Effect of Plasma Lipoproteins on Natural Killer Cell Activity in the Elderly Population

Tomiya Yasumasa,1 Kazuo Takahara,1 Takao Sadayasu,3 Hirokazu Date,4 Kazuhiko Isozumi,2 Ryouji Kouzuma,2 and Yasuhide Nakashima2

1First Department of Medical Technology, School of Health Sciences, and 2Second Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan. 3Seiwakai Clinic and 4Ashiya Central Hospital, Fukuoka Prefecture, Japan.

Background. Natural killer (NK) cells possess spontaneous cytotoxicity against tumors and virus-infected cells to play a major role in immunosurveillance and defense against the development of cancer, as well as bacterial and viral infections. The role of plasma lipoproteins in atherogenesis is well recognized, but the physiological relevance of their immunoregulatory properties is still questioned. In particular, it is unknown whether hypercholesterolemia should be considered a risk factor for diminished immunity in old age.

Methods. To evaluate effects of plasma lipoprotein levels on immune function, we assessed the relation between plasma lipoprotein profiles and NK cell activity. NK cell activity was assayed by release of 51Cr from K562 target cells, and concurrent plasma lipoprotein levels were measured in 47 samples of elderly males (mean age ± SD, 66.6 ± 1.7 years).

Results. Univariate regression analyses revealed direct relations between NK cell activity and high-density lipoprotein cholesterol (r = .46, p < .001), apolipoprotein (Apo) A-1 (r = .48, p < .001), and Apo A-2 (r = .46, p < .005). In addition, multiple regression analyses showed a direct independent relation between NK cell activity and Apo A-1 (β = 0.32 ± 0.09 mg/dl, p < .001).

Conclusion. NK cell activity is related directly to plasma Apo A-1 levels in elderly subjects. The mechanisms of this interaction are unknown, but Apo A-1 contributes to the composition of the antiatherogenic fraction of high-density lipoprotein and could also defend against infectious and malignant disease through a potential for NK cell activity.

The human natural killer (NK) cell is a T-cell receptor negative, large granular lymphocyte, which is phenotypically characterized as CD3 negative, CD16 positive, and Leu 19 positive. NK cells possess spontaneous cytotoxicity against tumors and virus-infected cells independent of major histocompatibility restriction or prior sensitization to these targets (1). In vivo, NK cells play a major role in the immunosurveillance and defense against the development of cancer (2), as well as bacterial and viral infections (3), and their function in resistance to growth and metastasis of tumors has been well established (4).

The role of plasma lipoproteins in atherogenesis is well recognized, but the physiological relevance of their immunoregulatory properties is still questioned. In vitro, NK cells expressed different lipoprotein receptors, and the effects of different lipoproteins on the proliferative and cytotoxic responses of these cells were investigated (5). However, in experimental studies, responses of NK cell activity to lipoproteins vary according to the concentrations of lipoproteins or modified lipoprotein, such as acetyl-modified low-density lipoprotein (LDL) or oxidation of lipoproteins, and in vitro artefacts, such as the choice of culture medium and supplements. In addition, NK cells stimulated with different lipoproteins secrete a variety of cytokines, such as interleukin-2, and these cytokines in an autocrine–paracrine manner affect NK cell activity on stimulation with optimal concentrations of lipoproteins (6). These experimental studies illustrate the complexity of the regulatory networks for immunoenhancement or immunosuppression by lipoproteins. Many other factors must be taken into account in determining whether hypercholesterolemia in elderly people should be considered a risk factor for diminished immunity in these experimental analyses.

In this study, to evaluate the role of plasma lipoproteins and the physiological relevance of their immunoregulatory properties, we studied the relation between the plasma lipoprotein profile and NK cell activity in elderly subjects who lived in the same community and underwent a medical checkup.

Methods

Subjects

The subjects were 47 elderly males living in the Ashiya district who underwent a medical checkup at Ashiya Central Hospital, Onga, Fukuoka Prefecture, Japan, during the period from May 1, 1997, to May 31, 2001. Only patients aged 65–69 years were enrolled in the study. None had clinical signs or symptoms of infectious disease, malignancy, leukemia, or an immune deficiency disorder, nor were any taking medication that could affect plasma lipoprotein profiles, such as HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase.
inhibitors or fibric acid derivatives. All underwent a physical examination followed by a blood analysis. A signed consent form was obtained from each subject.

**Lipoprotein and Apolipoprotein Profiles**

Blood was drawn between 8 AM and 9 AM after 15 minutes rest from subjects who had been fasting since 9 PM the previous evening. Total cholesterol and high-density lipoprotein (HDL) cholesterol concentrations were measured enzymatically by using L-Type Wako Cholesterol H (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) and Determiner-L HDL-C reagent (Kyowa Medex Co. Ltd., Tokyo, Japan), respectively, in a Chemistry Autoanalyzer BM8 (Jeol Ltd., Tokyo, Japan) in the laboratory at Ashiya Central Hospital. Triglyceride concentrations were measured by H2O2 colorimetric assay after the elimination of free glycerol, using Determiner-L TG 2 reagent (Kyowa Medex) in the same autoanalyzer. Interassay coefficients of variation for control products in these assays were less than 5.0% for total cholesterol and HDL cholesterol and less than 3.0% for triglyceride. LDL cholesterol was calculated by using the Friedewald equation (7).

Apolipoprotein (Apo) levels were measured by an immunonoturbidimetric method using Apo A-1, A-2, B, C-2, C-3, and the E Auto-N “Daichii” kits (Daichii Pure Chemicals Co., Ltd., Tokyo, Japan) in the laboratory of the Second Department of Internal Medicine, University of Occupational and Environmental Health, Japan. Interassay coefficients of variation for control products in these assays were less than 5.0% for Apo A-1, Apo A-2, Apo B, Apo A-1, Apo C-3, and Apo E, and they were less than 10.0% for Apo C-2.

**NK Cell Activity**

A short-term (3.5-hour) radiolabeled release assay using 51Cr-labeled K562 cells as targets was performed (8). The effector cells were collected from the peripheral blood of subjects and purified by Conray–Ficoll gradient sedimentation (d = 1.077). The purified NK cells were washed twice with phosphate-buffered saline (PBS) and then resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum. NK cells were adjusted to the concentration needed for the cytotoxic assay (1 × 10^6 cells/ml).

Briefly, K562 cells were labeled with 50–100 µCi of Na 51Cr for 1 hour at 37°C. Labeled cells were washed 3 times with PBS and resuspended at 1 × 10^6 cells/ml in RPMI-1640 medium supplemented with 10% fetal bovine serum. A fixed number of labeled K562 cells was mixed with effector cells at an effector:target cell ratio of 20:1. The combination of target and effector cells was seeded in triplicate into 96-well U-bottomed microtest plates. 51Cr release was measured in 100-µl samples of supernatants by using a γ-counter (LKB-1272: PerkinElmer Life Sciences, Inc., Boston, MA). The maximum release of radioactivity was determined by measuring the radioactivity released from 51Cr-labeled K562 cells stimulated with 1N-HCl, while spontaneous (control) release was determined from the radioactivity released by 51Cr-labeled K562 cells mixed with RPMI-1640 medium supplemented with 10% fetal bovine serum in place of effector cells. The percent lysis was calculated by the following formula:

\[
\text{% specific lysis} = \frac{(\text{experimental release} - \text{spontaneous release})}{(\text{maximum release} - \text{spontaneous release})} \times 100
\]

**Statistical Analysis**

Most numerical group data are presented as the mean (SD). Apo C-3 levels were natural-logarithmically (ln) transformed to approximate a natural distribution before analysis. Univariate relations were assessed by Pearson’s product-moment correlation coefficient. Multivariate relations were assessed by stepwise forward multiple linear regression. The associations are reported as bivariate correlation (r) and multiple regression (b) coefficients ± SE. The unit-independent standardized multiple regression coefficients (β) are also presented. In all tests, p values of .05 or less were considered to indicate statistical significance.

**RESULTS**

Table 1 depicts the ranges and means (SD) of age, anthropometry, lipoproteins, apolipoproteins, and NK cell activity in 47 male subjects. The mean age of the study group was 66.6 (1.7) years. Six subjects had obesity greater than 26.4 kg/m² of body mass index. Twenty-three had hyperlipidemia (total cholesterol ≥220 mg/dl or triglyceride ≥150 mg/dl).

Univariate correlations of lipoproteins with NK cell activity for the study group are shown in Table 2. Figures 1 and 2 illustrate the significant relations between NK cell activity and plasma HDL cholesterol (r = .46, p < .001, Figure 1), Apo A-1 (r = .48, p < .001, Figure 2A), and Apo A-2 levels (r = .46, p < .005, Figure 2B); other lipoprotein variables showed no significant association with NK cell activity. As a way to reveal the independent determinants of NK cell activity, stepwise multiple linear regression analyses were performed with the NK cell activity as the dependent variable, and total cholesterol, HDL cholesterol, triglycerides, Apo A-1, Apo A-2, Apo B, Apo C-2, Apo C-3, and Apo E as independent variables. An independent effect

---

**Table 1. Clinical Characteristics of the 47 Male Subjects**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Range</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>65–69</td>
<td>66.6 (1.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>49.0–81.2</td>
<td>62.3 (7.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>150.0–178.0</td>
<td>164.5 (6.0)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>14–34</td>
<td>22.2 (4.5)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>18.6–29.8</td>
<td>23.0 (2.4)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>139–276</td>
<td>204 (29)</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>45–183</td>
<td>123 (28)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>33–86</td>
<td>57 (14)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>44–243</td>
<td>118 (54)</td>
</tr>
<tr>
<td>Apo A-1 (mg/dl)</td>
<td>94–192</td>
<td>137 (23)</td>
</tr>
<tr>
<td>Apo A-2 (mg/dl)</td>
<td>15–40</td>
<td>27 (6)</td>
</tr>
<tr>
<td>Apo B (mg/dl)</td>
<td>47–171</td>
<td>103 (24)</td>
</tr>
<tr>
<td>Apo C-2 (mg/dl)</td>
<td>0.1–14.7</td>
<td>5.6 (3.9)</td>
</tr>
<tr>
<td>Apo C-3 (mg/dl)</td>
<td>5.4–18.1</td>
<td>9.8 (3.4)</td>
</tr>
<tr>
<td>Apo E (mg/dl)</td>
<td>2.8–10.3</td>
<td>4.8 (1.5)</td>
</tr>
<tr>
<td>NK cell activity (%)</td>
<td>17–77</td>
<td>49 (16)</td>
</tr>
</tbody>
</table>

**Notes:** LDL = low-density lipoprotein; HDL = high-density lipoprotein; Apo = apolipoprotein; NK = natural killer.
The intestine and liver are the main organs where lipoproteins (phospholipids and free cholesterol) and apolipoproteins. Solubility in aqueous media is achieved by packaging the hydrophilic complexes of fats and proteins called apolipoproteins. Plasma lipoproteins are hydrophilic and other important molecules. Cholesterol can be obtained from plasma lipoproteins. Plasma lipoproteins are hydrophilic and other important molecules. Cholesterol can be obtained from plasma lipoproteins. Plasma lipoproteins are hydrophilic and other important molecules. Cholesterol can be obtained from plasma lipoproteins.

**Table 2. Univariate Correlations of Lipoproteins With NK Cell Activity**

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.24</td>
<td>0.11</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.02</td>
<td>0.91</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.46</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.004</td>
<td>0.98</td>
</tr>
<tr>
<td>Apo A-1</td>
<td>0.48</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Apo A-2</td>
<td>0.46</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Apo B</td>
<td>0.15</td>
<td>0.31</td>
</tr>
<tr>
<td>Apo C-2</td>
<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td>Lp Apo C-3</td>
<td>0.25</td>
<td>0.09</td>
</tr>
<tr>
<td>Apo E</td>
<td>0.18</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Notes: LDL = low-density lipoprotein; HDL = high-density lipoprotein; Apo = apolipoprotein; Lp Apo C-3 = natural-logarithmically transformed Apo C-3 values; NK = natural killer.

on NK cell activity was confirmed for only Apo A-1 ($b = 0.32 \pm 0.09$ mg/dl; $\beta = 0.48$, $p < .001$).

**DISCUSSION**

We found that NK cell activity is directly related to plasma HDL cholesterol, Apo A-1, and Apo A-2 levels, and only Apo A-1 held independent predictive value for NK cell activity in elderly male subjects in this study.

Our aim was to determine whether hypercholesterolemia in elderly people should be considered a risk factor for diminished immunity, so we studied the relation between NK cell activity and plasma lipoprotein levels in subjects susceptible to infectious disease and malignancy and selected a target population aged 65 years. The narrow age range of the subjects was intended to help avoid the confounding effect of age on NK cell activity through an indirect effect on lymphocyte membrane lipid composition, modified plasma LDL (9), and anti-LDL autoantibodies (10,11).

In vitro, NK cells expressed various lipoprotein receptors; there were at least 3—1 for chylomicron (CM) and very low-density lipoprotein (VLDL), 1 for LDL, and 1 for HDL (5). The effects of the different lipoproteins on the proliferative and cytotoxic responses of these cells were investigated by De Sanctis and colleagues. CM, VLDL, and LDL at low concentrations increased NK cell activity, whereas HDL and acetylated LDL inhibited, in a dose-dependent fashion, the killing by NK cells of K562 cells (5). Our clinical data showed that HDL was the only lipoprotein that was positively correlated with NK cell activity, and, moreover, Apo A-1 levels were independently related to NK cell activity. This discrepancy may be attributable to the different conditions in vitro and in vivo.

In vivo, all types of cells (even the immunosurveillance cell; NK cell) require cholesterol as a structural component of biological membranes and precursor of steroid hormones and other important molecules. Cholesterol can be obtained from plasma lipoproteins. Plasma lipoproteins are hydrophilic complexes of fats and proteins called apolipoproteins. Solubility in aqueous media is achieved by packaging the nonpolar lipids (triglycerides and cholesterol esters) in an oily core encapsulated by a monolayer of more polar lipids (phospholipids and free cholesterol) and apolipoproteins. The intestine and liver are the main organs where lipoprotein complexes are formed and apolipoproteins are synthesized. CM is of intestinal origin and VLDL is of hepatic origin, and they represent two parallel systems, one for the transport of exogenous (dietary) lipids and the other for the transport of endogenous lipid synthesized by liver. LDL is a product of VLDL metabolism; LDL is removed from the circulation in part via high-affinity receptors for Apo B and Apo E receptors present in liver and extrahepatic tissues (12) and in part by receptor-independent (i.e., nonspecific) uptake (13). HDL is considered to facilitate cholesterol transport back to the liver (14) and to be a negative risk factor for coronary artery disease (15). Nascent HDL containing mostly Apo A-1 (16) is converted to the mature form by the acceptance of free cholesterol from the cell membranes of peripheral tissues and from other lipoproteins. As mentioned, circulating lipoproteins comprise a complex system that delivers cholesterol and fatty acids to peripheral tissues and returns cholesterol to the liver, where it is converted to bile acids and excreted. Therefore, the investigation of NK cell activity in response to plasma lipoprotein profiles should help to unravel the physiological relevance of their immunoregulatory properties.

Interestingly, Chen and Bonavida (17) have proposed that secreted protein derived from human NK cells is involved in the NK cell-mediated cytotoxic (NK-CMC) reaction. They confirmed that the protein derived from concanavalin A-stimulated human NK cells was Apo A-1, and both rabbit and another commercially derived anti-Apo A-1 inhibited the NK-CMC reaction. This experimental study supports our finding that Apo A-1 levels were independently and directly related to NK cell activity. Apo A-1 may be produced and secreted by activated NK cells and may be indispensable to the NK-CMC reaction.

Immune functions are affected by nutritional status (18,19). A positive chronic imbalance between energy intake and
expenditure leads to situations of obesity, which is associated with both elevated plasma leptin (the protein product of the obese gene) and lipoproteins levels. Leptin may play a key role in linking nutritional status with immune functions (19). However, the fact that the correlation was with HDL components, which are not elevated in obesity, would suggest our results are not secondary to the leptin.

The limitations of this study have to be pointed out. Our measurement of NK cell activity was conducted in a short-term (3.5-hour) culture system, yet the resuspension medium contained RPMI and 10% fetal bovine serum. Fetal bovine serum is rich in lipoproteins, and this might confound the results. However, assays of NK cell activity and lipoprotein levels in all samples were conducted by using the same methods. Results using this method show a direct independent relation between plasma Apo A-1 levels and NK cell activity. In addition, plasma Apo A-1 in vivo may have much more of an effect on NK cells because the time for interaction between NK cells and Apo A-1 is much longer than in a short-term assay of NK cell activity in vitro. We speculate that NK cells may be primed to have greater cytotoxic activity by plasma Apo A-1 in vivo, or NK cells with higher cytotoxicity secrete more Apo A-1 in vivo as in the study of Chen and Bonavida (17).

Plasma lipoproteins may affect another cell type (e.g., endothelial cells), inducing them to release cytokines that, in turn, modify NK cell function indirectly (20,21). In this context, to reveal precise mechanisms of the interaction between NK cells and plasma lipoproteins, especially Apo A-1 in elderly people, more research is needed to clarify how the cytokine networks of the cells surrounding NK cells regulate the NK cell function in response to plasma lipoproteins.

In this clinical study, we demonstrated that NK cell activity is influenced by plasma Apo A-1 levels in elderly subjects; Apo A-1 contributes to the composition of the antiatherogenic fraction of HDL and could also enhance the NK cell activity to play a major role in immunosurveillance and defense against the development of cancer as well as bacterial and viral infections.

ACKNOWLEDGMENTS

We thank Chizuko Oba (Second Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Japan) for technical assistance in the assay of apolipoproteins.

Address correspondence to Kazuo Takahara, MD, PhD, First Department of Medical Technology, School of Health Sciences, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi, Kitakyushu, 807-8555, Japan. E-mail: takahara@med.uoeh-u.ac.jp

REFERENCES


*Received September 3, 2002
Accepted October 29, 2002*