Adenovirus 36 as an Obesity Agent Maintains the Obesity State by Increasing MCP-1 and Inducing Inflammation

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Background. Although it is well known that adenovirus 36 (Ad36) is associated with obesity in humans as well as in animals, the detailed cellular mechanism is unclear.

Methods. Wild-type (WT) mice and monocyte chemoattractant protein-1 knockout (MCP-1−/−) mice were infected with Ad36, and their weights and inflammatory status were measured. Macrophage infiltration was examined in their reproductive fat pads and in a coculture system. The correlation between Ad36 antibody presence and MCP-1 levels was tested in human samples.

Results. We have shown that Ad36 infection stimulated an inflammatory state by increasing the level of MCP-1 through the activation of nuclear factor κB, which in turn induced the infiltration of macrophages into adipocytes. This induced inflammation resulted in viral obesity, which caused chronic inflammation and affected lipid metabolism. In contrast to WT mice, MCP-1−/− mice were protected from Ad36-induced inflammation and obesity. The MCP-1 levels in Ad36-antibody-positive human group were higher than those in the antibody-negative group.

Conclusions. These findings support the proposition that virus-induced inflammation is the cellular mechanism underlying Ad36-induced obesity. These results also suggest that MCP-1 plays a critical role in Ad36-induced obesity and that MCP-1 may be a therapeutic target in preventing virus-induced obesity.

Obesity has become the number one public health problem worldwide. The etiology of obesity is considered to be multifactorial, including endocrine, pharmacological, nutritional, environmental, and genetic agents. Over the past 30 years, studies of “infectobesity,” a new term to describe obesity of infectious origin, have identified 10 different pathogens that cause obesity in animal models such as mice, chickens, and nonhuman primates [1–3]. Several pathogens have been implicated as the cause of obesity in animal models; these include canine distemper virus, Rous-associated virus-7, Borna disease virus, SMAM-1 (an avian adenovirus), adenovirus 5 (Ad5), and Ad37. However, their role in human obesity has not been established fully [1]. Interestingly, only adenovirus 36 (Ad36) has been clearly associated with human obesity [4].

Ad36 is a nonenveloped icosahedral virus comprising double-stranded DNA and is one of 56 serotypes in 7 subgroups of human adenoviruses [5]. Generally, adenovirus infection causes coldlike symptoms, gastroenteritis, and conjunctivitis [6]. Recent reports from North America, Korea, Denmark, and Italy suggest that Ad36 is related to obesity in adults and children [7–13]. Ad36 infection accelerates the differentiation of preadipocytes to adipocytes in 3T3-L1 cells and human preadipocytes [14]. Ad36 infection also increases glucose uptake in human adipose-derived stem cells and human skeletal muscle cells, and this uptake is dependent on Ras signaling [15, 16]. One of the Ad36 viral genes, E4 orf-1, is thought to be involved in the mechanism underlying virus-induced
obesity by increasing adipocyte differentiation factors such as CCAAT-enhancer binding proteins β, peroxisome proliferator-activated receptor γ2, and glycerol 3-phosphate dehydrogenase [17]. Although some effects of Ad36 on human obesity are known, the cellular mechanisms responsible for virus-induced chronic obesity remain unknown. Recent reports have revealed that inflammation contributes to the maintenance of the obesity state [18, 19]. Ad36 infection also increases interleukin 6 (IL-6) expression in adipocytes in vitro, which may contribute to chronic low-grade inflammation [20]. Thus, we hypothesize that Ad36 infection up-regulates inflammation in adipose tissue and induces the progression to chronic obesity. Interestingly, adipose tissue in obese subjects is characterized by macrophage infiltration, which is stimulated by monocyte chemoattractant protein-1 (MCP-1) [21, 22]. Macrophages in adipose tissue are a source of proinflammatory factors [21, 23]. To identify whether Ad36 is involved in chronic inflammatory obesity, we assessed proinflammatory cytokines and infiltrated macrophages, and monitored the obesity state by measuring body weight, reproductive fat pad weight, and metabolic factors in wild-type (WT) and MCP-1 knockout (MCP-1 

METHODS

The methods used for virus preparation, the migration assay, the polymerase chain reaction (PCR) assay, the enzyme-linked immunosorbent assay (ELISA), immunohistochemistry, analysis of metabolic parameters, flow cytometry, and Western blotting are described in the Supplementary Methods.

Animals, Food Intake, and Body Weight

Five-week-old female C57BL/6 (WT) and MCP-1−/− mice were used in this study. WT mice were obtained from Orient Company, and MCP-1−/− mice were purchased from Jackson Laboratory. In total, 1 × 10⁷ plaque-forming units of Ad36 were used to infect WT and MCP-1−/− mice by intraperitoneal injection. Mice were maintained at 22–24°C under a constant 12-hour light/dark cycle in the animal facility at the Catholic University according to the institutional guidelines. They were fed a normal chow diet for 14 weeks. Food intake and body weight were measured once a week at 15:00–16:00.

Human Subjects

To identify the relationship between Ad36 antibody presence and MCP-1 levels in humans, we used 80 human samples that were analyzed in a previously published study [13]. These samples were collected from participants at the Health Promotion Center of the Ewha Womans University Medical Center (EWUMC). From 540 samples collected from equal numbers of normal, overweight, and obese subjects (each group contained 90 men and 90 women; mean age, 44.3 ± 7.04 years; age range, 30–59 years), we randomly selected samples from 20 subjects in the normal group, 30 in the overweight group, and 30 in the obese group. Informed written consent for their participation was obtained from each individual, and the study design was approved by the Institutional Review Board of EWUMC. A peripheral venous blood sample was collected from each subject after a 12-hour fast. After centrifugation, the serum was collected and stored frozen at −80°C until analysis.

Statistical Analysis

All data are expressed as means ± SD. The data were analyzed using Student t-test (SAS Institute Inc). Differences of P < .05 were regarded as significant.

RESULTS

Wild-Type (WT) Mice

C57BL/6 WT mice were infected with phosphate-buffered saline (PBS) (mock) or Ad36 at age 5 weeks. The mice were killed 1, 3, 7, 30, and 90 days after infection, the reproductive fat pads were weighed, and MCP-1 and tumor necrosis factor α (TNF-α) messenger RNA (mRNA) levels were measured. Ninety days after infection, body weight did not differ between mock- and Ad36-infected mice (Figure 1A). However, the weight and size of the reproductive fat pads were significantly greater in the Ad36-infected mice than in the mock-infected mice (Figure 1B), even though the food and water intake did not differ between the groups in our study (data not shown). Supplementary Data 1A–B also show that Ad36 can infect the reproductive fat pads, so Ad36 may directly affect the reproductive fat pads. One day after infection, the mRNA levels of the proinflammatory cytokines MCP-1 and TNF-α were 3 times and 2 times higher, respectively, in reproductive fat pads of Ad36-infected mice compared with mock-infected mice. At 90 days, the MCP-1 mRNA level was twice as high in Ad36-infected WT mice compared with mock-infected WT mice, and the TNF-α mRNA level was 6 times higher in Ad36-infected mice (Figure 1C). Similar results were obtained in Balb/C mice (see Supplementary data 1C–D). Moreover, the MCP-1 protein levels in the reproductive fat pads were higher in the Ad36-infected WT mice than in the mock-infected WT mice. TNF-α protein levels were also higher in the Ad36-infected WT mice than in the mock-infected WT mice (Figure 1D).

MCP-1 Knockout (MCP-1−/−) Mice

Ninety days after infection, body weight and the weight of reproductive fat pads did not differ significantly between Ad36-infected and mock-infected MCP-1−/− mice (Figure 2A and 2B). The weight and size of fat pads were slightly lower
in Ad36-infected MCP-1$^{-/-}$ mice than in mock-infected MCP-1$^{-/-}$ mice (Figure 2B). At 90 days, the mRNA levels of MCP-1 and TNF-α were lower in Ad36-infected MCP-1$^{-/-}$ mice than in mock-infected MCP-1$^{-/-}$ mice (Figure 2C). Moreover, the MCP-1 protein levels appeared to be lower in the Ad36-infected MCP-1$^{-/-}$ mice than in the mock-infected mice. TNF-α protein levels were also significantly lower in the Ad36-infected MCP-1$^{-/-}$ mice than in the mock-infected MCP-1$^{-/-}$ mice (Figure 2D).

**Inflammatory Status of WT and MCP-1$^{-/-}$ Mice**

To determine whether the protection against Ad36-induced obesity observed in MCP-1$^{-/-}$ mice was caused by the absence of MCP-1, WT mice and MCP-1$^{-/-}$ mice were killed, and immunohistochemical studies were performed to identify inflammation 90 days after infection. The inflammatory state was verified by hematoxylin and eosin (H&E) staining, and infiltrated macrophages were detected using F4/80 antibody. Infiltration of immune cells and macrophages into

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**Figure 1.** Ad36 infection increased the weight of reproductive fat pads and monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor α (TNF-α) messenger RNA (mRNA) levels in wild-type C57BL/6 mice. A, Body weight was recorded each week during the 90-day experiment (mock: PBS-treated group, black lozenge-shaped symbol; Ad36: Ad36-infected group, gray square-shaped symbol). B, Reproductive fat pads were weighed 1, 3, and 90 days after infection. Values are expressed as mean ± SD (n = 10, scale bar = 1 cm). C, The mRNA levels of MCP-1 and TNF-α in reproductive fat pads were analyzed by quantitative real-time PCR (AU, arbitrary unit; *P < .05, **P < .01, †P < .001). D, Protein levels of cytokines in reproductive fat pads were measured using ELISA (†P < .001).
reproductive fat pads was greater in Ad36-infected WT mice than in mock-infected WT mice (Figure 3A and 3B). Similar results were obtained in Balb/c mice infected with Ad36 (see Supplementary Data 1E). However, Ad36-infected MCP-1−/− mice did not show an increased infiltration of inflammatory cells in reproductive fat pads (Figure 3A and 3B). Macrophage infiltration in reproductive fat pads was also confirmed by real-time PCR. The F4/80 mRNA level was ∼3.5 times higher in Ad36-infected WT mice than in mock-infected WT mice, whereas no increase was observed in MCP-1−/− mice (Figure 3C).

Macrophages were classified into M1 and M2 types according to their function. M1 macrophages produce proinflammatory cytokines, such as MCP-1, TNF-α, and IL-6. CD64 on M1 macrophages increases in the inflammatory state [24]. By contrast, M2 macrophages, which are the major resident macrophages in lean adipose tissue, are characterized by relatively high expression of interleukin 10 (IL-10), MG-1, and CD206, which are involved...
in the repair or remodeling of tissues and the resolution of inflammation [25]. The mRNA levels of M1 macrophages, which cause a proinflammatory state, and M2 macrophages, which cause an anti-inflammatory state, were identified by real-time PCR (Figure 3D). The mRNA level of M1 macrophages (CD64) was ~2 times higher in Ad36-infected WT mice than in mock-infected WT mice. By contrast, the mRNA level of M2 macrophages (CD206) was significantly lower in Ad36-infected WT mice than in mock-infected WT mice. The mRNA levels of both CD64 and CD206 were lower in Ad36-infected MCP-1−/− mice (Figure 3D).

**Function of MCP-1 On Lipid Metabolism in Ad36-Induced Obesity**

We assumed that MCP-1 regulates lipid metabolism in Ad36-induced obesity through inflammation. Thus, we analyzed...
metabolic parameters in WT mice and MCP-1−/− mice (Table 1). Serum was obtained from the mice via heart puncture 90 days after infection. In WT mice, Ad36 infection significantly decreased nonesterified fatty acids (NEFA), glucose, and insulin concentrations, indicating increased insulin sensitivity. Lipid parameters such as HDL-C, LDL-C, and total cholesterol concentrations were lower in Ad36-infected WT mice than in mock-infected WT mice. Interestingly, the changes in metabolic parameters in MCP-1−/− mice were opposite the pattern observed in WT mice (Table 1). For example, NEFA and glucose levels increased and insulin levels increased slightly in Ad36-infected MCP-1−/− mice. Ad36-infected MCP-1−/− mice also had higher HDL-C, LDL-C, and total cholesterol concentrations than did mock-infected MCP-1−/− mice (Table 1).

Table 1. Metabolic Parameters Analyzed in WT Mice and MCP-1−/− Mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT Mice</th>
<th>K/O Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mock</td>
<td>Ad36</td>
</tr>
<tr>
<td>NEFA</td>
<td>420.0 ± 2.1</td>
<td>382.5 ± 2.1**</td>
</tr>
<tr>
<td>Glu</td>
<td>85.5 ± 0.7</td>
<td>59.0 ± 1.4**</td>
</tr>
<tr>
<td>HDL</td>
<td>53.9 ± 0.4</td>
<td>48.9 ± 0.6*</td>
</tr>
<tr>
<td>LDL</td>
<td>123.5 ± 0.7</td>
<td>104.5 ± 0.7**</td>
</tr>
<tr>
<td>TC</td>
<td>88.5 ± 0.7</td>
<td>80.5 ± 2.1*</td>
</tr>
<tr>
<td>TG</td>
<td>48.0 ± 2.8</td>
<td>52.5 ± 0.7</td>
</tr>
<tr>
<td>Insulin</td>
<td>3.6 ± 0.1</td>
<td>3.0 ± 0.2*</td>
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Abbreviations: Ad36, Ad36-infected group; Glu, glucose (mg/dL); HDL, high-density lipoprotein-cholesterol (mg/dL); insulin (µU/mL); K/O, MCP-1−/− mice; LDL, low-density lipoprotein-cholesterol (mg/dL); mock, PBS-treated group; NEFA, neutral free fatty acid (µEq/mL); TC, total cholesterol (mg/dL); TG, triglyceride (mg/dL); WT, wild-type mice. *P < .05, **P < .01.

DISCUSSION

Here, we provide evidence that Ad36-induced obesity can be maintained as a chronic state through inflammation. The correlation between obesity and inflammation has been known for decades from epidemiological and cellular studies [28, 29]. Recent epidemiological studies have confirmed and extended these findings by showing increased inflammation in obese subjects [14]. In addition, hypertrophic adipocytes may make proinflammatory cytokines such as MCP-1, TNF-α, resistin, and plasminogen activator inhibitor-1 [30, 31]. We found previously that Ad36 infection triggers the inflammatory pathway in human mesenchymal stem cells, which was confirmed by microarray analysis [32]. Therefore, on the basis of the above data, we assumed that there is a relationship between Ad36-induced obesity and inflammation. In this study, as in dietary obesity, Ad36-infected mice showed an increase in weight of reproductive fat pads and an inflammatory state that was maintained by macrophages driven by increased MCP-1 level. Our findings suggest that Ad36 infection induces obesity through inflammation and that MCP-1 may be a key regulator of Ad36-induced obesity.

It has previously been reported that MCP-1 is responsible for obesity, insulin resistance, and steatosis in MCP-1 transgenic mice and obese mice [21, 33], and inhibition of MCP-1 ameliorates insulin resistance and hepatic steatosis in obese mice [21]. Moreover, MCP-1–induced protein (MCPIP) can increase the expression of the C/EBPα family in 3T3-L1 cells [34]. MCP-1 also recruits macrophage colony-stimulating factors (MCSFs), which affect adipocyte hyperplasia and adipose tissue growth [35]. It is well known that MCP-1 plays a role in the recruitment of monocytes and macrophages into adipose tissue [21, 36], and that TNF-α increases the number of macrophages in adipose tissue and the stromal...
vascular fraction [21, 37]. In the present study, the mRNA levels of MCP-1 and TNF-α increased in the early stages of viral infection (Figure 1C), and the cytokine levels decreased with time. Ad36-induced obesity and inflammation in the early stage of infection might have induced further increases in MCP-1 and TNF-α mRNA and protein levels at later stages (Figure 1C and 1D). These proinflammatory cytokines may trigger the recruitment of macrophages. We found that more immune cells, especially macrophages, were recruited to reproductive fat pads of Ad36-infected WT mice (Figure 3). Interestingly, Ad36 infection increased M1 macrophage (“classically activated” macrophages) accumulation, whereas M2 macrophage (“alternatively activated” macrophages) accumulation decreased despite the Ad36 infection in WT mice (Figure 3D) [37]. M1 macrophages are involved in the Th1 pathway in the inflammatory state, and M2 macrophages are involved in anti-inflammatory responses [24, 25]. However, in Ad36-infected MCP-1−/− mice, body weight, reproductive fat pad weight (Figure 2A and 2B), and proinflammatory cytokine levels (Figure 2C and 2D) did not increase, and macrophage recruitment was not observed (Figure 3). In addition, there was a larger decrease in M1 and M2 macrophages in Ad36-infected MCP-1−/− mice compared with mock-infected MCP-1−/− mice (Figure 3D). Consistent with the in vivo data, our in vitro data also showed that Ad36 infection increased macrophage migration into adipocytes by activating NFκB, which induced the release of proinflammatory cytokines (Figure 4). Taking these data together, we conclude that Ad36 infection increases MCP-1, and this MCP-1 might function to induce adipogenesis via MCP-1-related factors, such as MCPIP and MCSF, in adipocytes. Furthermore, increased MCP-1 triggers macrophage infiltration into adipocytes and then induces inflammation. Finally, this inflammation may play a role in maintaining obesity.

Figure 4. Ad36 infection increased the migration of macrophages into adipocytes through NFκB activation in an in vitro coculture system with 3T3-L1 and RAW264.7 cells. A, Infiltrated macrophages (RAW264.7 cells) in cocultured 3T3-L1 cells were quantified by flow cytometry with F4/80 antibody. (mock, serum-free media-treated group; Ad36, Ad36-infected group; *P < .05, †P < .001). B, The protein levels of F4/80 and IκB-α were measured by western blotting, and the intensity of each band was normalized to that of α-tubulin using the ImageJ program (†P < .001). C, The messenger RNA (mRNA) levels of monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor (TNF-α) as proinflammatory cytokines in cocultured 3T3-L1 cells were measured by quantitative real-time PCR (AU, arbitrary unit; *P < .05, **P < .01).
Considering the above data, we also assumed that MCP-1 might affect lipid metabolic parameters, thus influencing the inflammatory state. Previous studies showed that Ad-36 decreases insulin and cholesterol levels, and the homeostatic model assessment (HOMA) index, and also decreases triglyceride and leptin levels [7, 38, 39]. Our data (Table 1) showed that Ad36 infection decreased NEFA, glucose, insulin, and cholesterol levels but did not decrease triglyceride level. These results suggest that Ad36 infection increases insulin sensitivity and reduces lipid concentrations in WT mice. By contrast, Ad36 infection decreased NEFA, glucose, insulin, and cholesterol levels, and the homeostatic model assessment (HOMA) index, and also decreases triglyceride and leptin levels [7, 38, 39]. Our data (Table 1) showed that Ad36 infection decreases NEFA, glucose, insulin, and cholesterol levels but did not decrease triglyceride level. These results suggest that Ad36 infection increases insulin sensitivity and reduces lipid concentrations in WT mice. By contrast, Ad36 infection decreased NEFA, glucose, insulin, and cholesterol levels, and the homeostatic model assessment (HOMA) index, and also decreases triglyceride and leptin levels [7, 38, 39]. Our data (Table 1) showed that Ad36 infection decreases NEFA, glucose, insulin, and cholesterol levels but did not decrease triglyceride level. These results suggest that Ad36 infection increases insulin sensitivity and reduces lipid concentrations in WT mice. 

Figure 5. Monocyte chemoattractant protein-1 (MCP-1) concentration correlated with Ad36 antibody presence in human samples. 
A, A statistically significant correlation between Ad36 antibody presence and MCP-1 concentration was observed in the obese group (\( **P < .01 \)). 
B, The Ad36-antibody-positive group had higher MCP-1 concentrations in the total human sample (\( \Delta P < .001 \)). All human samples tested (n = 80) were selected randomly from 540 human samples that have been reported elsewhere [13].

In conclusion, our results suggest that Ad36 infection induces early inflammation by recruiting macrophages to reproductive fat pads by increasing MCP-1, which is activated by NFκB. This induced inflammation causes obesity, which in turn induces chronic inflammation and affects lipid metabolism in viral obesity. In addition to the viral obesity mechanism, which involves the viral gene E4 orf-1 [17], these results provide some insight into one of the cellular mechanisms responsible for Ad36-induced obesity, in which the host immune responses to the viral infection, such as inflammation, may contribute to Ad36-induced obesity. Future progress in decoding the fundamental cellular mechanisms underlying the relationship between Ad36 and obesity may lead to new strategies and therapeutics for preventing and treating obesity.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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