The group-specific protein marker: a possible indicator of syphilis, not human immunodeficiency virus infection

D. Russell Pollard, PhD
Peter Gill, PhD
Ann Day, RT

We wished to compare the frequency of group-specific (Gc) phenotypes in the general population with that in people with human immunodeficiency virus (HIV) infection to find out whether the Gc protein is a marker for susceptibility to HIV infection. We determined the phenotype frequency in 1083 randomly selected serum samples obtained from the Canadian Influenza Survey Studies and compared it with that in 263 serum samples obtained from the Federal Centre for AIDS and the Syphilis Serology Proficiency Testing Laboratory. No association between Gc phenotype and HIV status was found. However, there was a strong association between the Gc protein 1f/1f phenotype and syphilis.

On a comparé les fréquences relatives des phénotypes de groupe (Gc) dans la population générale et chez les porteurs du virus immunodéficitaire humain (VIH) afin de savoir si la protéine Gc pourrait servir à reconnaître les personnes sujettes à cette infection. Les 1083 échantillons de sérum choisis au hasard parmi ceux prélevés pour la surveillance canadienne de l'influenza sont mis en regard de 263 échantillons de sérum obtenus du Centre fédéral d'étude du SIDA et du Laboratoire de contrôle du sérodiagnostic de la syphilis. Si les phénotypes Gc ne montre aucun rapport avec la sérologie VIH, le phénotype Gc 1f/1f en possède un très marqué avec la syphilis.

From the Bureau of Microbiology, Laboratory Centre for Disease Control, Department of National Health and Welfare, Ottawa

Reprint requests to: Dr. D. Russell Pollard, Bureau of Microbiology, Laboratory Centre for Disease Control, Tunney's Pasture, Ottawa, Ont. K1A 0L2

The availability of a clinical marker for susceptibility to human immunodeficiency virus (HIV) infection is of paramount importance to the global medical community.

The group-specific component (Gc protein) is an α_2-globulin that is found on cells and in serum.[1,2] Electrophoresis has shown that there are three major alleles in the Gc system: Gc^{1f}, Gc^{1s} and Gc^{2}.[3] A person's phenotype for the Gc protein is determined by the rules of mendelian inheritance; therefore, any person may have one of six common phenotypes. Some 30 rare variants of this system have been reported.[4]

The Gc protein was originally thought to be a blood-group protein, but subsequent investigations have identified it as the vitamin-D-binding factor.[5] When bound to vitamin D, Gc can transport large amounts of calcium and may be important in regulating the bioenergetics of cells. Little is known about the biologic importance of the Gc protein or the functional differences between the six subtypes. Gc interacts with IgG on the membrane of B lymphocytes and with the Fc (crystallizable fragment) receptors of IgG and may, in fact, be an Fc-gamma receptor. Certain Gc subtypes have been associated with rheumatoid arthritis,[6] multiple sclerosis[7] and liver disease,[8] but how the Gc protein contributes to the pathogenesis of these disorders has not been established.

Eales and colleagues[9] reported an association between different Gc alleles and the susceptibility to and the clinical manifestation of HIV infection. Subsequent studies[10,11] were unable to confirm this finding. It is most commonly suggested that the discrepancy between these later reports and the initial study is most readily explained by sampling error (chance), differences in the methods used to screen for the Gc protein, differences in the ethnic backgrounds of the sample populations or some combination of these factors.

In an attempt to test the hypothesis proposed by Eales and colleagues we compared the frequen-

cy of Gc phenotypes in the general population with that in people with HIV infection.

Methods

We obtained as controls 1083 randomly selected serum samples from the Canadian Influenza Survey Studies, which collect blood samples every 2 weeks until the influenza season begins to examine viral antibody titres in clinically healthy people of different ethnic backgrounds and sexual preference from three age groups: less than 15 years of age, 15 to 64 years of age and 65 years of age or more. These people were from five provinces, which included the main urban areas in eastern and western Canada.

In addition we obtained serum samples from the Federal Centre for AIDS, Ottawa, which receives samples from across Canada for confirmation of HIV infection, and from the Syphilis Serology Proficiency Testing Laboratory, Laboratory Centre for Disease Control, Ottawa, which receives samples for subsequent pooling to establish a proficiency test panel. Of the 263 samples 155 were HIV-positive; of the 133 syphilis-positive samples 47 were HIV-positive, and of the 130 syphilis-negative samples 108 were HIV-positive.

Gc subtyping was performed by means of isoelectric focusing on 1% agarose gel that contained 6% ampholytes at pH 4.5 to 5.4; the anode buffer was 0.05 M sulfuric acid and the cathode buffer 1.0 M sodium hydroxide. The gels were prefocused for 30 minutes at 1500 V, 30 W and 50 mA. Undiluted serum (15 μl) was added, and the gels were focused for 3000 Vh. Sample wicks were removed after 45 minutes, and 10% trichloroacetic acid was used as a fixative for examining the Gc bands; for verification the findings were compared with those of immunofixation on cellulose acetate strips saturated (1:5) with rabbit antihuman Gc globulin and stained with 0.2% Coomassie blue. Subtypes were scored independently by two investigators who were unaware of the clinical or serologic profile of the samples.

To preclude biologic false-positive results the microhemagglutination assay for *Treponema pallidum* antibodies (MHA-TP) was used for screening and the fluorescent treponemal antibody absorption test (FTA-ABS) for confirmation of a syphilis serodiagnosis on all tested samples.

Results

The frequency distribution of Gc phenotypes in the 1083 control samples did not differ from the distribution in 4864 control samples, as cited in a letter by Daiger and associates[11] (Table I); the latter samples represented the weighted averages of 13 Caucasian populations. The phenotype frequencies were in Hardy–Weinberg equilibrium, which predicts the constancy of genotype frequency from generation to generation in a population; this suggested that no selective pressures were involved. No differences in geographic location or age were detected.

There was no association between the frequency distribution of Gc subtypes and HIV infection. Furthermore, the frequency distribution in all of the HIV-positive samples did not differ from that in the control samples (Table I).

The distribution of Gc subtypes in the HIV-positive samples obtained from the Syphilis Serology Proficiency Testing Laboratory clearly differed from that in the other HIV-positive samples. Subsequent analysis of all the samples for reactivity in the MHA-TP and FTA-ABS tests revealed a strong association between the distribution of Gc subtypes and seropositivity for syphilis (Table I).

The 1f/1f phenotype was 10 times more frequent among people with syphilis than among those without syphilis; it was 5 times more frequent among those with syphilis than among the general population. The Gc^{1f} allele was twice as frequent among people with syphilis as among

Table I — Frequencies of group-specific (Gc) protein and Gc alleles in serum samples from control subjects and people with human immunodeficiency virus (HIV) infection, with or without syphilis

	Frequency								
	Phenotype						Allele		
Samples	2/2	2/1s	2/1f	1s/1s	1s/1f	1f/1f	Gc^{2}	Gc^{1s}	Gc^{1f}
Control									
European study[11] (n = 4864)	0.066	0.305	0.076	0.355	0.176	0.022	0.256	0.596	0.148
Present study (n = 1083)	0.076	0.283	0.083	0.344	0.185	0.029	0.259	0.578	0.162
HIV-positive (n = 155)	0.077	0.329	0.077	0.290	0.200	0.026	0.281	0.554	0.164
Syphilis*									
Positive (n = 133)†	0.045	0.331	0.090	0.203	0.218	0.113	0.259	0.483	0.270
Negative (n = 130)†	0.069	0.377	0.085	0.338	0.115	0.015	0.294	0.578	0.114

*Homogeneity χ^2 for syphilis by phenotype = 19.347, p = 0.002, 5 degrees of freedom.
†Of the syphilis-positive and syphilis-negative samples, 47 and 108 respectively were HIV-positive.

those without syphilis. Neither population was in Hardy-Weinberg equilibrium.

Discussion

People with the Gc^{1f} allele appear to be at greater risk for syphilis than those without this allele. This association is similar to the association that Eales and colleagues[9] observed between different Gc alleles and the susceptibility to and the clinical manifestation of HIV infection. Because of the large proportion of HIV-positive people who are also seropositive for syphilis (more than 30% in this study), concomitant syphilitic infection or positive serologic results for syphilis might have been a confounding factor in their study.

Whether the Gc^{1f} allele represents a genetic marker for susceptibility to a viral or bacterial infection remains unresolved and merits further study. Mounting evidence is showing a statistically significant association between seropositivity for syphilis and for HIV, as demonstrated by the association between *T. pallidum* serologic markers and HIV infection.[12]

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