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Abnormal blood rheology and chronic low grade inflammation: possible risk factors for accelerated atherosclerosis and coronary artery disease in Lewis negative subjects

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Abstract

Objective—To test the hypothesis that abnormal hemorheology and chronic low-grade inflammation are more prevalent in Lewis negative individuals, possibly contributing to premature atherosclerosis.

Methods and Results—We enrolled 223 healthy subjects (154 females, mean age: 64yrs). Conventional risk factors, markers of inflammation and hemorheological profiles were measured; Lewis blood group was determined by serology. Conventional risk factors (age, gender, BMI, blood pressure, lipid profile, smoking habit) did not differ among Lewis phenotypes. However, markers of inflammation (WBC, hs-CRP, ESR) were significantly elevated and rheological parameters (RBC aggregation, plasma viscosity) were abnormal in Lewis negative subjects, especially when compared to the Le(a−b+) group.

Conclusions—With a prevalence of 33% in select populations, our data support the hypothesis that Le(a−b−) represents a pro-inflammatory phenotype that may contribute to the elevated cardiovascular risk in this group.

Keywords

Lewis negative phenotype; atherosclerosis; inflammation; blood rheology

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Disclosures
Authors have no conflict of interest to disclose.

Introduction

The synthesis of Lewis A (Le^a) and Lewis B (Le^b) antigens is determined by the activity of multiple fucosyltransferase enzymes (FUT) encoded on chromosome 19 (19p13.3)[1]. Soluble Le^a and Le^b are released by exocrine epithelial cells into body fluids and adsorb passively onto the red blood cell (RBC) membrane[2] thereby enabling the use of immunoassays to determine an individual's Lewis phenotype: negative Le(a−b−); A+ Le(a+b−); B+ Le(a−b+) or A+B+ Le(a+b+). Lewis negative individuals lack alpha (1–3/1–4) fucosyltransferase activity due to inactivating point mutations affecting the FUT3 locus[3].

Epidemiologic studies found the Lewis negative phenotype to be independently associated with a two-fold higher prevalence of coronary artery disease and a four-fold higher risk for fatal coronary events[4, 5]. While a few subsequent studies failed to prove such association[6], several others re-confirmed Le(a−b−) as an independent risk factor for atherosclerosis[7, 8]. The underlying mechanisms responsible for the increased cardiovascular risk have not been determined.

Abnormal hemorheological parameters lead to impaired hemodynamic profiles both in the macro- and microcirculation[9]. As a consequence, an extended area of the vascular endothelium is subjected to low-oscillatory shear stress inducing arterial wall remodeling, endothelial dysfunction[10] and adhesion molecule overexpression[11], ultimately promoting inflammation and atherosclerotic plaque formation[12]. The association between Lewis phenotypes, chronic low-grade inflammation and disturbed blood rheology has not been explored. The present study evaluated the hypothesis that the accelerated atherosclerosis in Lewis negative individuals is associated with a genetically determined pro-inflammatory state that exists on a permissive background of abnormal hemorheology.

Methods

A blinded cross-sectional study was designed to evaluate our hypothesis. Blood samples were collected from 318 randomly selected males and postmenopausal females participating in one of two randomized controlled trials at the University of Southern California (USC): 1) B-Vitamin Atherosclerosis Intervention Trial (BVAIT; n=145)[13] and 2) Women's Isoflavone Soy Health trial (WISH; n=173)[14]. For both trials, exclusion criteria included known atherosclerotic vascular disease, diabetes mellitus, untreated hypertension, chronic kidney disease, untreated hypo- or hyperthyroidism and five or more alcoholic beverages per day. For the current study, additional exclusion criteria included acute illness within four weeks and transfusion of blood products within 90 days of sampling. All participants provided written informed consent and the Institutional Review Board of USC approved all protocols.

After collecting demographic information, risk factor profile and hemorheological data from 100 individuals blinded to their Lewis phenotype, the study protocol was expanded to include the following surrogate inflammatory markers: white blood cell count (WBC), high-sensitivity C reactive protein (hs-CRP), plasma fibrinogen concentration and insulin level. In addition, plasma homocysteine was determined in all BVAIT subjects. An additional 123

subjects were enrolled in this second study phase. Given the low prevalence of Lewis negatives in the general population, a third phase of sampling was performed aiming to enrich for Le(a−b−) subjects. All 95 trial participants in this third phase were tested for their Lewis status but only blood from the five Le(a−b−) subjects was utilized. Individuals who typed Le(a−b+) (n=80) or Le(a+b−) (n=10) were not added to the analytic dataset.

BVAIT and WISH participants were recruited at the 30-month and pre-randomization visits, respectively. Samples for hs-CRP, fibrinogen and insulin measurements were stored at −80°C for a maximum of three months and were assaye d in batches. All other tests were completed within six hours of collection. Complete blood counts were determined using an automated hematology analyzer (Micros, Horiba-ABX, Irvine, CA); microhematocrit (Hct) for each suspension was also confirmed using a tabletop centrifuge. A Coulter Plasma Viscometer II (Coulter Electronics, Luton, UK) was utilized to measure plasma viscosity (PV) at 25°C. RBC aggregation and aggregability (i.e., the intrinsic tendency of RBC to aggregate in a standard 70kDa dextran medium) were determined with a Myrenne MA-2 aggregometer (Myrenne GmBH, Roetgen, Germany) according to the current guidelines for hemorheological laboratory techniques[15]. Erythrocyte sedimentation rate (ESR) was measured in a subset of subjects at native and at 40% Hct using standard Westergren tubes. Plasma fibrinogen was determined by the STart benchtop hemostasis system (Diagnostica Stago, Parsippany, NJ) for 79 individuals. hs-CRP (Zymutest, Hyphen BioMed, Neuville-sur-Oise, France) and insulin levels (Linco Research, St. Charles, MO) were measured by ELISA in 120 and 97 samples, respectively. Homocysteine was determined by reverse phase high performance liquid chromatography in 87 B-VAIT samples. RBC Lewis phenotype was determined by two independent investigators by serology according to standard laboratory procedures[16]. Monoclonal anti-Le^a and anti-Le^b antibodies were purchased from Ortho Clinical Diagnostics, Rochester, NY.

Demographic and laboratory characteristics were compared between Lewis phenotype groups using analysis of variance for continuous, and chi-square tests for categorical measures. The associations between hemorheological parameters and Lewis phenotype were evaluated with adjustment for age, gender, race/ethnicity and smoking habits using analysis of covariance (ANCOVA). These comparisons of mean differences of hemorheological measures among Lewis phenotype groups were also adjusted for a 3-level trial group variable that included WISH (all participants were sampled at pre-randomization), B-vitamin-treated BVAIT, and placebo-treated BVAIT. Variables not normally distributed were log transformed for ANCOVA; results are presented as mean±SEM by Lewis phenotype.

Results

318 individuals were Lewis phenotyped; 49 (15.4%) were Lewis negative, 68 (21.4%) were Le(a+b−) and 201 (63.2%) tested Le(a−b+). Consistent with literature[17], Lewis negative phenotype was most prevalent among African Americans (25.0%) followed by Hispanics (19.0%), Caucasians (13.9%) and Asians (13.9%). The hemorheological profile was evaluated for 223 subjects with the following Lewis phenotype distribution: 19.7% Le(a−b−); 26.0% Le(a+b−); 54.3% Le(a−b+).

As shown in Table 1, the average age, BMI, fasting plasma glucose, lipid profile, systolic/diastolic blood pressure and the number of current/ex-smokers were equivalent among the three Lewis groups. The prevalence of the individual Lewis phenotypes did not differ among subjects enrolled from the WISH and BVAIT trials. Mean values for platelet count (data not shown) and hemoglobin/Hct were similar among Lewis phenotypes while WBC count was elevated in Lewis negatives ($p=0.05$). ESR values were strikingly different between the groups both at native and at 40% Hct: ESR was in the normal range for all Le(a−b+) subjects while almost one-half of Le(a−b−) individuals had values exceeding the upper limit of the age-adjusted normal range (<30mm/hr; $p<0.001$; Table 2). Results obtained by the Myrenne aggregometer were consistent with the ESR data. Mean group differences were attenuated when testing RBC aggregability but plasma viscosity was significantly elevated in Lewis negatives compared to the Lewis positive population ($p<0.0001$). A trend for elevated fibrinogen and hs-CRP values was also noted such that Le(a−b−)>Le(a+b−)>Le(a−b+); the difference in hs-CRP reached borderline statistical significance between the Lewis negative and Le(a−b+) groups ($p=0.05$). Although diabetes mellitus was an exclusion criterion and BMI was similar across the groups, fasting insulin levels were elevated in Le(a−b−) individuals. There was a trend for elevated homocysteine values in Lewis negatives but the difference did not reach statistical significance (Table 2).

Discussion

The present study is the first to demonstrate an abnormal hemorheological profile in healthy individuals with Lewis negative phenotype. Our findings revealed increased WBC count, RBC aggregation both at native (ESR) and at 40% Hct (ESR and Myrenne) in this group compared to Lewis (a−b+) participants (Table 2). The increased ESR, plasma viscosity, hs-CRP and plasma fibrinogen in Lewis (a−b−) participants are consistent with a low-grade inflammatory state. The significant ($p<0.05$), but less prominent, elevation of Le(a−b−) RBC aggregability suggests relatively subtle differences in RBC surface properties. Despite normal fasting glucose values, insulin level was significantly increased in the Lewis negative group, a finding that would not alone promote abnormal blood rheology. Overall, our results are consistent with prior reports of increased obesity[18], hypertriglyceridemia[19], insulin resistance[20] and higher prevalence of non-insulin dependent diabetes mellitus[19] in the Le(a−b−) population. However, the underlying pathological mechanisms responsible for these observations remain unclear.

Localized low-oscillatory shear stress provokes endothelial cell activation thereby increasing luminal E-selectin and P-selectin expression[11]. These selectins nurture focal leukocyte adhesion, transmigration, inflammation and, over years, plaque formation[21]. Lewis antigens comprise Type-1 (Le^a, Le^b) and Type-2 (Le^x, Le^y) carbohydrates assembled by the sequential addition of fucose monosaccharides onto precursor oligosaccharides[22]. Remarkably, sialyl-Le^x and sialyl-Le^a are both high-affinity circulating selectin ligands thereby limiting leukocyte adhesion to the activated endothelium[23]. We therefore hypothesize that soluble sialyl-Le^x and sialyl-Le^a in the plasma of Lewis positive subjects function as a naturally occurring selectin receptor antagonist. Conversely, Lewis negative individuals lack these circulating fucosylated oligosaccharides thereby reducing the number of defense mechanisms mitigating the chronic inflammatory process of atherosclerosis.

Considering that the prevalence of Le(a−b−) phenotype exceeds 10% in Caucasians and is considerably higher among African Americans and Hispanics, future research might target investigation of early screening to identify healthy individuals with inherently increased risk for premature cardiovascular adverse events.

Study limitations

Our analysis is limited by its cross-sectional design. ESR, fibrinogen, hs-CRP and insulin data were only available for a subset of subjects due to study design. Circulating sialyl-Le^x and sialyl-Le^a levels were not directly assessed but will be added to follow-up study protocols. A poorly understood but well described phenomenon is when patients with certain diseases and during pregnancy lose Lewis antigens from their RBC surface[24, 25] despite normal FUT3 activity. This renders RBCs Lewis negative by serology. No participants had known malignancy at the time of enrollment and all females were postmenopausal by study design.

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Table 1

Demographic data and conventional risk factors for the study population.

	Le(a−b−) n=44	Le(a+b−) n=58	Le(a−b+) n=121	p value*	p value**
Age (years)	65.5±1.4	63.9±1.2	63.5±0.9	0.50	0.24
Females	32 (73%)	46 (79%)	76 (63%)	0.07	0.24
BMI (kg/m^2)	27.7±0.8	26.1±0.7	26.9±0.5	0.28	0.43
Fasting glucose (mg/dL)	98.7±1.7	95.3±1.5	97.7±1.0	0.25	0.62
Total cholesterol (mg/dL)	214.2±5.4	223.7±4.7	215.3±3.2	0.28	0.86
LDL cholesterol (mg/dL)	132.3±4.8	138.6±4.2	132.3±2.9	0.43	0.99
HDL cholesterol (mg/dL)	58.8±2.5	63.6±2.2	59.1±1.5	0.19	0.93
Triglycerides (mg/dL)	115.2±8.4	107.0±7.2	119.3±5.0	0.38	0.70
Systolic BP (mmHg)	125.8±2.4	119.9±2.1	124.5±1.4	0.11	0.66
Diastolic BP (mmHg)	75.2±1.3	76.1±1.1	76.8±0.8	0.57	0.31
Smoking (ex and current)	21 (48%)	24 (41%)	47 (39%)	0.61	0.32
Trial***					
BVAIT: B-Vitamin treated	15 (34%)	11 (19%)	37 (31%)	0.15	
BVAIT: Placebo treated	11 (25%)	14 (24%)	38 (31%)		
WISH	18 (41%)	33 (57%)	46 (38%)		

Values in tables are mean±SEM or n (percent)

* p value for comparison of differences among Lewis phenotype groups ANOVA or chi-square test

** p value for comparison of Lewis(a−b−) vs. Lewis(a−b+) group using ANOVA

*** Trial distribution by Lewis phenotype frequencies, n (%), chi-square p-value

Table 2

Comparison of hemorheological profiles and inflammatory markers among individuals with different Lewis phenotypes, adjusted for age, gender, ethnicity, smoking and trial group.

	Le(a−b−) n=44	Le(a+b−) n=58	Le(a−b+) n=121	p value*	p value**
WBC (cell/μl)±SEM	5.65±0.25	4.97±0.23	5.22±0.20		
Log WBC (cell/μl)±SEM#	1.70±0.04	1.59±0.04	1.62±0.03	0.05	0.08
Hematocrit (%)±SEM	41.88±0.48	41.54±0.44	41.86±0.38	0.74	0.98
ESR (native hct; mm/hr)±SEM	28.37±2.56	18.24±2.33	14.83±2.50		
Log ESR (native hct; mm/hr)±SEM#	3.21±0.15	2.75±0.14	2.48±0.14	0.0001	0.0002
ESR (40% hct; mm/hr)±SEM	29.38±2.61	17.67±2.38	13.82±2.55		
Log ESR (40% hct; mm/hr)±SEM#	3.30±0.14	2.80±0.13	2.55±0.14	<0.0001	<0.0001
M in plasma ±SEM+	23.48±0.75	19.13±0.69	18.03±0.59		
Log M in plasma ±SEM#+	3.14±0.04	2.94±0.04	2.88±0.03	<0.0001	<0.0001
M1 in plasma ±SEM+	35.94±1.10	28.43±1.01	26.91±0.87		
Log M1 in plasma ±SEM#+	3.57±0.04	3.34±0.04	3.28±0.03	<0.0001	<0.0001
M in dextran ±SEM+	24.32±0.75	22.87±0.69	22.55±0.59	0.06	0.03
M1 in dextran ±SEM+	36.72±1.00	34.38±0.92	34.43±0.79	0.05	0.03
Plasma viscosity (mPa.s)±SEM	1.67±0.01	1.60±0.01	1.58±0.01	<0.0001	<0.0001
Fibrinogen (g/L)±SEM	4.11±0.23	3.74±0.24	3.65±0.23		
Log Fibrinogen (g/L)±SEM	1.38±0.06	1.30±0.06	1.27±0.06	0.16	0.10
hs-CRP (mg/L)±SEM	2.83±0.67	2.28±0.64	1.60±0.62		
Log hs-CRP (mg/L)±SEM#	0.38±0.27	0.29±0.27	−0.12±0.26	0.18	0.05
Insulin (μU/mL)±SEM	13.37±1.38	11.30±1.44	9.70±1.38		
Log Insulin (μU/mL)±SEM#	2.47±0.10	2.38±0.11	2.20±0.10	0.05	0.02
Homocysteine (μmol/L)±SEM	13.06±1.71	9.30±1.83	9.98±1.02		
Log homocysteine (μmol/L)±SEM#	2.34±0.08	2.20±0.08	2.26±0.04	0.41	0.35

* p value for comparison of Lewis negative vs. Lewis A vs. Lewis B groups using ANOVA

** p value for comparison of Lewis negative vs. Lewis B group using ANOVA

+ Myrenne M value represents RBC aggregation at stasis; M1 index represents RBC aggregation at very low shear.

variables compared following log transformation

Number of subjects different for these parameters ($n_{a-b-}/n_{a+b-}/n_{a-b+}$): ESR = 19/30/29, fibrinogen = 21/25/33, hs-CRP = 31/36/53, insulin = 29/30/38; fasting homocysteine = 15/12/60

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