

An investigation of the immune response of patients suffering from anorexia nervosa

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Summary

Patients suffering from anorexia nervosa rarely appear to develop the common cold or influenza. This study examines the immunological response of fifteen female anorexia nervosa patients of both the vomiting and carbohydrate-abstaining type and compares them with a control population matched for age and occupation. Both anorectics and control populations received the admune influenza vaccine. Initially both groups had similar haemagglutination inhibition titres against the three different viral antigens: A/HK; A/PC; A/Eng. However, the anorectics showed over a 2-month period a higher titre of antibody especially to the Hong Kong virus: this was significant. Cellular immune responses were measured using a tuberculin and a macrophage inhibition test, no significant difference between the two groups was observed. These results which support the clinical findings are discussed.

Introduction

Patients suffering with anorexia nervosa rarely appear to develop the common cold or influenza. Furthermore, contrary to previously held opinion, they seldom die from infective illness (Crisp, 1967, 1970; Dally, 1969). According to Dally (1969) some patients complain that after regaining weight they lose this immunity and become more susceptible to infection. In contrast to the supposed resistance to, or avoidance of viral infections, for these patients are often special isolates, Dally reported that five patients out of a population of 140 developed tuberculosis during starvation or shortly after regaining their target weight. More recently, Crisp, Palmer and Kalucy (1976) have reported that two

out of twenty-eight male cases had active pulmonary tuberculosis in contrast to there being no such illness amongst 300 female patients. The two males were characterized by regular vomiting and purging and displayed the profound metabolic disturbance associated with this particular pathological form of anorexia nervosa (Crisp, 1970). To examine these clinical findings, humoral antibody response to influenza, haemagglutination inhibition (HAI) and cell-mediated immunity (CMI) against tuberculin were measured in a group of patients suffering from anorexia nervosa and a control group of healthy subjects.

Materials and methods

Study population

The test group consisted of 15 female anorectics receiving either in- or out-patient treatment. Their age range was from 16 to 36 years (mean 21.7 years). The control population comprised school children, undergraduates and technical staff and were age (mean 20.6 years) and sex matched. All participants were normal with respect to respiratory function as demonstrated by medical history, physical examination, chest X-ray and vital capacity. None of the subjects had a history of influenza in the preceding 12 months. Subjects with a history of allergy were excluded. During the course of the study, records of infection were kept; none of the subjects developed influenza.

Vaccines. Each 0.5-ml dose of Admune influenza vaccine contained: A/England/42/72, 400 i.u., A/Port Chalmers/1/73, 400 i.u., and A/Hong Kong/1/68, 360 i.u. In addition, each subject received intradermally 0.1 ml tuberculin purified derivative (PPD) of *Mycobacterium tuberculosis*.

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Extraction of sera and treatment to remove non-specific inhibitor. Blood was collected into sterile universal bottles and after clot retraction serum was pipetted off and centrifuged at 1500 r.p.m. for 10 min, transferred to a sterile bijou and stored at -20°C . To remove non-specific inhibitors, serum was diluted 1 in 4 with receptor destroying enzyme (RDE) Wellcome Reagents, and incubated for 18 hr at 37°C , the enzyme was inactivated by incubating the serum at 56°C for 1 hr.

Red blood cell. Human type 'O' Rh +ve RBC were used and on each test occasion taken from the same subject. The cells were washed three times using veronal buffer and 0.1% bovine plasma albumin (BPA) then counted using a Celloscope 401 to give a dilution of 60×10^8 cells/l (0.5% suspension).

Virus culture. Fertile, specific pathogen-free (SPF) hen eggs were incubated at 38°C for 9 days in 55–60% humidity. Viruses were inoculated allantoically and harvested 3 days later.

Antibody assay. Haemagglutination and haemagglutination inhibition tests were carried out using microtitre plates (Flow Laboratories Ltd). The method used was that of Heirholzer, Suggs and Hall (1969).

Macrophage inhibition. The test for inhibition of macrophages was carried out with peritoneal macrophages obtained from unimmunized guinea-pigs using the method of Rocklin, Meyer and David (1970).

Protocol. Initially, 10 ml of blood were taken by venepuncture and HAI estimation carried out, thereafter each subject was given, by intramuscular injection, 0.5 ml commercial inactivated influenza vaccine. A further 10 ml venous blood were collected on the 10th and 28th day after vaccination. On day

48, a tuberculin test was carried out and 48 hr later the site of injection was examined and the reactive zone measured and recorded. On day 50, 20 ml venous blood were taken to determine final haemagglutinating antibody titre and cellular immune response (CIR) to tuberculin.

Results

Eight of the patients (8/15; 53%) and 10 of the controls (10/15; 67%) were positive to the tuberculin test. No significant difference was observed in the macrophage inhibition test of the two groups. The results for haemagglutination titres showed considerable individual variation, therefore the results have been pooled and the means for each group (patients and controls) calculated.

The amount of carbohydrate ingested was variable within the anorectic population. There were three main groups: in-patients on a high carbohydrate diet, out-patients before treatment on a low carbohydrate diet and out-patients after treatment on a normal carbohydrate diet. This variation in carbohydrate intake was not associated with the haemagglutination titre response. In addition, although some of the patients had previously maintained low weight by means of vomiting and purging, thus fundamentally changing their metabolic status in relation to the carbohydrate abstaining group of anorectics (Crisp, 1970), this was not associated with any difference in the immune response.

The haemagglutinating inhibition titres are set out in Table 1 and illustrated in Fig. 1. The levels of antibody against all three influenza antigens on day 0 were similar, this is particularly significant because it means both groups had obviously a past history of infection and their baseline levels were

TABLE 1. The geometric mean titre, mean and standard deviation for the control group and those patients suffering from anorexia nervosa

	Day	Control Group			Anorexia Nervosa Group		
		GMT	\bar{X}	s.d.	GMT	\bar{X}	s.d.
A England 42/72	0	53	1.723	0.297	55	1.743	0.520
	10	211	2.325	0.518	139	2.144	0.582
	28	279	2.455	0.443	444	2.636	0.453
	50	279	2.445	0.260	463	2.666	0.494
A Hong Kong 1/68	0	84	1.923	0.267	76	1.883	0.618
	10	254	2.405	0.271	201	2.305	0.588
	28	320	2.505	0.254	670	2.826	0.502*
	50	292	2.465	0.298	845	2.927	0.578*
A Port Chalmers 1/73	0	21	1.321	0.311	22	1.341	0.392
	10	127	2.104	0.405	55	1.743	0.439*
	28	201	2.304	0.387	279	2.445	0.604
	50	211	2.325	0.391	335	2.525	0.687

GMT = Geometric mean titre (antilog of \bar{X}).

\bar{X} = mean of log titre values.

s.d. = standard deviation of mean of log titre value.

* $P < 0.05$ using students *t*-test.

the same. By day 10 the control subjects had higher titres of HA antibodies and the titres for antibodies against A/Port Chalmers/1/73 were significantly higher ($t = 2.344$; $P < 0.05$) than the antibody levels in the test group. Thereafter, on day 28 and 50 the situation reversed with the test group of patients showing higher titres of antibody to A/England/42/72

and A/Port Chalmers/1/73 viruses, although the difference was not significant there was an increase in the expected direction. The increase in titre in response to A/HK/1/68 at day 28 and 50 was, however, statistically significant ($t = 2.2098$; $P < 0.05$) ($t = 2.7491$; $P < 0.05$).

Discussion

Anorexia nervosa is an illness which predominantly affects young women and is characterized by a tendency to manipulate diet to lose weight (Crisp, 1967). Amenorrhoea usually supervenes at an early stage. Two distinct groups are found clinically. The first, and more common, comprises those who lose weight by avoiding carbohydrate which, in extreme cases, may lead to a concomitant reduction in protein and fat intake. The second smaller group induce vomiting to lose weight or maintain a low weight. This group tend also to have periods of overeating, at which time their diet may contain large quantities of carbohydrate.

The nutritional disorder in anorexia nervosa is associated with manipulation of carbohydrate rather than lack of protein and is, therefore, not comparable to kwashiorkor. Both Russell (1968) and Crisp (1970) have shown that anorectics eat a diet containing adequate amounts of protein. Berkman and Powell (1947) reported that serum albumin and globulin were mostly within normal range although one-third of the thirty-four patients, presumably vomiters, had levels lower than normal. Both Brothwood (1964) and Russell (1968) confirmed generally normal levels of plasma proteins although Russell showed a slight reduction in the rate of degradation of albumin and that protein depletion is more a feature of male anorexia nervosa. He suggested that, in females, fat propensity may protect them for protein depletion although the finding could be explained by the greater tendency of male anorectics to vomit as a means of controlling their weight.

In children suffering from kwashiorkor, Cohen and Hansen (1962) measured albumin and γ -globulin. They found that whilst albumin was reduced by about 50%, the level and turnover of γ -globulin was relatively unaffected by the state of nutrition. In cases of kwashiorkor complicated by infection, however, the globulin synthesis rate was three times higher than that observed in unaffected children. The production of large amounts of γ -globulins by protein-depleted subjects suggests that γ -globulin-forming cells preferentially utilize available amino acids, as a result, the synthesis of other biologically important proteins may be seriously limited and this may be the reason why the clinical manifestations of kwashiorkor are often precipitated by infection. Brown and Katz (1965) have challenged these

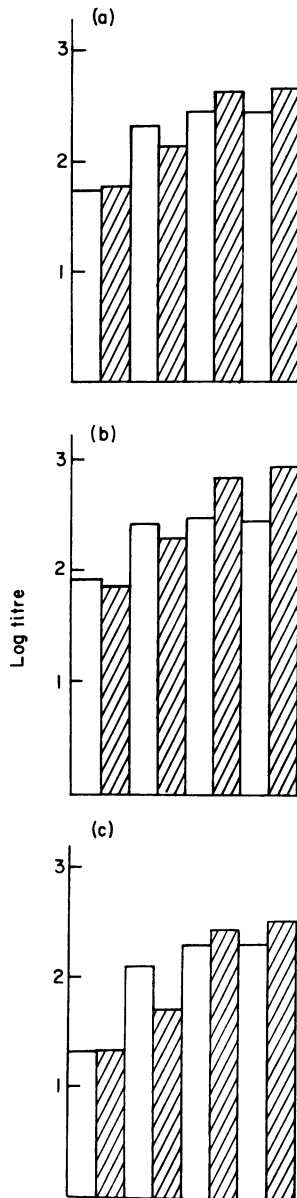


FIG. 1. Geometric mean titres of humoral antibody response to three influenza antigens. (a) England 42/72, (b) Hong Kong 1/68 and (c) Port Chalmers 1/73. The shaded bars are the test group, the unshaded are the control group.

findings; but McFarlane *et al.* (1970) found normal serum IgA, IgM and IgG levels in children with kwashiorkor. In Najjar, Stephan and Asfour's (1969) study of immunoglobulins in marasmic infants, it was found that infants of 3–6 months had significantly higher levels of immunoglobulins compared with healthy infants of the same age. In older infants of 7–30 months, levels of IgA and IgM were significantly higher than the corresponding levels in well nourished infants.

Many authors have commented on the low levels of reproductive hormones in anorexics of low body weight. Russell (1970), reviewing this work, reported that on re-feeding, gonadotrophins and oestrogens tended to increase to almost normal levels but little cyclical activity was noted. Crisp and his colleagues have reported that low serum levels of reproductive hormones in anorexia at low body weight revert to normal values on re-feeding, although this capacity depended, in part, on their pre-morbid weight (Crisp *et al.*, 1973; Palmer *et al.*, 1975).

The possible relationship between gonadotrophic and gonadal steroid hormone and immunity is biologically interesting and relatively unexplored. Indications of the effect of oestrogens on immunity in animal experiments have been reported by Nicol *et al.* (1964). He found that oestrogenic hormones stimulate reticulo-endothelial cells in the uterus (Nicol, 1935), and further investigation indicated that the reticulo-endothelial system (RES) as a whole responded to stimulation by natural and synthetic oestrogenic compounds (Nicol *et al.*, 1964). Nicol and his colleagues also showed RES activity varied at different stages of the oestrus cycle and during pregnancy in both rats and mice. It seems feasible therefore that the observed differences between the test and control group may be directly related to serum oestrogen levels and this possible relationship is worth further investigation.

In unpublished studies, Armstrong-Esther, Kean and Meerabux (1973) found raised serum levels of IgM and transferrin and decreased levels of IgA and IgG in women taking oral contraceptives. There appeared to be no significant change in the titre of IgA or IgM during the menstrual cycle but there was an increase in IgG. It is possible, therefore, that the effects of hormones, especially oestrogens, on IgG concentrations may be different from those on IgA or IgM. This could occur by action on the catabolic rate of IgG, which unlike that of IgA and IgM is directly proportional to its concentration and may involve a saturable protection system (Brambell, Hemmings and Morris, 1964). Several hormones may act upon the half-life role of the immunoglobulins; steroids reduce the levels of IgG, IgM and IgA, and IgG survival time is shortened (Waldmann, Bluese and Stroler, 1974).

Thyroxine also reduced the IgG survival time in guinea-pigs (Dixon *et al.*, 1962), and it is therefore possible that the ovarian hormones may play a similar role.

Other possible mechanisms for the effect of oestrogen on immune responses may be related to RNA-antigen complexes (Franzl, 1962), since RNA synthesis is oestrogen-sensitive (Gorski and Nicolette, 1963). Pre-menstrual decline in oestrogens also leads to a decline in blood levels of vitamin A (Laurence and Sobel, 1953). Since vitamin A has adjuvant properties (Jurin and Tannock, 1972) and increases the titres of certain antibodies, this may be an important factor in patients suffering from anorexia nervosa. The glucocorticoids also have a well known immuno-suppressive effect (Nicol and Snell 1956; Elliott and Sinclair, 1968); it is possible that oestrogen may favour immunological synthesis by decreasing the production of glucocorticoids. Oestrogen also prolongs the biological half-life of the adrenal corticosteroids (Yates and Urquhart, 1962; Tait and Burstein, 1964), their increased levels during pregnancy may be part of a biological mechanism which according to Anderson (1971) prevents the development of maternal immunity against the fetus. By comparison, the stimulatory effect of oestrogens upon the RES has been well demonstrated by Nicol *et al.* (1964) in a series of animal experiments. These workers have also proposed that the human RES may be activated during the follicular phase of the menstrual cycle when repair and proliferation take place, and during the luteal phase as a protection against infection as the endometrium degenerates. Such fluctuations of activity in the RES during the menstrual cycle were first observed by Eufinger (1932). The latter would be of obvious importance as a defence mechanism and the former may increase the effectiveness of the immunological surveillance mechanism (Burnet, 1970) and thus decrease the risk of malignancy which is concomitant with any proliferative process.

The results of the study show an interesting difference between the two groups and this may be related to the prepubertal status of the patients rather than their protein/caloric intake.

Acknowledgments

Admune influenza vaccine was kindly supplied by Duncan Flockhart and Co. Ltd, London. Viruses for anti-haemagglutination antibody estimation were kindly supplied by Dr G. C. Schild, National Institute for Medical Research, Mill Hill, London. Fertile specific pathogen-free hen eggs for culture were obtained from Orchard Farm, Great Missenden, Buckinghamshire.

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