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An association between Gc (vitamin D-binding protein) alleles and susceptibility to rheumatic fever

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SUMMARY

Rheumatic fever is associated with exaggerated activity of B cells with massive production of antibody to the Group A streptococcus. Gc (vitamin D-binding protein) is constitutively expressed on B-cell membranes in association with membrane immunoglobulin, and could be involved in cell activation. We therefore looked for associations between the three major Gc alleles and susceptibility to rheumatic fever in a homogeneous Arab population. Patients with tuberculosis or rheumatoid arthritis and control donors, were studied in parallel. Allele frequencies in the controls, rheumatoid and tuberculosis patients were identical to those found in a previous study of normal Arab donors. However, there was a striking association between Gc2 and rheumatic fever. This allele was twice as common in these patients as in controls ($p = 0{\cdot}0024$), and was present in 56·4% of all rheumatic fever patients.

INTRODUCTION

Vitamin D-binding protein (also known as Group-specific component or Gc) is an alpha 2-glycoprotein of 56,000–58,000 MW belonging to the albumin/fetuin gene family (Cooke & David, 1985). It is abundant in serum where it acts as a transport protein for vitamin D sterols, particularly the major, but relatively inactive circulating form, 25-(OH) vitamin D_3 (Haddad & Walgate, 1976). When bound to this sterol Gc can also transport calcium. The most active form of vitamin D_3 (1-25-$(OH)_2$ cholecalciferol, or calcitriol) shows little affinity for Gc, but enters cells by an unknown mechanism before interacting with the receptor in the nucleus (McDonnell *et al.*, 1987). However, it is possible that serum or membrane Gc is relevant to the entry of 25(OH) cholecalciferol into cells such as monocytes, which can express a 1-hydroxylase and so convert 25(OH) D_3 into the active 1-25-$(OH)_2D_3$ derivative, which then exerts potent effects on macrophage maturation and activation (Rook *et al.*, 1986). Gc also has a high affinity for actin, to which it tends to be complexed when tissue damage has resulted in systemic actin release (Lind *et al.*, 1986). This property may be relevant to its occurrence in the membranes of several cell types, including B and T lymphocytes, monocytes (Petrini, Emerson & Galbraith, 1983) and trophoblast (Nestler *et al.*, 1987). Its presence in membranes and its ability to bind actin suggest a cytoskeletal function. Moreover, it is a substrate for phospholipid and Ca^{2+}-dependent protein kinase (Wooten *et al.*, 1985), so is likely to be involved in cell activation. Specifically immunological roles are suggested by the association of membrane Gc with Fc receptors (Petrini *et al.*, 1984) and membrane immunoglobulin (Petrini *et al.*, 1983).

Serum Gc shows polymorphism due to the existence of three major alleles (Gc2, Gc1F and Gc1S) (Constans & Viau, 1977) and about 100 rare ones. The conservation of this molecule throughout the animal kingdom, and the maintenance of the diversity in humans, suggests that the different alleles confer different advantages but these do not appear to relate to their known function of binding vitamin D. Associations have been reported between these alleles and susceptibility to rheumatoid arthritis (Papiha & Pal, 1985), multiple sclerosis (Gonatas, Greene & Waksman, 1986), liver disease (Brown, Carter & Sood, 1979), and Kuru (Kitchin, Bearn & Alpers, 1972). We report here that Gc2 is strongly associated with susceptibility to rheumatic fever in an Arab population, but no associations were found with rheumatoid arthritis or tuberculosis, in spite of the clear evidence that vitamin D_3 metabolism in granuloma macrophages plays a key role in the pathology of the latter (Rook *et al.*, 1986).

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MATERIALS AND METHODS

Subjects

All serum donors for all control and patient groups were Arabs. Rheumatic fever (RF) donors were 39 Arab children (26 males,

13 females) with acute rheumatic fever attending the paediatric clinic at Mubarak Teaching Hospital in Kuwait. These, like the control group, were from various Arab countries, particularly Kuwait, Jordan and Saudi Arabia. None of the patients were siblings. The diagnosis was based on the revised Jones criteria (Stollerman *et al.*, 1965) and 15 of them developed rheumatic heart disease.

The tuberculosis (TB) patients were 41 Arabs (34 males, seven females) with radiologically and bacteriologically proven pulmonary tuberculosis.

The rheumatoid arthritis (RA) patients were 66 Arabs (24 males, 42 females) attending the rheumatology clinic with established rheumatoid arthritis varying in duration from 2 to 18 years.

The control donors were 90 Arabs (70 males, 20 females) who were university and hospital staff.

Gc subtypes

Gc subtypes were identified by isoelectric focusing on thin-layer polyacrylamide gels as described elsewhere (Eales *et al.*, 1987). The ampholyte (pH 4·5–5·4) was obtained from Pharmacia, Uppsala, Sweden. Gels were prefocused for 30 min at 30 watts, 1500 volts, with 1·0 mol/litre sodium hydroxide and 1 mol/litre o-phosphoric acid as cathode and anode buffers, respectively. Sample application wicks were saturated wth 20 μl of serum or plasma, and samples were allowed to focus for 3000 volt/hr. The Gc bands, previously localized with the aid of appropriate antisera, were visualized by fixation with 10% trichloroacetic acid. Standards of known Gc phenotype were included between each pair of unknown samples. All samples were run on two different systems simultaneously to eliminate possible problems due to the equipment reported previously (Eales, Nye & Pinching, 1988).

RESULTS

The three phenotypes containing Gc2 were more common in ARF than in control sera. This was significant for Gc2, 1S ($P=0·0044$). There were no significant differences between the phenotype frequencies of the RA or TB sera and the controls. The gene frequencies show that Gc2 is twice as frequent (33·3%) in RF sera than in the controls (16·7%, $P=0·0024$). The compensatory decrease occurred mostly in 1F ($P=0·048$). The frequencies of the three alleles in TB and RA were essentially identical to those seen in the control group.

Table 1. The frequencies of Gc phenotypes and alleles

Phenotype	Normal ($n=90$)	TB ($n=41$)	RF ($n=39$)	RA ($n=66$)
2·2	5	0	3	4
	(5·6)	(0)	(7·7)	(6·1)
2·1S	12	10	14*	4
	(13·3)	(24·4)	(35·9)	(6·1)
2·1F	8	3	5	5
	(8·9)	(7·3)	(12·8)	(7·6)
1S·1S	38	13	10	30
	(42·2)	(31·7)	(25·6)	(45·5)
1F·1S	16	13	7	16
	(17·8)	(31·7)	(17·9)	(24·2)
1F·1F	11	2	0	7
	(12·2)	(4·9)	(0)	(10·6)
Allele				
2	(16·7)	(15·9)	(33·3)**	(12·9)
1S	(57·8)	(59·8)	(52·6)	(60·6)
1F	(125·6)	(24·4)	(15·4)***	(26·5)

P values calculated relative to the control group, by Fisher's exact test. Values in parentheses are percentages. *$P=0·0044$; **$P=0·0024$; ***$P=0·048$.

DISCUSSION

Our data for normal donors are in remarkably close agreement with a previous study of 342 normal Arabs (Nevo & Cleve, 1983). These authors found Gc2 in 18·6% (our value, 16·7%). Gc1S in 60·2% (our value 57·8%), and Gc1F in 21·2% (our value 25·6%). Similarly the lack of association between Gc alleles and tuberculosis is in agreement with an earlier study (Papiha, Agarwal & White, 1983) and, since vitamin D metabolism is directly involved in the pathogenesis of this disease (Rook *et al.*, 1986), the negative finding could indicate that the relevant functions of the Gc alleles do not concern vitamin D transport.

Papiha & Pal (1985) reported an excess of Gc2 in rheumatoid arthritis patients, though this was of doubtful significance and was not found in the Arab population studied here.

The finding that Gc2 is twice as frequent in rheumatic fever cases as in the normal population is striking (relative risk 2·25). Thus 56·4% of all RF patients expressed this gene, compared to 27·8% of the controls. This is not an artefact due to a non-genetic familial association, because none of the patients were siblings and, moreover, they were from several different Arab countries.

Rheumatic fever is usually thought to be due to an autoimmune response provoked by cross-reactive antigens present in Group A streptococci (Barrett, 1984), though the details of the pathogenesis remain unknown. There is hyperproduction of antibody to the Streptococci as well as to other antigens in these patients (Ayoub, 1984; Rejholec, 1957), and there is also a claim that they have an unusual B-cell alloantigen (Patarroyo, 1983). Since Gc is found on B-cell membranes where it may provide a link between surface immunoglobulin and the cytoskeleton (Petrini *et al.*, 1983; Petrini *et al.*, 1984) it is possible that the relevance of Gc alleles in rheumatic fever depends on direct modulation of a B-cell function. It is conceivable that a streptococcal component can interact directly with membrane-bound Gc2. A further possibility is that membrane Gc2 is related to the B-cell alloantigenic specificity reported by Pattarroyo (1983), though these authors have some evidence that this is associated with two polypeptides of 29,000 and 33,000 MW.

It is also possible that the association we have detected is due to another linked gene on the same region of chromosome 4 (Cooke *et al.*, 1986). The gene for IL-2 is one such possibility (Seigel *et al.*, 1984) since this cytokine is implicated in the activation of B cells.

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