Atherosclerosis is associated with immune activation and systemic immune responses and signs of inflammation.1–3 Clinical investigations point to inflammatory/immune activation of plaque as a cause of acute coronary syndromes,4,5 and seroepidemiological studies have suggested links between atherosclerosis and microbial infections.4,6,7 Therapy with immunoglobulin has been investigated in a wide range of immune-mediated disorders,8–10 but the mode of action of immunoglobulin is still unclear. We have reported that the Fc portion of immunoglobulin has anti-inflammatory and immunomodulating actions in experimental and clinical studies of myocardial diseases.10–12 Recently, we also found that immunoglobulin treatment, given simultaneously with the development of the disease, suppresses atherosclerosis in apolipoprotein E-deficient mice via the action of the Fc portion.13 However, it remains unclear whether this treatment at a subsequent stage of atherosclerosis would be effective, so the current study was designed to evaluate this issue.

Methods

Experimental Atherosclerosis

The apo E-deficient 129ola × C57BL/6 hybrid mice were the generous gift of Dr Edward M. Rubin (University of California, Berkeley, CA, USA). They were mated with C57BL/6 mice to produce F1 hybrids. The F1 apo E+/– mice were then backcrossed to C57BL/6 mice for 10 generations. Mice homogeneous for the apo E-null allele on a C57BL/6 background were subsequently generated. The mice were kept in a temperature-controlled facility on a 14.10-h light-dark cycle with free access to food and water. After being weaned at 4 weeks of age, mice were fed a normal chow diet until 6 weeks of age, when the animals were switched to a high-fat diet (HFD) containing 20% fat and 0.3% cholesterol as previously described.13,14

We performed animal experiments in accordance with the Declaration of Helsinki, and these were approved by our institutional ethics committee for animal experiments.

Immunoglobulin Treatment

After confirming by pathological examination the presence of atherosclerotic lesions in the apo-E deficient mice fed HFD over 5 weeks, intact human immunoglobulin (Venoglobulin-IH, Mitsubishi Pharma; a polyethylene glycol-treated human immunoglobulin) or F(ab′)2 fragments of immunoglobulin (Gamma-Venin, Aventis; a polyethylene glycol-treated human immunoglobulin) were intraperitoneally administered on alternate days at a dose of 1 g · kg⁻¹ · day⁻¹ for another 4 week period (intact immunoglobulin, n=6; F(ab′)2 fragments, n=6; controls, n=6). Littermate controls were injected with 1 g · kg⁻¹ · day⁻¹ of human serum albumin (HSA) intraperitoneally.

Conclusions

Immunoglobulin treatment, even at a later stage of atherosclerosis, suppresses the development of lesions associated with the reduced expression of immune-activated cells in fatty streak plaques, demonstrating the benefits of immunoglobulin therapy for prevention of atherosclerosis. (Circ J 2005; 69: 1543–1546)

Key Words: Atherosclerosis; F(ab′)2 fragments; Fc portion; Immunoglobulin

Effects of Late Administration of Immunoglobulin on Experimental Atherosclerosis in Apolipoprotein E-Deficient Mice

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Background

Although immunoglobulin treatment, beginning simultaneously with the initiation of atherosclerosis, suppresses experimental atherosclerosis in apolipoprotein E-deficient mice, it remains unclear whether the treatment at a subsequent stage of atherosclerosis would be effective.

Methods and Results

Experimental atherosclerosis was induced in mice fed a high-fat diet containing 0.3% cholesterol. After confirming the presence of atherosclerotic lesions at 11 weeks, the mice were treated with an intraperitoneal injection of either intact type of immunoglobulin or F(ab′)2 fragments of immunoglobulin (both, 1 g · kg⁻¹ · day⁻¹) on alternate days over 4 weeks. Fatty streak lesion was suppressed by intact immunoglobulin administration, but not by F(ab′)2 fragments of immunoglobulin. Immunohistochemical analysis showed that macrophage and CD4⁺ T-cell accumulation in the fatty streak lesion was suppressed in mice that received intact immunoglobulin but not in those that received F(ab′)2 fragments.

Conclusions

Immunoglobulin treatment, even at a later stage of atherosclerosis, suppresses the development of lesions associated with the reduced expression of immune-activated cells in fatty streak plaques, demonstrating the benefits of immunoglobulin therapy for prevention of atherosclerosis. (Circ J 2005; 69: 1543–1546)
shown in previous studies, immunoglobulin antigenecity between different species does not appear to be a problem, and furthermore both agents have the same chemical structure as the Fab portion of immunoglobulin.

**Tissue Processing**

Mice were killed by bleeding after puncturing the right ventricle. The blood was collected and allowed to clot. After the serum was separated, lipid profiles were analyzed. The vasculature was perfused with sterile phosphate-buffered saline. The root of the aorta was dissected under a macroscope and frozen in OCT embedding medium for serial cryosectioning covering 1.0 mm of the root. The first section was harvested when the first cusp became visible in the lumen of the aorta. Four sections of 10μm thickness were harvested per slide, and thus 20 slides per mouse were prepared. All sections were immersed for 2 min in 60% isopropanol, stained for 15 min in a saturated oil red-O solution at 37°C, counterstained with hematoxylin, and then mounted under coverslips with glycerol gelatin.

**Quantitative Assessment of the Atherosclerotic Lesions**

The oil red-O-stained sections were analyzed at a magnification of ×10, as previously described. The image was captured directly from the RGB camera attached to a light microscope and displayed on a microcomputer to quantify the cross-sectional surface area of the lesion and the cross-sectional surface area of the vessel. The fractional area of the lesion was calculated by dividing the whole vessel area, including the lumen, intima, media and adventitia, as previously described. For each animal, 20 sections (ie, every 4th section) were examined, and the mean fractional area was calculated.

**Immunohistochemistry**

Aortic root cryosections from mice treated with immunoglobulin, F(ab’)2 fragments, or HSA were processed for immunohistochemistry as described previously. In brief, anti-macrophage (anti-Ms, M3/84, 1:400, PharMingen, San Diego, CA, USA), anti-CD4 (GK1.5, 1:50, PharMingen), anti-CD8 (53–6.7, 1:50, PharMingen), and anti-I-Ab (25-9-17, 1:25, PharMingen) antibodies were applied to acetone-fixed cryosections. After being washed, the sections were then exposed to a second antibody (horseradish peroxidase-conjugated antibodies), and the antibody binding was visualized with diaminobenzidine. Sections were counterstained with 1% methyl green. The percentage of positively stained cells per cells infiltrating the lesions was calculated for each antibody, as previously described (ie, lesions of the aortic root were analyzed). Data were obtained by dividing the number of positively stained cells by all methyl green-stained cells inside the internal elastic lamina. Three to 5 random microscopic fields were analyzed at ×200.

**Serum Lipid Measurement**

Serum was separated by centrifugation and stored at −80°C. Serum total cholesterol and triglyceride levels were measured with assay kits (Wako) according to the manufacturer’s instructions.

**Statistical Analysis**

Values were expressed as means ± SD. Statistical analysis of the data was determined by one-way ANOVA, followed by the Fisher protected least-significant-difference test. A value of p<0.05 was considered statistically significant.

**Results**

**Effects of Immunoglobulin**

**Physiological Parameters**

As shown in Table 1, treatment with the immunoglobulin preparations did not signifi-
ulin, but not in mice that received F(ab')2 fragments. Atherosclerotic lesions were suppressed in mice that received immunoglobulin, but not in mice that received F(ab')2 fragments.

Table 3 Effects of Immunoglobulin and F(ab')2 Fragments on Inflammatory Cells in Lesions

<table>
<thead>
<tr>
<th></th>
<th>HSA</th>
<th>Immunoglobulin</th>
<th>F(ab')2 fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mø (%)</td>
<td>14.3±6.1</td>
<td>2.8±2.1*</td>
<td>5.7±5.0</td>
</tr>
<tr>
<td>CD4+ (%)</td>
<td>18.1±7.7</td>
<td>5.9±3.2*</td>
<td>10.4±3.9</td>
</tr>
<tr>
<td>CD8+ (%)</td>
<td>7.2±4.0</td>
<td>3.9±3.0</td>
<td>5.5±2.1</td>
</tr>
<tr>
<td>I-Ab+ (%)</td>
<td>3.8±1.9</td>
<td>3.3±1.7</td>
<td>4.0±2.0</td>
</tr>
</tbody>
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*p<0.01 vs HSA. Data are mean±SD.

Discussion

This study clearly demonstrated that immunoglobulin treatment, even when initiated at a later stage of an experimental model of atherosclerosis, suppresses the development of the lesions associated with reduced expression of immune-activated cells in fatty streak plaques.

Intravenous immunoglobulin has been used to treat primary and secondary antibody deficiency for more than 25 years.6,7 It is a safe preparation with no long-term side effects. The therapy was first demonstrated to be effective for an autoimmune disorder (idiopathic thrombocytopenic purpura)8 and since then, it has been established as effective in the treatment of Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, myasthenia gravis, dermatomyositis and Kawasaki’s syndrome, and in the prevention of graft-vs-host disease in recipients of allogenic bone marrow transplants.9–17 Benefits have been reported for many other autoimmune and systemic inflammatory conditions, but controlled trials are often lacking.

The mode of action of immunoglobulin is complex, involving modulation of the expression and function of Fc receptors, interference with the activation of complement and the cytokine network, provision of anti-idiotype anti-bodies, and effects on the activation, differentiation, and effector functions of T cells and B cells.6,9

At present, atherosclerosis is considered to be the result of generalized inflammation associated with immune activation.1–3 We have already reported that immunoglobulin administration, beginning simultaneously with development of the disease, suppressed the lesions in experimental atherosclerosis via the actions of the Fc portion.13 In the clinical setting, however, the disease is subclinical; that is, most patients with atherosclerosis have small or moderate lesions when they begin treatment. Accordingly, in the current study, our aim was to clarify the effects of immunoglobulin in apo E-deficient mice that already had atherosclerotic lesions. Our results showed that immunoglobulin treatment at a subsequent stage of atherosclerosis suppressed the further development of lesions associated with the reduced expression of macrophage and CD4+ cells in the fatty streak plaque.

Some of the beneficial effects of immunoglobulin therapy in autoimmune and immune-mediated disorders have been attributed to the Fc portion of the drug.10,11,13,16 Indeed, from our data, treatment with the intact type, but not with F(ab')2 fragments, significantly suppressed the severity of atherosclerotic lesions associated with reduced expression of macrophages or CD4+ cells. Recent studies suggest that one mode of action of immunoglobulin is via...
Fcγ receptor IIb, an inhibitory receptor, and that a large dose of immunoglobulin increases the expression this receptor. Accordingly, it may be that plaque is stabilized by this inhibitory receptor.

To date, there are no immunomodulating agents available for the prevention of atherosclerosis in the clinical setting even though immune abnormalities are postulated as part of the disease’s pathogenesis. Although there are some difficulties in the practical application of this therapy, exploration of the efficacy of this agent in human atherosclerosis appears warranted.

In conclusion, immunoglobulin administration, even at a subsequent stage in the progression of the disease, has the potential to reduce the development of lesions associated with the reduced expression of immune-activated cells in fatty streak plaques.

References