Serum immunoglobulins in a rural and an urban population of Greece with special reference to immunoglobulin A

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SUMMARY The serum concentration of immunoglobulins G, A, and M was studied in 390 inhabitants of a rural community and in 204 residents of an urban area of Greece. It was found that serum IgA levels were significantly higher in the rural area where intestinal infections were more prevalent. No significant difference in serum IgG was found between the two groups. IgM was found to be lower among residents of the rural area. The present findings suggest a possible ecological association between increased IgA and chronic exposure to intestinal infection.

The demonstration that IgA is the principal class of immunoglobulin in external secretions and that it is the main immunoglobulin made by plasma cells in mucous membranes of the gastrointestinal tract (Crabbé *et al.*, 1965) has stimulated great interest in recent years. The description of alpha chain disease (α -CD) by Seligmann *et al.* (1968), which presents as a benign or neoplastic aberration of the IgA secretory system, has led to the observation that this disease affects mainly individuals living in areas with a high degree of intestinal infestations and low socioeconomic background (Seligmann *et al.*, 1971). This peculiar geographic distribution of patients with α -CD and 'Mediterranean lymphoma', which is closely related to α -CD (Doe, 1975), strongly suggests that environmental factors providing a sustained antigenic stimulation of the IgA secretory system may initiate a diffuse proliferation of plasma cells in the gut mucosa. This process can be reversible or eventually resume a malignant character (Rimbaud and Matuchansky, 1973; Seligmann, 1975). It is noteworthy that, of the four cases with α -CD seen in Greece, two lived in villages with poor hygienic conditions and the other two came from very low income families (Manousos, 1975; Economidou *et al.*, 1976; Economidou, unpublished observations).

The situation with regard to the origin of serum IgA in humans is not quite clear. The intestinal IgA

immunocytes produce varying proportions of monomers and polymers (Brandtzaeg and Baklien, 1977), and although the main secretory product is oligomeric it is believed that the gut-associated lymphoid tissue is probably a significant source of the monomeric serum IgA in man (Lamm, 1976).

Thus we decided to examine the serum immunoglobulin levels in two populations with different degrees of exposure to intestinal pathogens in order to test the hypothesis that chronic intestinal infection may cause changes in secretory IgA production which will be reflected in the serum IgA levels.

Material and methods

A total of 594 persons of both sexes aged 17 to 70 years was studied; 390 lived in a rural area of central Greece (Thessaly). This area was chosen because of its poor hygienic conditions (the water supply is pumped from open wells and there is no sewage system) and because two of the index cases of α -CD originated from these village communities. A group of 204 permanent inhabitants of Athens matched for age and sex served as controls. The persons tested were selected at random. They were given a complete physical examination and their previous history of infection with special reference to brucellosis, typhoid, paratyphoid infections, and history of frequent attacks of diarrhoea was recorded as well as their body weight and height. Subjects with a history of chronic illness which might have influenced the immunoglobulin levels, for example,

renal failure, cirrhosis, rheumatoid arthritis, alcoholism, etc., were not included in this series. Also subjects showing evidence of acute infection were excluded.

All blood samples were screened for protein abnormalities by serum electrophoresis. Quantitative determination of IgA, IgG, and IgM was performed by the radial immunodiffusion technique (Mancini *et al.*, 1965) using commercially obtained immunoplates and standard serum (Melyo Laboratories, Springfield, Va, USA). Serum immunoelectrophoresis and the immunoselection plate technique for the detection of free alpha chains (Doe *et al.*, 1972) was carried out in selected cases. The hepatitis B surface antigen (HBsAg) was detected by the reverse haemagglutination technique (Hepanosticon, Organon).

Statistical analysis was performed after logarithmic transformation of the immunoglobulin values using standard analysis of variance techniques (Armitage, 1971). In duplicate measurements, the analytical error for IgG, IgA, and IgM was 5.8%, 4.9%, and 10.7% respectively (Carlson, 1960).

Table 1 Distribution of subjects according to age, sex, and urban-rural residence

Age group (years)	Urban		Rural	
	Males	Females	Males	Females
-29	30	40	79	70
30-39	20	18	39	28
40-49	32	21	68	38
50+	20	23	39	29
Total	102	102	225	165

Table 2 History of infection in subjects of urban or rural residence

Residence	Number examined	Percent with histories of:			Percent prevalence of HBsAg
		Salmonellosis	Brucellosis	Frequent diarrhoea	
Urban	204	1.5	0.0	0.5	0.5
Rural	390	6.7	5.4	7.2	5.9

Table 3 Geometric mean values and log-normal range of serum immunoglobulins according to urban or rural residence

Immunoglobulin (g/l)	Urban		Rural	
	Males	Females	Males	Females
IgG	11.48 (7.83-16.83)	12.02 (8.20-17.62)	11.75 (7.24-19.05)	12.02 (7.41-19.50)
IgA	1.86 (0.65- 5.37)	1.82 (0.68- 4.88)	2.00 (0.84- 4.74)	2.00 (0.94- 4.23)
IgM	1.20 (0.38- 3.77)	1.95 (0.54- 6.98)	1.05 (0.38- 2.91)	1.70 (0.52- 5.55)

Results

The distribution of the 594 examined subjects according to age, sex, and urban or rural residence is shown in Table 1. Histories of infection from all persons summarised in Table 2 indicate that in the rural area the frequency of gastrointestinal infection and episodes of diarrhoea is much higher than in the urban area. The initial screening of the serum samples by electrophoresis on cellulose acetate revealed a paraprotein spike in two persons living in different villages. The paraproteins were characterised as monoclonal IgA λ and IgG λ respectively. All sera with raised immunoglobulin levels were also further analysed by immunoelectrophoresis, and sera with increased IgA were studied by the immunoselection plate technique for the detection of free alpha chains. This investigation did not reveal any additional cases of monoclonal immunoglobulins. Among the 594 examined subjects, two, both males from the rural area, were found to have no detectable levels of IgA. These cases with selective IgA deficiency were excluded from all further analysis since this abnormality is considered to be genetically determined (Goldberg *et al.*, 1968; Nell *et al.*, 1972).

Serum concentrations of IgG, IgA, and IgM according to sex and rural or urban residence were analysed after logarithmic transformation of the individual values because the respective frequency distributions were positively skewed. The geometric mean values and the log-normal range for each immunoglobulin class are shown in Table 3.

With respect to serum IgG, there was clearly no sex effect, and no significant difference was found between rural and urban residents.

With respect to serum IgA there was again no sex difference. However it was found that the rural sample had significantly higher serum IgA than the urban sample (t value 2.06, with 590 df; $p < 0.05$).

By examining the IgM data it was observed that the IgM concentration was significantly higher in females in both the rural and urban populations ($p < 0.001$). On the other hand, residents of urban areas had a higher mean serum IgM concentration than residents of rural areas, and this was true for both men ($p < 0.02$) and women ($p < 0.02$). The

effect of height on IgM concentration was also examined, and a weak positive correlation between these two factors was found ($r = +0.13$ for males and $r = +0.10$ for females).

Discussion

In Greece, the mortality from infectious and parasitic diseases and the prevalence of parasitoses is higher in the rural than in the urban parts of the country (Constandinidis *et al.*, 1974; Kaladiti *et al.*, 1977). Extensive studies in large groups of the population have shown that there is a significantly greater frequency of markers for hepatitis B infection in the rural than in the urban areas (Blumberg *et al.*, 1975; Trichopoulos *et al.*, 1976).

The above cited evidence implied that the rural populations of the country are exposed to intestinal infections more often than the urban ones. This was clearly confirmed by our investigation, which showed a higher prevalence of HBsAg carrier state and a more frequent reporting of salmonellosis, brucellosis, and undiagnosed attacks of diarrhoea in the rural area studied. For this reason we thought it sufficient to compare the levels of serum immunoglobulins in the two groups instead of analysing individual cases with and without intestinal infections.

The levels of IgG were not found to be raised in the village communities studied in spite of the evidence for a higher prevalence of infection. The increased levels of IgG and IgM which have been observed in certain communities of developing countries have been attributed mainly to exposure to malaria, trypanosomiasis, or other parasitic infestations which are not endemic in Greece (Zegers *et al.*, 1973; McGregor *et al.*, 1970).

The occurrence of higher IgM levels in females than in males has been reported several times (McGregor *et al.*, 1970; Butterworth *et al.*, 1967) and has been observed in the present study. The existence of a weak positive correlation between height and IgM concentration, which has been demonstrated in the analysis of our data, might explain the difference noted, since in Greece urban residents are taller than inhabitants of rural areas (Georgiadis *et al.*, 1969).

Our main finding was that serum IgA was significantly higher in the rural area. This suggests that there might be an ecological correlation between serum IgA and intestinal infection.

The occurrence in certain countries of Mediterranean lymphoma and α -CD has been attributed to chronic intestinal infections prevalent in these areas (Seligmann *et al.*, 1971; Ramot *et al.*, 1965). Eidelman *et al.* (1966) performed serial intestinal

biopsies in healthy people from these countries and showed an increased lymphocytic and plasma cell infiltration within the lamina propria. These observations have provided support for the current view about the role of chronic intestinal infections in initiating diffuse plasmacytic proliferation in the intestinal mucosa which may eventually progress to the development of Mediterranean lymphoma or α -CD in genetically predisposed subjects (Seligmann, 1975; Rappaport *et al.*, 1972). However, the relation of intestinal infection to serum IgA has not been specifically studied in large population samples of healthy people. The results of the present work provide some support to the concept that chronic antigenic exposure of the gut mucosa causes a sustained stimulation of the IgA secretory system which may be reflected by increased serum IgA levels.

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