Autoantibodies Against N-Homocysteinylated Proteins in Humans

Implications for Atherosclerosis

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Background and Purpose—Homocysteine (Hcy)-thiolactone mediates protein N-homocysteinylination in humans. Protein N-linked Hcy comprises a major pool of Hcy in human blood, greater than the “total” Hcy pool. N-homocysteinylated proteins are structurally different, compared with native proteins, and are thus likely to be recognized as neoself antigens and induce an autoimmune response. This study was undertaken to provide evidence for anti–Nε-Hcy-Lys-protein antibody and to examine associations between the antibody level, Hcy, and stroke in humans.

Methods—ELISA was used to quantify anti–Nε-Hcy-Lys-protein antibodies in human serum.

Results—We found that autoantibodies that specifically recognize Nε-Hcy-Lys epitope on Hcy-containing proteins occur in humans. Serum levels of anti–Nε-Hcy-Lys-protein autoantibodies positively correlate with plasma total Hcy levels, but not with plasma cysteine or methionine levels. In a group of exclusively male patients with stroke, mean level of anti–Nε-Hcy-Lys-protein autoantibodies was 50% higher than in a group of healthy subjects.

Conclusion—These findings support a hypothesis that Nε-Hcy-Lys-protein is a neoself antigen, which may contribute to immune activation, an important modulator of atherogenesis. (Stroke. 2004;35:1299-1304.)

Key Words: atherosclerosis ■ dose–response relationship, immunologic ■ homocysteine ■ immune system ■ immune tolerance ■ proteins ■ stroke

Plasma total homocysteine (tHcy) is a risk factor for cardiovascular disease and stroke in humans and predicts mortality independently of traditional risk factors in patients with coronary artery disease. Plasma tHcy is also a risk factor for neurodegenerative disorders, including dementia and Alzheimer disease. Cell culture and animal studies have shown that Hcy induces cell death and potentiates amyloid β-peptide toxicity in neurons. Molecular mechanisms by which homocysteine (Hcy) exerts its effects are largely unknown.

To gain insight into the molecular basis of Hcy toxicity in humans, physiological consequences of metabolic conversion of Hcy to Hcy-thiolactone during protein biosynthesis have been studied. Because of its similarity to the protein amino acid methionine, Hcy enters initial steps of protein synthesis and is converted by methionyl-tRNA synthetase (MetRS in Equation 1) to homocysteinylation-adenylate.

\[
\text{MetRS} + \text{Hcy} + \text{ATP} \rightleftharpoons \text{MetRS-Hcy} \sim \text{AMP} + \text{PP}_i
\]

However, Hcy does not complete the protein biosynthetic pathway, but is edited by the conversion to Hcy-thiolactone (Equation 2), a reaction catalyzed by MetRS in all organisms investigated, including human.

\[
\text{Hcy} + \text{PP}_i \rightleftharpoons \text{Hcy-thiolactone} + \text{ADP} + \text{Pi}
\]

Accumulation of Hcy-thiolactone can be detrimental because of its ability to modify proteins by forming adducts in which Hcy is N-linked to ε-amino group of protein lysine residues (Figure 1). Such Nε-Hcy-Lys-proteins loose their function and become susceptible to further damage by oxidation. Human plasma levels of N-linked protein Hcy are directly related to plasma tHcy levels. Each individual human blood protein examined so far (including hemoglobin, serum albumin, γ-globulins, low-density lipoprotein (LDL), high-density lipoprotein (HDL), transferrin, antitrypsin, and fibrinogen) has been found to contain small amounts of N-linked Hcy, which constitute a major pool of Hcy in human blood, larger than tHcy pool.

Immune activation is known to modulate atherogenesis. Some antigens (such as modified artery wall proteins and oxidized or glycated LDL), which can cause immune activation, have been identified. However, it is not yet known...
whether N-homocysteinylated proteins can be recognized as neoself antigens by the human immune system. The present study provides evidence that autoantibodies against Ne-Hcy-Lys-proteins occur in human plasma. We also show that the autoantibody positively correlates with plasma tHcy and with stroke in humans.

**Materials and Methods**

**Human Subjects**

The subjects were recruited from patients attending the Neurology Clinic at the University of Medical Sciences, Poznań, Poland. The mean age was 66 years, with a range from 46 to 79 years. There were 128 subjects: 51.5% were women, 54 subjects had had stroke in humans.

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**Preparation of N-Homocysteinylated Lysine, Its Derivatives, and Proteins**

N-homocysteinylation reactions were carried out as described. Lysine or its derivatives (10 mmol/L) were modified with 20 mmol/L N-homocysteinylthioactone, and assaying radiolabeled proteins has been determined from parallel reactions with identical concentration of [35S]Hcy-thiolactone, and assaying radiolabeled proteins by precipitation with trichloroacetic acid. Both methods gave similar results. Under these conditions, each Hcy-thiolactone-modified human blood protein contained 1 mol/L Hcy/mol protein. When needed, thiol groups in N-homocysteinylated proteins were blocked with iodoacetamide (IAA).

Keyhole limpet hemocyanine (KLH; Sigma) was dissolved at 20 mg/mL in 0.1 mol/L potassium phosphate buffer, pH 7.4, 0.2 mmol/L EDTA, and modified with 0.2 mmol/L L-Hcy-thiolactone-HCl for 16 hours at 37°C. The extent of modification has been determined by monitoring increase in protein thiol groups with Ellman’s reagent (Sigma). Alternatively, the extent of modification has been determined from parallel reactions with identical concentration of [35S]Hcy-thiolactone, and assaying radiolabeled proteins by precipitation with trichloroacetic acid. Both methods gave similar results. Under these conditions, each Hcy-thiolactone-modified human blood protein contained 1 mol/L Hcy/mol protein. When needed, thiol groups in N-homocysteinylated proteins were blocked with iodoacetamide (IAA).

**Rabbit Immunization**

Five hundred micrograms of Ne-Hcy-Lys-KLH adduct (131 mol/L Hcy/mol protein) was dissolved in 0.5 mL PBS and emulsified with 0.5 mL complete Freund’s adjuvant (Sigma) and injected intradermally into several sites in New Zealand White rabbits (Covance Research Products, Denver, PA). Blood was collected at monthly intervals and monitored by ELISA for IgG binding to microplate wells coated with Ne-Hcy-Lys-hemoglobin or Ne-Hcy-Lys-albumin.

**Purification of Antibodies**

IgG fraction was isolated from serum by protein A-Agarose (Sigma) chromatography. Diluted serum was applied onto the affinity column normally into several sites in New Zealand White rabbits (Covance Research Products, Denver, PA). Blood was collected at monthly intervals and monitored by ELISA for IgG binding to microplate wells coated with Ne-Hcy-Lys-hemoglobin or Ne-Hcy-Lys-albumin.

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**Table 1. Subject Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Stroke Patients (n=54)</th>
<th>Healthy Controls (n=74)</th>
<th>P†</th>
</tr>
</thead>
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<tr>
<td>Age, years</td>
<td>63.4±8.4</td>
<td>66.3±7.4</td>
<td>0.038</td>
</tr>
<tr>
<td>Men/women, n/n</td>
<td>37/17</td>
<td>29/45</td>
<td>0.001‡</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.3±3.4</td>
<td>25.5±3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>34 (63.0)</td>
<td>25 (33.8)</td>
<td>0.001‡</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>11 (20.4)</td>
<td>1 (1.4)</td>
<td>0.0003‡</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>32 (59.3)</td>
<td>23 (31.1)</td>
<td>0.001‡</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>6.3±1.3</td>
<td>6.7±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.02±1.07</td>
<td>6.09±1.20</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>4.06±0.89</td>
<td>4.04±0.80</td>
<td>NS</td>
</tr>
<tr>
<td>Homocysteine, μmol/L*</td>
<td>1.13±0.29</td>
<td>1.33±0.34</td>
<td>0.005</td>
</tr>
<tr>
<td>Methionine, μmol/L* (adjusted original scale)</td>
<td>29.4±0.35</td>
<td>27.5±0.41</td>
<td>0.009</td>
</tr>
<tr>
<td>Cysteine, μmol/L</td>
<td>18.9</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td>Homocysteine, μmol/L*</td>
<td>272.7±91.6</td>
<td>283.7±89.3</td>
<td></td>
</tr>
<tr>
<td>Methionine, μmol/L* (adjusted original scale)</td>
<td>3.11±0.45</td>
<td>3.12±0.25</td>
<td></td>
</tr>
</tbody>
</table>

NS indicates not significant. P>0.05.

*To equalize variances, log-transformed data were analyzed.
†Two-sample t test.
‡x² test.
Determination of Antibodies to Ne-Hcy-Lys-Hemoglobin or Ne-Hcy-Lys-Albumin by ELISA

PolisSorp 96-well plates (Nunc) were coated with 0.2 mL 10 µg/mL Ne-Hcy-Lys-hemoglobin or Ne-Hcy-Lys-albumin in 0.1 mol/L sodium carbonate buffer, pH 9.6, for 2 hours at 24°C. After removal of the antigen, each well was blocked with 0.25 mL 3% bovine serum albumin in PBS at room temperature for 1 hour, and then aspirated to dryness. To determine the antibody titer, 150 µL human or rabbit sera at various dilutions in PBS, 0.05% Tween 20, 3% BSA was added to each microtiter plate well and the plates were incubated for 18 hours at 4°C. The serum was aspirated from each well, and the wells were washed 4 times with PBS, 0.05% Tween 20. To quantify the amount of IgG bound, 150 µL of a 1000-fold dilution of goat anti-rabbit or anti-human IgG conjugated with horseradish peroxidase (Sigma) was added to each well and incubated for 1 hour at 24°C. The liquid was then aspirated from each well and the wells were washed 4 times with PBS, 0.05% Tween 20. Two hundred microliters of peroxidase substrate (citrate/phosphate buffer, pH 5.0, 1.5 mg/mL o-phenylenediamine, 0.03% H2O2) was added to each well and incubated at 24°C for 30 minutes. The reactions were stopped with 50 µL of 2 mol/L H2SO4 and read at 492 nm using a microplate reader.

Each data point has been obtained from at least duplicate measurements. In some experiments, anti-rabbit and anti-human IgG coated with alkaline phosphatase were used as a secondary antibody; the bound conjugated IgG was then assayed by hydrolysis of p-nitrophenyl-phosphate monitored at 405 nm. Nonspecific IgG binding has been corrected for by subtracting controls in which Ne-Hcy-Lys was used to compete out specific binding, or in which IAA-treated N-homocysteinylated protein was used as an antigen. Both controls gave similar results.

Determination of Antibody Specificity by Competitive ELISA

Competitive inhibition studies were performed by incubating 0.1 mL of a fixed dilution of serum or affinity purified IgG with 0.1 mL of various concentrations of putative competitor for 18 hours at 4°C. The amount of the primary IgG bound was then quantified as above. Values for controls without antigen, primary antisera, or secondary antibody, in the presence or absence of a competitor, were subtracted from complete duplicate assays.

Small amounts of N-linked Hcy, from 0.04 to 0.6 mol %, can be present in native proteins.13 These levels of endogenous N-linked Hcy in native proteins did not interfere with the assays, in which antigens containing 100 mol % of N-linked protein Hcy were used.

Determination of Plasma tHcy, Cysteine, and Methionine

Plasma tHcy, methionine, and cysteine were measured by high performance liquid chromatography (Gyrocotech) with electrochemical detection (CullArray 5600; ESA), as described by Accini et al.17

Results

Anti–Ne-Hcy-Lys-Protein Autoantibody Is Present in Human Serum

Each human serum tested showed some titer of autoantibody against Ne-Hcy-Lys-protein (Figure 2A). Sera from 2 rabbits showed low titers of autoantibody against Ne-Hcy-Lys-protein (Figure 2B). Because N-Hcy-proteins are known to be immunogenic in rabbits,18 we prepared sera from a rabbit immunized with Ne-Hcy-Lys-KLH to serve as positive controls. As shown in Figure 2B, titers of the anti–Ne-Hcy-Lys-protein antibody increased ~10-fold on day 34 after immunization. The titers remained at a similar elevated level on day 59 and then dropped on day 135 to ~30% of the maximum levels (Figure 2B).

Figure 2. Antibody binding curves of human and rabbit sera to human Ne-Hcy-Lys-hemoglobin. Microtiter plate wells were coated with 10 µg/mL human Ne-Hcy-Lys-hemoglobin. A, Antibody-binding curves were determined with 10 to 320-fold dilutions of sera from 6 human subjects. Goat anti-human IgG-horseradish peroxidase was used as a secondary antibody. B, Antibody-binding curves were determined with 25 to 400-fold dilutions of sera from 2 rabbits before (●, ○) and 34 days (■), 59 days (●), and 125 days (▲) after a single intradermal injection with Ne-Hcy-Lys-KLH. Goat anti-rabbit IgG-horseradish peroxidase was used as a secondary antibody.

Anti–N-Hcy-Protein Autoantibody Recognizes Ne-Hcy-Lys Epitope

To determine whether the IgG binding to microtiter plate wells coated with human Ne-Hcy-Lys-hemoglobin is specific, human and rabbit sera were examined by competitive ELISA. For each of the 4 human sera examined, IgG binding exhibited identical specificities. Data for a representative serum are shown in Figure 3. Ne-Hcy-Lys-albumin, Ne-Hcy-Lys-hemoglobin (Figure 3A), Ne-Hcy-Lys-transferrin, and Ne-Hcy-Lys-antitrypsin (not shown) were effective competitors. Native albumin, hemoglobin (Figure 3A), transferrin, or antitrypsin (not shown) did not compete. Proteins containing Ne-Hcy-Lys epitope with its thiol blocked with IAA also did not compete (Figure 3A), suggesting that free thiol of N-linked Hcy is important for autoantibody binding.

Ne-Hcy-Lys, Ne-Hcy-Ne-acetyl-Lys, and Ne-Hcy-LysAla were effective competitors for autoantibody binding to antigen-coated microtiter plate wells (Figure 3B). High specificity of human autoantibody was further demonstrated by our finding that Ne-acetyl-Ne-Hcy-Lys, in which Hcy is attached to the ε-amino group of lysine instead of the α-amino group, did not compete with the human IgG binding (Figure 3B). LysAla, Ne-acetyl-Lys, Hcy (Figure 3B), thiolactone, or lysine (not shown) also did not compete with the human IgG binding. These data suggest that human IgG specifically recognizes Ne-Hcy-Lys epitope on Ne-Hcy-Lys-protein (Figure 1).
Figure 3C and 3D shows parallel competition studies using rabbit serum (collected 35 days after immunization with N\textsubscript{e}-Hcy-KLH) as a primary antibody, goat anti-rabbit IgG as a secondary antibody, and human \textit{N}\textsubscript{e}-Hcy-Lys-hemoglobin as an antigen. Human \textit{N}\textsubscript{e}-Hcy-Lys-hemoglobin, \textit{N}\textsubscript{e}-Hcy-Lys-albumin, \textit{N}\textsubscript{e}-Hcy-Lys-LDL (Figure 3C), \textit{N}\textsubscript{e}-Hcy-Lys-transferrin, and \textit{N}\textsubscript{e}-Hcy-Lys-antitrypsin (not shown) were good competitors. Native human proteins did not compete with rabbit IgG binding to human \textit{N}\textsubscript{e}-Hcy-Lys-hemoglobin-coated microtiter plate wells. \textit{N}-\textsubscript{e}-(Cys-S-S-Hcy)-Lys-albumin (containing the antigen epitope with its thiol blocked by a disulfide bond with cysteine) competed less efficiently than \textit{N}\textsubscript{e}-Hcy-Lys-albumin (not shown). Thus, the thiol group of the \textit{N}\textsubscript{e}-Hcy-Lys epitope is important for rabbit IgG binding.

\textit{N}\textsubscript{e}-Hcy-N\textsubscript{e}-acetyl-Lys, but not \textit{N}\textsubscript{e}-acetyl-N\textsubscript{e}-Hcy-Lys, prevented rabbit antibody from binding to human \textit{N}\textsubscript{e}-Hcy-Lys-hemoglobin (Figure 3D). This shows that the rabbit IgG specifically recognizes Hcy linked by amide bond to \(\varepsilon\)-amino group of protein lysine residue; Hcy linked by amide bond to \(\alpha\)-amino group was not recognized. In addition, short peptides containing \textit{N}\textsubscript{e}-Hcy-Lys, were also recognized by the antibody, as shown by their ability to compete with antibody binding to the antigen (Figure 3D). Hcy, Hcy-thiolactone, lysine, or unmodified lysine derivatives did not compete (not shown).

Taken together, the competitive ELISA experiments demonstrate that both human and rabbit IgGs exhibit similar high specificity in the recognition of the \textit{N}\textsubscript{e}-Hcy-Lys epitope on \textit{N}\textsubscript{e}-Hcy-Lys-proteins.

A rabbit antibody, which recognizes extensively N-homocysteinylated LDL, hemoglobin, and albumin, but not native proteins, has been described. However, it is unclear whether that antibody can also recognize moderately N-homocysteinylated proteins or the \textit{N}\textsubscript{e}-Hcy-Lys itself.

**Plasma Hcy Is a Determinant of Anti–\textit{N}\textsubscript{e}-Hcy-Lys-Protein IgG Autoantibody Levels**

In normal human subjects, there was \(\approx\)32-fold variation in serum levels of anti–\textit{N}\textsubscript{e}-Hcy-Lys-protein IgG, from 0.0035 to 0.123 AU\textsubscript{y}\textsubscript{y}/min/\(\mu\)L serum. Plasma tHcy levels varied 3.6-fold, from 8.5 to 30.6 \(\mu\)mol/L. As shown in Figure 4A, serum levels of anti–\textit{N}\textsubscript{e}-Hcy-Lys-protein IgG were positively correlated with plasma tHcy levels (correlation coefficient \(r=0.50, P<0.001\)).

We have also examined a relationship between anti–\textit{N}\textsubscript{e}-Hcy-Lys-protein IgG and plasma levels of cysteine or methionine. Although there were significant variations in plasma cysteine (132 to 484 \(\mu\)mol/L) or methionine (11.1 to 38.5 \(\mu\)mol/L), there was no correlation between anti–\textit{N}\textsubscript{e}-
Two novel Hcy metabolites, Hcy-thiolactone\(^9,10\) and N-homocysteineylated proteins,\(^{10,13}\) have recently been discovered in human blood. The level of protein N-linked Hcy in individual protein is roughly proportional to the abundance of the protein. Protein N-linked Hcy constitutes a major pool of Hcy in human blood, with N-linked Hcy in hemoglobin and albumin comprising 75% and 22%, respectively, of this pool. The pool of N-linked protein Hcy (\(\sim 15.6 \mu\text{mol/L} \text{Hcy}\)) contains more Hcy than the tHcy pool (\(\sim 7 \mu\text{mol/L} \text{Hcy}\)) in normal human subjects.\(^{13}\)

In the present study we have addressed immunological consequences of the formation of N-homocysteinylated protein and found that autoantibodies against \(\text{Ne-Hcy-Lys}\)-proteins are present in human serum. The antigen specificity of the human autoantibodies was identical to that of a rabbit antibody raised against \(\text{Ne-Hcy-Lys}-\text{protein}\), which suggests that a significant part of the immune response in rabbits and humans is directed against the same \(\text{Ne-Hcy-Lys}\)-epitope. In fact, \(\text{Ne-Hcy-Lys}\) itself was a competitor for IgG binding, as were \(\text{Ne-Hcy}\)-adducts of hemoglobin, albumin, transferrin, antitrypsin, and LDL. Native proteins were not competitors. Thus, human autoantibody specifically recognizes the \(\text{Ne-Hcy-Lys}\)-epitope on \(\text{Ne-Hcy-Lys}\)-proteins. Our findings also suggest that \(\text{Ne-Hcy-Lys}\)-protein is a novel neoself antigen in humans. Other neoself antigens identified in humans include proteins altered by oxidation or glycation.\(^{15,16}\)

Chief risk factors for atherogenesis, such as dyslipidemia and diabetes, contribute to inflammatory conditions through complex mechanisms, such as lipid peroxidation, glycoxidation, and increased secretion of proinflammatory cytokines.\(^{15}\) That inflammation is important in atherogenesis is strongly supported by studies showing that increased plasma levels of inflammation markers, such as C-reactive protein, interleukin 6, serum amyloid A, interleukin 1 receptor antagonist, and soluble adhesion molecules, are independent predictors of coronary events.\(^{19}\) Preliminary studies suggest that moderate hyperhomocysteinemia is also associated with a marker of immune activation, neopterin.\(^{20}\) Our finding that anti–\(\text{Ne-Hcy-Lys}\)-autoantibodies occur in humans suggests a novel mechanism by which Hcy, a risk factor for cardiovascular disease and stroke,\(^1,2\) may contribute to immune activation. How can Hcy cause immune activation? We have previously shown that plasma tHcy is positively correlated with

**TABLE 2. Adjusted Means of Hcy and Anti–\(\text{Ne-Hcy-Lys}\)-Protein IgG for Stroke Patients and Healthy Controls According to Gender**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Stroke Patients (N)</th>
<th>Healthy Controls (N)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine, (\mu\text{mol/L})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Adjusted Mean</td>
<td>Adjusted Original</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>3.04±0.35</td>
<td>20.9 (37)</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>2.71±0.21</td>
<td>15.0 (17)</td>
<td></td>
</tr>
<tr>
<td>Anti–(\text{Ne-Hcy-Lys})-protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{IgG-1000, A}_{492}/\mu\text{L serum})</td>
<td>3.93±0.89</td>
<td>51.0 (37)</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>3.24±1.08</td>
<td>26.0 (17)</td>
<td></td>
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</table>

NS indicates not significant. \(P>0.05\).

*Calculated using log-transformed data.

†Two-sample t test.
plasma N-linked protein Hcy, both of which vary considerably among human subjects. In the present study, we show that plasma tHcy is also positively correlated with serum anti–Nε-Hcy-Lys-protein autoantibody (Figure 4). We believe that these associations are not fortuitous, and can be explained by the following mechanism. Elevation in Hcy levels leads to inadvertent elevation in Hcy-thiolactone8–10 (Equation 2), which in turn mediates the incorporation of Hcy into proteins,8–14 and, thus, the formation of neoself antigens (Nε-Hcy-Lys-protein; Figure 1). Raising levels of neoself Nε-Hcy-Lys-protein might then trigger an immune response in humans as they do in rabbits (Ferguson et al18 and our results).

Our findings that higher levels of anti–Nε-Hcy-Lys-protein autoantibodies are present in male, but not female, stroke patients, compared with healthy controls, most likely reflect higher levels of Hcy in male stroke patients (Table 2). However, it is also likely that the antibody itself might play an important role in atherosclerosis, as demonstrated for autoantibodies recognizing other neoself antigens, such as glycated or oxidatively modified proteins.15,16,19 Like other autoantibodies, the anti–Nε-Hcy-Lys-protein antibody could be beneficial or deleterious. For instance, anti–Nε-Hcy-Lys-protein autoantibodies may clear Nε-Hcy-Lys-proteins from circulation, which would be beneficial. However, if Nε-Hcy-Lys-proteins were present on endothelial cells, this could lead to the formation of antigen–antibody complexes on the surface of the vascular vessel. Endothelial cells coated with anti–Nε-Hcy-Lys-protein autoantibodies would be taken up avidly by the macrophage via the Fc receptor, which would result in injury to vascular surface. If the neoself antigen Nε-Hcy-Lys, which initiates the injury, was present continuously, repeatedly attempts to repair the damaged vascular wall would lead to an atherosclerotic plaque.

Human autoantibodies described here recognize Nε-Hcy-Lys epitope itself as well as Nε-Hcy-Lys adducts on protein. N-homocysteinylated many blood proteins has been documented. It is likely that proteins in other organs can also undergo N-homocysteinylated. Thus, autoantibodies directed against Nε-Hcy-Lys neoeptipe could react with Nε-Hcy-Lys-proteins in many tissues, possibly accounting for deleterious effects of hyperhomocysteinemia on many organs.1,2

In conclusion, our data suggest a novel mechanism by which Hcy elicits autoantibody formation with a variety of pathophysiological effects. Although our findings do not establish causality of Nε-Hcy-Lys-protein or anti–Nε-Hcy-Lys-protein autoantibodies in atherogenesis, they underscore the importance of examining Hcy-linked immune activation in human disease.

Acknowledgments

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