can lead to unregulated accumulation of cholesterol in macrophages and the formation of foam cells.[2] Consequently, it has been proposed that ox-LDL is one of the physiological ligands for scavenger receptors.[2] Numerous studies have provided evidence that ox-LDL is present in vivo. For example, ox-LDL can be extracted from athersoclerotic lesions,[1,4-5] and epitopes of ox--LDL have been demonstrated immunohistochemically in atherosclerotic lesions in humans and animal models.[4,6-10] Autoantibodies reactive with ox-LDL are present in plasma and lesions of human and animals,[4,10-12] and small amounts of minimally oxidized LDL can be demonstrated in plasma.[12-16]

Many of the proatherogenic properties of ox-LDL cause these effects. LDL oxidation is very complex and heterogenous. During oxidation both the protein and the lipid moieties of LDL particle can be modified.[4]

The aim of this study was to examine the expression of autoantibody against 2 different sources of oxidized LDL in patients with early stage acute myocardial infarction (AMI) and in normal subjects, and the other purpose was to study the correlation of this autoantibody expression in normal subjects and in patients with AMI.

MATERIALS AND METHODS

Patients

Sera were collected from the following 4 groups of people.

Group I: 26 normal subjects with no lipid disorder or cardiovasular disease.

Group II: 27 patients with high serum LDL (150~220 mg/dl)

Group III: 25 patients with high serum triglyceride (200~560 mg/dl)

Group IV: 41 patients (aged from 29 to 91 years old; 33 males and 8 females) with early stage acute myocardial infarction. The sera were collected within 36 h after chest pain, Electrocardiograms showed acute nontransmural or transmural infarction, and creatine phosphokinase (CK) levels were all elevated (ranging from 500 to 3500 units) with elevated creatine phosphokinase MB (ranging from 30 to 250 units). Only 30 out of 41 AMI patients were studied for autoantibody against 2 different sera.

All 4 groups were tested for antibody against high LDL serum.

Materials

Ultracentrifuge tube was purchased from Beckman, California, U.S.A.

Methods

Preparation of LDL from human sera and LDL oxidized by $CuSO_4$

LDL was isolated and purified from 2 groups of sera by ultracentrifugation according to the method of Redgrave et al.[17] One was with high-LDL serum (above 170 mg/dl) and the other was in a normal LDL range (below 120 mg/dl). The purified LDL was oxidized by 10 mM $CuSO_4$ for 18 h, then stopped with addition of an ethylenediaminetetraacetate (EDTA) solution. The oxidized LDL was detected with a trinitrobenzenesulfonic acid assay[18] and separated by 1% agarose gel electrophoresis.

ELISA method of anti-oxidized LDL antibody assay

The 2 groups of oxidized LDL were dissolved in a 0.15 M $NaHCO_3$ solution, then coated with 5 mg/ml of an ELISA (enzyme-linked immunosorbent assay) plate, individually. After the blocking procedure with 3% BSA, the 50x-diluted sera from AMI patients and normal subjects were integrated against 2 different groups of ox-LDL, respectively, then incubated for 1 h at 37 °C. After the washing procedure, the anti-human second antibody (conjugated with alkaline phosphatase) was added and incubated at 37 °C for 1 h again. Finally, the results were shown by absorbance at a 405-nm wavelength under substrate integration. Sera from high-triglyceride (H-TG, 200~560 mg/dl) and high-LDL (H-LDL, 150~220 mg/dl) subjects were also assayed against the group of ox-LDL from high-LDL serum according to the above methods.

Statistical Methods

Student's *t*-test was used for statistical analysis in