Inhibition of Monocyte Adhesion to Endothelial Cells and Attenuation of Atherosclerotic Lesion by a Glucagon-like Peptide-1 Receptor Agonist, Exendin-4

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OBJECTIVE—Exogenous administration of glucagon-like peptide-1 (GLP-1) or GLP-1 receptor agonists such as an exendin-4 has direct beneficial effects on the cardiovascular system. However, their effects on atherosclerogenesis have not been elucidated. The aim of this study was to investigate the effects of GLP-1 on accumulation of monocytes/macrophages on the vascular wall, one of the earliest steps in atherosclerosis.

RESEARCH DESIGN AND METHODS—After continuous infusion of low (300 pmol · kg−1 · day−1) or high (24 nmol · kg−1 · day−1) dose of exendin-4 in C57BL/6 or apolipoprotein E−deficient mice (apoE−/−), we evaluated monocyte adhesion to the endothelia of thoracic aorta and arteriosclerotic lesions around the aortic valve. The effects of exendin-4 were investigated in mouse macrophages and human monocytes.

RESULTS—Treatment with exendin-4 significantly inhibited monocyte adhesion in the aortas of C57BL/6 mice without affecting metabolic parameters. In apoE−/− mice, the same treatment reduced monocyte adhesion to the endothelium and suppressed atherosclerosis. In vitro treatment of mouse macrophages with exendin-4 suppressed lipopolysaccharide-induced mRNA expression of tumor necrosis factor-α and monocyte chemotactic protein-1, and suppressed nuclear translocation of p65, a component of nuclear factor-κB. This effect was reversed by either MDL-12330A, a cAMP inhibitor or PKI14-22, a protein kinase A–specific inhibitor. In human monocytes, exendin-4 reduced the expression of CD11b.

CONCLUSIONS—Our data suggested that GLP-1 receptor agonists reduced monocyte/macrophage accumulation in the arterial wall by inhibiting the inflammatory response in macrophages, and that this effect may contribute to the attenuation of atherosclerotic lesion by exendin-4. Diabetes 59:1030–1037, 2010

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The present study was designed to determine the effect of exendin-4 on atherosclerosis, with a special focus on accumulation of monocytes/macrophages in the vascular wall using en face immunohistochemistry of the endothelial surface in combination with confocal microscopy. The results indicated that exendin-4 directly suppressed the progression of atherosclerosis by downregulation of various inflammatory and adhesion molecules on monocytes/macrophages.

RESEARCH DESIGN AND METHODS

Animals. The study protocol was approved by the Animal Care and Use Committee of Juntendo University. Male C57BL/6 mice (7 weeks old) were purchased from Oriental Yeast (Tokyo, Japan) and housed in specific pathogen-free barrier facilities at Juntendo University. Male apolipoprotein E-deficient (apoE−/−) mice (6 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in specific-pathogen-free barrier facilities at the Institute of Nihon Bioreresearch (Gifu, Japan). Mice were maintained under 12-h light/dark cycle, fed a standard rodent diet (from CLEA Japan at Nihon Bioreresearch), and provided with water ad libitum, except where noted. Mice were treated with either high-dose (24 mmol·kg body wt−1·day−1) or low-dose (300 mmol·kg body wt−1·day−1) exendin-4 (Sigma-Aldrich, Tokyo, Japan), or with saline through a mini-osmotic pump (Alzet, model 1004; DURECT, Cupertino, CA) that delivered the solution continuously for up to 28 days. At the age of 8 weeks, the osmotic pump was implanted under the skin of the back of each mouse after local anesthesia. The skin incision was closed with wound clip.

Preparation of cells and Western blotting analysis. We prepared cell extracts from various samples. Livers and lungs were isolated from mice and snap-frozen in liquid nitrogen. Islets were isolated by a standard collagenase digestion method as described previously (16). Mouse aortic vascular endothelial cells were isolated and cultured as described previously (17). The cultured cells were verified as endothelial cells by positive immunostaining with anti-veal Willebrand factor antibody (Dako, Carpenteria, CA) and negative immunostaining with anti-α-smooth muscle actin (Sigma-Aldrich). Mouse aortic vascular smooth muscle cells were isolated and cultured as described previously (18). The cultured cells were verified as smooth muscle cells by immunostaining for α-smooth muscle actin (Sigma-Aldrich). Macrophages from the mesenteric lymph nodes were harvested from the mice with cold PBS at 3 days after intraperitoneal injection of 3% thioglycollate media. The pooled macrophages from each mouse were cultured in RPMI 1640, supplemented with 0.2% FCS, 10 mmol/l HEPES, 1 mmol/l sodium pyruvate, 2 mmol/l L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 50 μmol/l 2-mercaptoethanol under 95% relative humidity and 5% CO2 at 37°C. All samples were sonicated on ice and centrifuged at 15,000g at 4°C for 20 min. The supernatants were collected and Western blot analysis was performed using anti-GLP-1R antibody (ab3907; Abcam, Cambridge, U.K.) or rabbit anti-glyceraldehyde-3-phosphate dehydrogenase antibody (Cell Signaling Technology, Beverly, MA) as described previously (16).

IGTT and insulin tolerance test. The intraperitoneal glucose tolerance test (IPGTT) was performed at the age of 12 weeks (4 weeks after implantation of the osmotic pump). Briefly, 1.0 g/kg body wt glucose was injected intraperitoneally after overnight fasting. Blood glucose level was measured with a glucometer (One-Touch Ultra; Life Scan, Burnaby, Canada). Plasma insulin levels were measured using an ELISA kit (Morinaga, Kanagawa, Japan). The insulin tolerance test was performed at the age of 12 weeks with 0.75 units/kg body wt insulin (Humulin; Eli Lilly, Indianapolis, IN) after 6 h of fasting. Blood samples were collected from the retro-orbital venous plexus in awake mice to measure blood glucose and plasma insulin concentrations.

Immunohistochemistry. After mice were killed by intraperitoneal injection of sodium pentobarbital (1 mg/kg; Abbott Laboratories), tissue preparation was performed by perfusing the animal with saline and 10% buffered formalin. Fixation was performed by immersion of the isolated thoracic aorta with 10% buffered formalin at 4°C. For en face immunohistochemistry of the endothelial surface, the thoracic aorta was cut open longitudinally along the ventral side with scissors and placed on a glass slide. Then immunohistochemistry was performed using anti-mouse Mac-2 monoclonal antibody (Dako, Cederlane, Burlington, ON, Canada). Next, each specimen was placed on a slide glass with the intimai side up, and covered with a coverslip. Specimens were viewed under a microscope (E800; Nikon, Tokyo, Japan) connected to an XYZ controller and a digital camera (Sony, Tokyo, Japan). To count the number of endothelium-adoherant monocytes, we set a rectangular area with sides that were twice the length of the long and short diameters of the vessel opening of the intracostal arteries, respectively, and that were centered on the opening. The total number of Mac-2–immunopositive cells within the entire rectangular areas were counted in each aorta. The cell density in each area was then calculated as the cell count (determined by an examiner blinded to the treatment regimen) divided by the total area (19–21).

For fluorescent staining, the samples were embedded in optimal cutting temperature compound, then sectioned, air dried, and washed in PBS. After immersion in blocking solution of 10% goat serum in PBS for 30 min at room temperature, the sections were incubated overnight at 4°C in a humidified chamber for labeling with rabbit polyclonal anti-GLP1 receptor antibody (1:50, LS-A1205; MBL International, Woburn, MA), and rabbit anti–Mac-2 monoclonal antibody (1:200; Dako). The specimens were placed in the appropriate goat sequential antibody conjugated with Alexa Fluor Dyes (Invitrogen, Carlsbad, CA) and diluted 1:300 in PBS for 30 min at room temperature. The specimen was placed on a glass slide, DAPI-containing mounting medium (Vector Laboratories, Burlingame, CA) was added, and then the tissue was covered with a cover glass. Samples were viewed by confocal laser scanning microscopy (Fluoview FV1000; Olympus, Tokyo, Japan).

Quantification of atherosclerotic lesions in the aortic sinuses. The heart and the aorta were flushed with normal saline followed by 10% buffered epidermal growth factor, and 0.5 ml GA-1000 (Cambrex BioScience Walkersville, Charles City, IA) under 95% relative humidity and 5% CO2 at 37°C. All samples were sonicated on ice and centrifuged at 15,000g at 4°C for 20 min. The supernatants were collected and Western blot analysis was performed using anti-GLP-1R antibody (ab3907; Abcam, Cambridge, U.K.) or rabbit anti-glyceraldehyde-3-phosphate dehydrogenase antibody (Cell Signaling Technology, Beverly, MA) as described previously (16).
formalin as described previously (20). For quantitative analysis of atherosclerotic lesions in the aortic sinus, the heart was cut in two halves and the top half was embedded in optimal cutting temperature compound, then cross-sectioned at 4-μm thickness at 50-μm interval with a cryostat. Twelve consecutive sections were taken sequentially from just above the aortic valve throughout the aortic sinus and allowed to dry at room temperature for 30 min. Sections were stained with oil red O staining as described above. Then, the images were captured with ImagePro Plus software. The mean lesion area of those 12 sections was calculated and expressed in square millimeters.

**Ex vivo treatment of macrophages.** The isolated macrophages were washed once and then incubated with or without 0.03, 0.3, and 3 nmol/l exendin-4 or 10 μmol/l forskolin (a cAMP activator; Sigma-Aldrich) for 1 h, followed by incubation with or without lipopolysaccharide (LPS, 1 μg/ml; Sigma-Aldrich) for 1 h. To inhibit the exenatide signal, macrophages were incubated with 5 μmol/l MDL-12330A (Sigma-Aldrich), a specific adenylate cyclase inhibitor, and 10 μmol/l PKI14-22 (Sigma-Aldrich), a protein kinase A (PKA) inhibitor, for 30 min before adding exendin-4. Control macrophages were incubated with the vehicle (DMSO, final concentration 0.1%). After treatment, total RNA was prepared for further analysis. Nuclear protein extracts were isolated from peritoneal macrophages and the content of nuclear factor-κB (NF-κB) p65 was determined using a specific ELISA kit using the method recommended by the manufacturer (Imgenex, San Diego, CA) (22).

**Isolation of tissue RNA and real-time quantitative RT-PCR.** Total RNA was extracted from peritoneal macrophages using the RNA easymicro Kit (Qiagen, Tokyo, Japan) and the instructions provided by the manufacturer. First-strand cDNA was synthesized using 1 μg of total RNA with oligo-dT primers and superscript reverse transcriptase (Invitrogen) as described previously (23). The resulting cDNAs were amplified using the SYBR Green PCR kit (Applied Biosystems, Foster City, CA). Quantitative PCR was performed with an ABI Prism 7700 sequence detection system (Perkin Elmer Life Sciences, Boston, MA). The relative abundances of mRNAs were calculated by

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**FIG. 2.** Exendin-4 reduced monocytic adhesion to the endothelium in C57BL/6 mice. A: Changes in body weight during treatment with exendin-4 in C57BL/6 mice (n = 6 each). B: Blood glucose concentrations during IPGTT after 24-day treatment with exendin-4 (n = 6 each). C: Plasma insulin levels during IPGTT after 24-day treatment with exendin-4 (n = 6 each). D: Results of insulin tolerance test in each group after 24-day treatment with exendin-4 (n = 6 each). E: The density of adherent Mac-2-positive cells on endothelial cells at branching areas in each group of mice after 28-day treatment (n = 6) with representative en face views of immunohistologic staining with Mac-2 antibody. Data are mean ± SEM. *P < 0.05 versus high-dose group, +P < 0.05 versus low-dose group. (A high-quality digital representation of this figure is available in the online issue.)
TABLE 1
Results of laboratory tests in C57BL/6 mice and apoE−/− mice after 28-day treatment with exendin-4

| C57BL/6 | Control | Low Ex4 | High Ex4 |
|---------|---------|---------|----------|
| Total cholesterol (mg/dl) | 66.7 ± 2.6 | 70.5 ± 1.4 | 73.9 ± 2.2* |
| LDL cholesterol (mg/dl) | 6.62 ± 0.42 | 6.33 ± 0.25 | 7.30 ± 0.59 |
| HDL cholesterol (mg/dl) | 56.6 ± 2.0 | 61.0 ± 1.2 | 63.3 ± 1.5* |
| TGs (mg/dl) | 42.9 ± 4.4 | 41.3 ± 3.3 | 37.6 ± 5.9 |
| CM (mg/dl) | 5.95 ± 1.02 | 7.24 ± 1.13 | 4.35 ± 1.02 |
| Sd-LDL (mg/dl) | 1.18 ± 0.07 | 1.16 ± 0.04 | 1.22 ± 0.09 |
| A1C (%) | NA | NA | NA |

| ApoE−/− | Control | Low Ex4 | High Ex4 |
|---------|---------|---------|----------|
| Total cholesterol (mg/dl) | 522.3 ± 26.3 | 472.6 ± 22.8 | 437.2 ± 30.0* |
| LDL cholesterol (mg/dl) | 136.5 ± 8.2 | 126.4 ± 7.5 | 138.4 ± 4.9 |
| HDL cholesterol (mg/dl) | 16.9 ± 0.8 | 16.1 ± 1.0† | 19.0 ± 0.9 |
| TGs (mg/dl) | 52.7 ± 4.2 | 54.7 ± 4.1 | 62.3 ± 4.2 |
| CM (mg/dl) | 63.9 ± 4.4 | 61.6 ± 2.3 | 55.5 ± 4.2 |
| Sd-LDL (mg/dl) | 27.0 ± 1.7 | 25.3 ± 1.7 | 29.2 ± 1.1 |
| A1C (%) | 3.46 ± 0.20 | 3.64 ± 0.17 | 3.38 ± 0.12 |

Data are mean ± SE. Blood samples were collected from C57BL/6J mice (n = 6) and apoE−/− mice (n = 13 except for HbA1C; n = 6 for HbA1C) in the fasting state after 28-day treatment with exendin-4. *P < 0.05 vs. control group. †P < 0.05 for low Ex4 vs. high Ex4. Low Ex4, low-dose exendin-4; High Ex4, high-dose exendin-4; TG, triglycerides; CM, chylomicron; Sd-LDL, small, dense LDL; HbA1c, A1C; NA, not applicable.

RESULTS

GLP-1 receptor is abundantly expressed in monocytes/macrophages. As a first step to elucidate the antiatherosclerotic effects of exendin-4, we first investigated the expression of GLP-1 receptor in cells associated with atherosclerosis. Similar to lung and pancreatic β-cells (24), mice peritoneal macrophages and vascular smooth muscle cells abundantly expressed GLP-1 receptor protein, and the expression level was higher than in freshly isolated endothelial cells. Similar to the expression level in macrophages, GLP-1 receptor was abundantly expressed in THP-1 cells, which are derived from human monocytes, and freshly isolated human monocytes. In contrast to the freshly isolated endothelial cells, abundant expression of GLP-1 receptor was detected in HUVECs (Fig. 1A and B).

In addition, immunohistochemical staining showed GLP-1

FIG. 3. The metabolic effect of exendin-4 in apoE−/− mice. A: Changes in body weight during exendin-4 treatment in apoE−/− mice (n = 13). B: Blood glucose concentrations during IPGTT after 24-day treatment with exendin-4 (n = 6). C: Plasma insulin levels during IPGTT after 24-day treatment with exendin-4 (n = 6). D: Results of insulin tolerance test in each group after 24-day treatment with exendin-4 (n = 6). Data are mean ± SEM. *P < 0.01 versus high-dose group, +P < 0.01 versus low-dose group.
receptor expression in cells that expressed Mac-2, a marker of macrophages located in the atherosclerotic lesions of the aortic valve of apoE<sup>−/−</sup> mice (Fig. 1C). These results may suggest that GLP-1 can directly act on monocytes or macrophages and affect the progression of atherosclerosis.

**Exendin-4 reduces monocyte adhesion in C57BL/6 mice without affecting glucose tolerance.** To investigate the effect of GLP-1 receptor activation on atherosclerosis, C57BL/6 mice received continuous infusion of 300 pmol·kg<sup>−1</sup>·day<sup>−1</sup> (low dose) or 24 nmol·kg<sup>−1</sup>·day<sup>−1</sup> (high dose) exendin-4 for 28 days. During the treatment period, neither dose affected body weight (Fig. 2A). After the 24-day treatment, both doses of exendin-4 improved glucose tolerance without affecting insulin secretion (Fig. 2B and C). The results of the insulin tolerance test were similar in the two groups (Fig. 2D). Treatment with high-dose exendin-4, but not the low dose, slightly increased total cholesterol and HDL cholesterol compared with control group (Table 1). The density of monocytes that adhered to the endothelial cells of the thoracic aorta was markedly suppressed in both the low- and high-dose treatment groups, compared with control (Fig. 2E).

**Exendin-4 reduces monocyte adhesion and atherosclerotic lesions in apoE<sup>−/−</sup> mice.** To explore the role of GLP-1 receptor activation on the progression of atherosclerosis, we treated apoE<sup>−/−</sup> mice with low- or high-dose exendin-4. Treatment with high-dose exendin-4 modestly reduced body weight gain and glucose tolerance and decreased serum total cholesterol level without affecting LDL cholesterol level (Fig. 3, Table 1). On the other hand, treatment with low-dose exendin-4 only modestly reduced glucose level at 30 min after glucose injection without affecting other parameters investigated (Fig. 3, Table 1). The density of monocytes that adhered to endothelial cells of the thoracic aorta was significantly lower in the low- and high-dose groups than the control group (Fig. 4A). Quantification of mRNA expression in the thoracic aorta showed that exendin-4 treatment significantly downregulated intercellular adhesion molecule-1 (ICAM-1) and tended to downregulate VCAM-1 (Fig. 4B). In parallel with the decreased monocyte adhesion to endothelial cells, the oil red O-positive area at the aortic valve was significantly reduced in the high-dose group compared with the control group (Fig. 4C). The area of the atherosclerotic lesions in the low-dose group also tended to be smaller than the control group, however the difference was not significant.

**Exendin-4 reduces the inflammatory response through cAMP signaling pathway in macrophages.** The data obtained from apoE<sup>−/−</sup> mice and C57BL/6 mice suggested that exendin-4 could have beneficial effects against atherosclerosis without affecting the metabolic parameters and that it could potentially prevent the progression of atherosclerosis by its direct action on the cells involved in atherogenesis. The abundant expression of GLP-1 receptor in monocytes/macrophages and the inhibitory effects of exendin-4 on monocyte adhesion on endothelial cells encouraged us to investigate the effects of exendin-4 on the inflammatory response.

LPS is known to induce inflammatory response. Indeed, incubation with 1 µg/ml of LPS for 1 h induced ~10-fold increases in the expression levels of TNF-α and monocyte chemotactrant protein-1 (MCP-1), a representative cytokine and a chemokine in isolated macrophages, respectively (data not shown). Thus, we investigated the effects of various concentrations of exendin-4 (0.03–3 nmol/l) in counteracting this response. Exendin-4 at all concentrations significantly suppressed LPS-induced increases in the expression levels of TNF-α and MCP-1 in macrophages (Fig. 5A). GLP-1 receptor is well-known G<sub>s</sub>-protein–coupled receptor, thus the activation of GLP-1 receptor results in increased cAMP concentration due to activation of adenylate cyclase (25). To explore the mechanism of exendin-4–induced suppression of TNF-α and MCP-1 expression in macrophages, we preincubated peritoneal macrophages with MDL-12330A, a specific adenyl cyclase inhibitor, or forskolin, an adenyl cyclase activator. The addition of MDL-12330A completely suppressed the inhibitory effect of exendin-4 on the expression levels of TNF-α and MCP-1 (Fig. 5B). On the other hand, forskolin significantly suppressed LPS-induced TNF-α and MCP-1 expression in macrophages, and the levels of suppression
by forskolin were similar to those of exendin-4 (Fig. 5C). These results suggest that the inhibitory effects of exendin-4 on the expression of TNF-α and MCP-1 are largely dependent on the activation of adenylate cyclase. Next, we investigated the downstream pathway of cAMP using PKI14-22, a specific PKA inhibitor. Similar to ML-12330A, the inhibitory effect of exendin-4 was significantly reversed by PKI14-22 (Fig. 5D), suggesting the involvement of PKA in the anti-inflammatory effect of exendin-4.

Although NF-κB is a major regulator of the expression of TNF-α and MCP-1, vasoactive intestinal peptide and pituitary adenylate cyclase–activating polypeptide are known to inhibit NF-κB–dependent gene activation by activation of PKA in cultured mononuclear cell line THP-1 (26). Thus, we investigated the effect of exendin-4 on LPS-induced nuclear translocation of NF-κB p65 in macrophages. Without any stimulation, nuclear NF-κB p65 was not detected in peritoneal macrophages, however, stimulation with LPS robustly induced nuclear translocation of NF-κB p65 (data not shown). Such translocation was markedly suppressed by exendin-4, and this inhibitory effect was completely abolished by ML-12330A (Fig. 5E).

These results indicate that exendin-4 inhibits nuclear translocation of NF-κB p65 by activating cAMP, in parallel with the expression of TNF-α and MCP-1.

Finally, we investigated the effect of exendin-4 on human monocytes. As stated above, exendin-4 attenuated the expression of ICAM-1 in apoE−/− mice. Thus, we investigated the effects of exendin-4 on their counterreceptors, CD11b, in isolated human peripheral monocytes. Exposure to 0.3 and 3 nmol/l, but not 0.03 nmol/l, exendin-4 for 24 h significantly reduced the surface expression of CD11b, as assessed by flow cytometry (Fig. 5F). These results suggest that activation of the GLP-1 receptor has the antiatherogenic effects on human circulating monocytes.

**DISCUSSION**

In the present study, we provide evidence that exendin-4, a GLP-1 receptor agonist, prevents the progression of atherosclerosis in apoE−/− mice without major effects on metabolic parameters. Our data suggest that exendin-4 markedly reduced the accumulation of monocytes/macrophages in the vascular wall at least in part by suppressing the inflammatory response in macrophages through the activation of the cAMP/PKA pathway.

The results showed that the exendin-4 decreased monocyte adhesion to endothelial cells in two nondiabetic mice, C57BL6 and apoE−/− mice. In both mouse strains, exendin-4 reduced glucose level during IPGTT, however, because the two strains are nondiabetic, the effect of exendin-4 on glucose level should play only a minor effect on its antiatherosclerotic properties.

In this study, we confirmed the expression of GLP-1 receptor in endothelial cells, smooth muscle cells, macrophages, and monocytes. Because these cells play critical roles in the progression of atherosclerosis, GLP-1 receptor
activation in these cells may be involved in atherosclerosis. Indeed, several groups reported the beneficial effects of GLP-1 receptor activation on endothelial cells (10–13). On the other hand, we confirmed in the present study the direct anti-inflammatory effect of GLP-1 on monocytes/macrophages. Indeed, treatment with exendin-4 at concentrations observed during treatment of humans (27,28) reduced the expression of inflammatory mediators TNF-α and MCP-1 in activated macrophages. TNF-α and MCP-1 are among the important cytokines and chemokines whose atherogenic effect has been established. Both TNF-α– and MCP-1–deficient mice have significantly reduced atherosclerotic lesions (29,30). Furthermore, forced expression of TNF-α and MCP-1 in leukocytes promotes advanced atherosclerotic lesions (29,31). Thus, in addition to the effect of GLP-1 on endothelial cells, its effect on monocytes/macrophages may also have a major impact on the attenuation of atherosclerosis.

It was reported previously that the main effects of GLP-1 are mediated through the activation of adenylyl cyclase and the production of cAMP (25). Using adenylyl cyclase inhibitor and activator, we also demonstrated in this study that stimulation of cAMP by exendin-4 is critical for the attenuated production of proinflammatory mediators from macrophages. This result is consistent with previous studies in which increased intracellular levels of cAMP inhibited TNF-α production and its transcription in macrophage (32–34). These data suggest that exendin-4 regulates inflammatory response of macrophages via the cAMP/PKA pathway, which inhibits proinflammatory cytokine production as reported recently (35,36). Our results showed that PKA activation and inhibition of NF-κB p65 translocation mediate overexpression of inflammatory cytokines by increased cAMP level elicited by GLP-1 receptor activation.

The adhesion of circulating monocytes to the intimal endothelial cells is thought to be one of the earliest events in the complex pathologic process of atherosclerosis (14,15). This can be mediated by the interaction of specific adhesion molecules on vascular endothelial cells with their integrin counterreceptors on monocytes. CD11b is an important adhesion molecule on monocytes. Activation of monocytes by cytokines, chemokines, hypercholesterolemia, and hyperglycemia leads to increased expression of this integrin, and increased monocyte expression of CD11b correlates with adhesion of these cells to the endothelium in patients with hypercholesterolemia (37). Our results showed a potential suppressive effect of exendin-4 on the surface expression of CD11b on human monocytes. On the other hand, we demonstrated that exendin-4 decreased the expression of ICAM-1, which interacts with CD11b on monocytes in apoE−/− mice. These effects may contribute at least in part to the reduced monocyte adhesion to the endothelium in vivo. However, additional experiments are required to clarify the mechanism of exendin-4–induced inhibition of CD11b expression.

In conclusion, our data suggest that GLP-1 receptor activation significantly reduced the accumulation of monocytes/macrophages in the vascular wall and eventually inhibited atherosclerosis genesis by regulating inflammation in macrophages via the cAMP/PKA pathway and the integrin-related gene expression on monocytes. These unique effects of GLP-1 receptor activation may help design new therapies for cardiovascular disease in patients with type 2 diabetes.

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