cell antibody and other tests that are sometimes diagnostically useful were not done in all cases, but available sera were subsequently assayed when possible. The only selection factor in this survey was that serum from the patient had to be available for antibody determinations. Anti-IF antibody status did not influence selection; for example, 84·6% of antibody-positive and 77·1% of antibody-negative patients were also tested for anti-parietal cell antibody.

Racial designation was based on the patient's own self-identification to the hospital admitting office, which was noted along with birth date and sex on the patient's hospital stamp.

Blocking (type I) anti-IF antibody was determined by a sensitive radioisotopic technique [18]; care was always taken to avoid the assay artifact induced by cobalamin injection given the patient. Anti-parietal cell antibody had been determined by Dr Kenneth Tung of the University of New Mexico by immunofluorescent antibody assay in the patients tested before 1981 as part of an earlier study [17]; the technique, its performance and the criteria used to determine positive and negative results have been described previously [17,19]. The immunofluorescent antibody assay using the Autoantibody Screen Kit (Zeus Scientific Co., Branchburg, NJ), which uses rat stomach sections for antigen, was done in patients seen after then. The technique and interpretation of results were exactly as described in the manufacturer's directions. Sera were tested in a 1:20 dilution and always compared with a strongly positive (4+) control serum. Any apple-green staining whatsoever (trace to 4+) was considered positive, and further serial dilutions could be done as needed. With this kit, 5% of healthy controls give positive results, a frequency in good agreement with the published literature [2–6]. Reassay of sera showed good agreement between the two anti-parietal cell antibody assays. Twelve of 14 sera gave identical results; one serum was positive by only one assay while another was positive only by the other assay. Moreover, testing showed that positive sera stored at −20°C for periods of 5 years or more maintained their positivity.

Table 1. Anti-intrinsic factor antibody prevalence in patients with pernicious anaemia

	Positive anti-intrinsic factor antibody		
	Women	Men	Total
White	32/59 (54%)	17/30 (57%)	49/89 (55%)
Latin American	55/77 (71%)	32/49 (65%)	87/126 (69%)
Black	64/72 (89%)	25/34 (74%)	89/106 (84%)
Asian	1/1 (100%)	2/2 (100%)	3/3 (100%)
Total	152/209 (73%)	76/115 (66%)	228/324 (70%)

Table 2. Anti-parietal cell antibody prevalence in patients with pernicious anaemia

	Positive anti-parietal cell antibody		
	Women	Men	Total
White	26/45 (58%)	11/23 (48%)	37/68 (54%)
Latin American	37/61 (61%)	25/43 (58%)	62/104 (60%)
Black	32/64 (50%)	13/27 (48%)	45/91 (49%)
Asian	1/1 (100%)	1/2 (50%)	2/3 (67%)
Total	96/171 (56%)	50/95 (53%)	146/266 (55%)

Statistical analysis

Standard methods of statistical analysis were applied, primarily Student's *t*-test and the χ^2-test, using the PC Statistician computer program (Human Systems Dynamics, Northridge, CA).

RESULTS

Antibodies and race

Anti-IF antibody was found in 70% of the 324 patients (Table 1). As also noted previously [16], the frequency was significantly higher in blacks than in Latin-American patients ($\chi^2 = 6{\cdot}89$, $P < 0{\cdot}01$) or white patients of European origin ($\chi^2 = 23{\cdot}54$, $P < 0{\cdot}001$). Latin Americans also had a higher frequency than whites ($\chi^2 = 4{\cdot}53$, $P < 0{\cdot}05$). While the antibody appeared slightly more frequently in women than in men, the overall difference between the sexes was not statistically significant. Nevertheless, black women had the highest incidence among all subjects (89%).

Anti-parietal cell antibody was present in only 55% of the 266 patients tested (Table 2). Although women had slightly higher frequencies than men and blacks had the lowest prevalence of this antibody among the racial groups, none of the racial or sex differences was statistically significant. It is striking, nevertheless, that less than half of the black patients had the antibody, compared with their unusually high rate of anti-IF antibody.

The two antibodies were present together in 42% of all patients, while 15% of patients had neither antibody (Table 3).

Table 3. Comparison of anti-intrinsic factor antibody (IFA) and anti-parietal cell antibody (PCA) findings in patients with pernicious anaemia

	+IFA +PCA	+IFA −PCA	−IFA +PCA	−IFA −PCA	Total
White	28 (41%)	15 (22%)	9 (13%)	16 (24%)	68
Latin American	40 (38%)	29 (28%)	22 (21%)	13 (13%)	104
Black	42 (46%)	35 (39%)	3 (3%)	11 (12%)	91
Asian	2 (67%)	1 (33%)	0	0	3
Total	112 (42%)	80 (30%)	34 (13%)	40 (15%)	

Interestingly, only 13% had anti-parietal cell antibody but not anti-IF antibody, whereas 30% had anti-IF antibody without having anti-parietal cell antibody. The latter disparity in favour of anti-IF antibody was more pronounced among blacks than among the other racial groups ($\chi^2 = 20{\cdot}68$, $P < 0{\cdot}01$). Significant differences between men and women were not found ($\chi^2 = 1{\cdot}53$).

Antibodies and age

Patients with anti-parietal cell antibody were older at the time of diagnosis than those lacking the antibody ($59{\cdot}6 \pm 16{\cdot}2$ *vs* $54{\cdot}8 \pm 17{\cdot}8$ years, $t = 2{\cdot}288$, $P = 0{\cdot}022$); those with anti-IF antibody were also older at diagnosis than those without, but not significantly so ($59{\cdot}9 \pm 16{\cdot}8$ *vs* $56{\cdot}5 \pm 16{\cdot}4$ years, $t = 1{\cdot}662$). Furthermore, the 112 patients who had both antibodies together were older than the 40 patients who had neither antibody ($61{\cdot}0 \pm 16{\cdot}2$ *vs* $53{\cdot}1 \pm 17{\cdot}9$ years, $t = 2{\cdot}586$, $P = 0{\cdot}01$); the 112 patients with both antibodies were also slightly older than patients who had only one of the two antibodies ($t = 2{\cdot}126$, $P = 0{\cdot}033$ when compared with the 80 patients with anti-IF but not anti-parietal cell antibody, and $t = 1{\cdot}874$, $P = 0{\cdot}06$ when compared with the 34 patients with the reverse combination). These age-related differences were not attributable to race or the fact that the black patients were younger than the whites [16]. The observed age difference related to antibody persisted among blacks, who were younger than whites and had the highest anti-IF antibody and lowest anti-parietal cell antibody frequencies. Thus, for example, the 42 blacks with both antibodies were older than the 11 blacks without either antibody ($58{\cdot}2 \pm 17{\cdot}3$ *vs* $45{\cdot}5 \pm 15{\cdot}6$ years, $t = 1{\cdot}830$, $P = 0{\cdot}029$).

Antibodies and serum gastrin

Serum gastrin elevations were not associated with either anti-IF or anti-parietal cell antibody status. As in earlier surveys [17,20], the 143 women tested in the present group had higher gastrin levels than the 73 tested men (1475 ± 1462 *vs* 908 ± 718 $\mu l/l$, $t = 3{\cdot}127$, $P = 0{\cdot}002$). Interestingly, a racial difference in gastrin levels, independent of sex, was also apparent. The 92 Latin Americans tested had lower gastrin levels than the 54 whites (962 ± 780 *vs* 1585 ± 1885 $\mu l/l$, $t = 2{\cdot}793$, $P = 0{\cdot}006$) and 67 blacks (1486 ± 1197 $\mu l/l$, $t = 3{\cdot}340$, $P = 0{\cdot}001$).

DISCUSSION

The results indicate that the greater frequency of anti-parietal cell antibody than anti-IF antibody in pernicious anaemia no longer holds true, at least in the patients seen in our hospital. The change from data reported in the past [2–6] is striking, particularly with respect to the decreased prevalence of anti-parietal cell antibody. Several possible reasons may contribute to this new pattern.

One reason could be technical. The anti-IF antibody assay has been made more sensitive by greatly increasing the serum-to-IF antigen ratio [18]. Conversely, it is conceivable that the anti-parietal cell antibody assay may be less sensitive than assays used by others in the past. However, the immunofluorescence test for this antibody is by now a standard technique. Moreover, similar results were obtained by two separate assays; the results at our hospital agreed closely with those obtained earlier in Dr Tung's laboratory.

A more likely explanation for the changes in prevalence has to do with the demographics of our patient population [21]. The increased prevalence of anti-IF antibody was confined largely to black patients and, to a much lesser degree, Latin-American patients. The prevalence of this antibody among the white patients at our hospital was comparable to that described in the past [2,3,6–12]. Thus, the anti-IF antibody observations are explicable more easily by the racial differences of the patients with pernicious anaemia seen at our hospital, than by technical factors like the improved assay.

Race and sex differences, however, do not explain the markedly lower prevalence of anti-parietal cell antibody evident in this study. Although the difference was most striking among the black patients, the low prevalence applied to all racial or ethnic groups and to both sexes.

Age may be a partial explanation, however, because the antibody-negative patients tended to be younger than those with anti-parietal cell antibody. It is worth noting, in this regard, that patients with 'juvenile' pernicious anaemia rarely have anti-parietal cell antibody, although they have a high frequency of anti-IF antibody [22,23]. The same has been observed in a group of younger patients in Scandinavia with a strong family history [24]. The lower frequency of anti-parietal cell antibody, thus, seems attributable more to a tendency to antibody negativity in younger patients than to differences between American and European patients.

The frequency of patients with pernicious anaemia who had anti-IF antibody but lacked anti-parietal cell antibody was also striking in the present study. This immunological combination occurred more than twice as often as patients having the anti-parietal cell antibody but not anti-IF antibody (80 *vs* 34 patients), and stands in sharp contrast to the rarity of such a combination in previous studies. It belies a claim that 'IF antibodies only rarely occur in the absence of parietal cell antibodies' [25].

The new patterns described here suggest that their relation to pernicious anaemia may now need reassessment on two levels. The more fundamental one has to do with the nature and mechanisms of the disease (or diseases?) called pernicious anaemia. Strickland & Mackay [26], in classifying atrophic gastritis and assigning pernicious anaemia to type A gastritis, placed great emphasis on the anti-parietal cell antibody in this classification. The changed pattern observed here, in particular the diminished prevalence of anti-parietal cell antibody, raises the possibility that the nature of the atrophic gastritis or its immunologic manifestations may be different.

Since the cause of the gastric lesion of pernicious anaemia is still unknown, only speculation can be provided at this time. However, the disease and perhaps even its origins may differ in

some patients. For example, black women, and to a much lesser extent Latin Americans, often develop the disease at a younger age and have a very high frequency of anti-IF but not anti-parietal cell antibody. Together with patients with 'juvenile' pernicious anaemia [22,23] and the Scandinavian patients with 'hereditary' pernicious anaemia [24], all of whom have in common features like younger age, higher rate of anti-IF antibody and lower rate of anti-parietal cell antibody, they may constitute a distinct subset of accelerated pernicious anaemia, as proposed elsewhere [27]. The association of anti-parietal cell antibody negativity with young age observed here fits with such a possibility.

Reassessment of current thinking about antibodies in pernicious anaemia, furthermore, seems to be justified at the level of clinical diagnosis. The anti-parietal cell antibody, long known to have poor diagnostic specificity for pernicious anaemia, now appears to have lost its sensitivity as well. Its value as a diagnostic tool, therefore, is clearly less than that of the more specific, and now also more sensitive, diagnostic tool of anti-IF antibody assay.

Whether the higher gastrin levels in women with pernicious anaemia than in men that we described earlier [17,20] and that others have confirmed [12] provide any additional clues to the gastritis underlying the pernicious anaemia remains to be seen. The present incidental finding of lower gastrin levels in Latin-American patients is intriguing but impossible to set in context in our present state of limited knowledge. Study of the underlying process of gastritis with more sophisticated techniques is necessary to resolve such questions.

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