Interleukin 6 Reduces Lipoprotein Lipase Activity in Adipose Tissue of Mice in Vivo and in 3T3-L1 Adipocytes: A Possible Role for Interleukin 6 in Cancer Cachexia

Andrew S. Greenberg, Richard P. Nordan, Joseph McIntosh, Juan Carlos Calvo, Robert O. Scow, and David Jablons

Laboratory of Cellular and Developmental Biology, National Institute of Diabetes, Digestive and Kidney Disease [A. S. G., R. O. S.]; Laboratory of Genetics [R. P. N.] and Surgery Branch [J. M., D. J.], National Cancer Institute; and Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Development [J. C. J.], NIH, Bethesda, Maryland 20892

ABSTRACT

To investigate whether interleukin 6 (IL-6) might be a potential mediator of the depleted fat reserves observed in malignancy-associated cachexia, we measured lipoprotein lipase (LPL) activity in adipose tissue of mice after administration of IL-6 or tumor necrosis factor and in cultured adipocytes after addition of these cytokines. Injection of IL-6 i.p. reduced adipose tissue LPL activity by 53% within 4.5 to 5.5 h. Injection of tumor necrosis factor elevated serum IL-6 levels and reduced adipose tissue LPL activity by 70%. Both human and murine IL-6 reduced heparin-releasable LPL activity in 3T3-L1 adipocytes in a dose-dependent manner; half-maximal inhibition of LPL activity was achieved with 5000 hybridoma growth factor units/ml. Thus, IL-6 reduces adipose LPL activity and may contribute to the loss of body fat stores associated with some cases of cancer cachexia. Since tumor necrosis factor increases circulating IL-6, some of its effects may be mediated or potentiated by IL-6.

INTRODUCTION

Animals and humans with chronic infections or cancer may develop the syndrome of cachexia which is characterized by severe wasting of both protein and fat stores. The syndrome is often associated with elevated serum triglycerides (1–3) and reduced serum LPL activity (4–6). LPL, produced in adipocytes and found at the endothelial cell surface, hydrolyzes circulating triglycerides to fatty acids, which serve as the major source of acyl groups used in fat storage in the adipocyte (7). Cerami et al. (8, 9) discovered a factor produced by endotoxin-stimulated macrophages that suppresses adipocyte LPL activity. This factor, termed cachetin for its presumptive role in lowering LPL activity and promoting the cachetic syndrome, was later found to be identical to TNF (10). When injected into animals, cachetin/TNF lowers adipose tissue LPL activity (11).

We recently reported that administration of recombinant TNF to humans and mice induces the appearance of circulating IL-6 (12, 13). IL-6 is a cytokine that appears to act as an important systemic regulatory hormone in response to infection and trauma (14); increased serum concentrations of IL-6 have been measured in patients with sepsis (15), acquired immunodeficiency syndrome (16), and plasma cell leukemia (17). Increased concentrations of circulating IL-6 are detectable in animals bearing tumors of multiple different histologies (13, 18, 19), and serum IL-6 levels increase in direct proportion to the amount of tumor burden (13, 19).

Since TNF increases serum IL-6 levels and since IL-6 activity is elevated in the presence of malignancy and infections, we investigated whether IL-6 might be a mediator of the depleted fat stores of the cachetic state. In this paper, we demonstrate that IL-6 significantly lowers adipose tissue LPL activity in vivo and directly inhibits LPL activity in cultured adipocytes.

MATERIALS AND METHODS

Cytokines. rHIL-6, a kind gift of Drs. Gordon Wong and Robert Donahue of Genetics Institute (Cambridge, MA), had less than 5 endotoxin units/mg protein and an activity of 4–6 × 10^6 plasmacytoma units/mg. rHIL-6 was dissolved in HBSS containing 2% human albumin, and the activity was assayed by the B9 hybridoma assay (see below). rHTNF, a kind gift of the Cetus Corp. (Emeryville, CA), had an activity of 2 × 10^7 units/ml in the L929 assay (see below) and an endotoxin content of <0.1 ng/mg protein. TNF was dissolved in HBSS with 2% human serum albumin, and activity was confirmed using the L929 assay (20).

Native murine IL-6 was purified by a modification of the procedure of Nordan et al. (21). Briefly, serum-free supernatant from the P388D1 murine macrophage cell line was applied to an affinity column containing the rat anti-murine IL-6 monoclonal antibody, D6906B4 (22), coupled to Sepharose 4B. After extensive washing with phosphate-buffered saline, the murine IL-6 was eluted with 0.2 M glycine-HCl/0.02% Tween 20, pH 2.0. The affinity-purified murine IL-6 was applied to a C-4 reverse phase HPLC column (214TP54, Vydyac) and eluted with a gradient of 40% to 50% acetonitrile/0.1% trifluoroacetic acid over 20 minutes. IL-6, which eluted as a single peak, was lyophilized and reconstituted in phosphate-buffered saline/0.02% Tween 20 and stored at 4°C. Activity was determined with the B9 assay.

B9 Biosay of IL-6 Activity. Recombinant, purified murine, and serum IL-6 activities was assayed by [3H]Thd uptake assay using the IL-6-dependent murine hybridoma subclone B9 as described previously (22, 23). Culture medium consisted of RPMI 1640 supplemented with 10% fetal calf serum, 5 × 10^-5 M 2-mercaptoethanol, and 50 μg/ml gentamicin. B9 cells were maintained in culture medium supplemented with IL-6 at 100 HGF units/ml. For the assay, 2000 washed B9 cells were cultured with 2-fold serial dilutions of the test sample in a final volume of 200 μl of culture medium. After an 84-h incubation at 37°C in a humidified atmosphere of 5% CO2-95% air, the cultures were pulsed for 4 h with 0.5 μCi [3H]Thd (6.7 Ci/mmol) and harvested over glass fiber filters. The incorporation of [3H]Thd into DNA was determined in a liquid scintillation counter. Preparations of known activity of rHIL-6 were diluted in heat-inactivated bovine calf serum and tested concurrently as standards. One hybridoma growth factor unit of IL-6 is defined as that amount which gives half-maximal stimulation of thymidine incorporation into B9 cells. The specificity of the assay was verified by inhibition with a neutralizing rabbit anti-murine IL-6 antiserum (24).

Animal Methods. Female C57BL/6 mice, 12 weeks of age, were obtained from The Jackson Laboratory, Bar Harbor, ME, or from the...
Small Animal Section, National Cancer Institute, Bethesda, MD. The animals were fed ad libitum.

rHTNF and rHL6 were prepared in HBSS and injected i.p. in groups of six animals for each experiment. Animals received an injection of either 1 ml of HBSS alone (vehicle), or 1 ml of HBSS containing 100 µg of rHL6, or 1 ml of HBSS containing 10 µg of rHTNF. Tailbleeds were performed 4 h after i.p. injection to determine serum cytokine activity. Blood was collected in serum separator tubes (SST, Becton Dickinson), and the sera were assayed immediately for IL-6, TNF, or both, or frozen at -70°C prior to analysis.

To measure in vivo adipose tissue LPL activity, animals were sacrificed by cervical dislocation 4.5 to 5.5 h after injection, and the entire periovarian and peritoneal fat pads were removed.

Adipose Tissue Lipoprotein Lipase Activity. Lipolytic activity in the fat pads was determined by a modification of the methods of Peterson et al. (25) and Langer et al. (26). The adipose tissue was weighed and homogenized with a Polytron tissue grinder in 9 volumes (w/v) of homogenization solution consisting of ice-cold 0.025 M ammonium chloride buffer (pH 8.2) containing 5 IU/ml heparin, 10 µg/ml leupeptin, 1 µg/ml pepstatin, 0.04% (w/v) sodium dodecyl sulfate, and 5 mM EDTA. The homogenates were centrifuged at 27,000 × g for 20 min at 4°C. The fat cake was gently pierced, and 10 µl of infranatant were removed and mixed with 10 µl of homogenization solution without Triton and sodium dodecyl sulfate and assayed for LPL activity in duplicate.

A stock lipid emulsion containing 0.005 mmol of tri[9,10-3H]triolein (1.0 Ci/mmol), 1.13 mmol of trioleoylglycerol, 60 µg of 1-α-phosphatidylcholine, and 9 ml of glycerol was prepared according to the method of Nilsson-Ehle and Schotz (27). Before assay an activated substrate mixture was prepared by mixing 1 volume of the stock emulsion with 5 volumes of heat-inactivated fasted rat serum (heated at 60°C for 10 min) and 19 volumes of an assay solution that resulted in a final concentration of 12% bovine serum albumin, 300 IU heparin/ml, 0.2 M NaCl, and 0.2 M Tris-HCl (pH 8.1) (25); the mixture was incubated at 37°C for 15 to 30 min. For assay, 100 µl of the activated substrate mixture, containing 2.0 µCi of tri[3H]oleoylglycerol/ml and 23 nmol of trioleoylglycerol, were added to the adipose tissue extract, brought to a total volume of 200 µl with 0.9% NaCl, and incubated at 37°C for 30 min. Radiolabeled fatty acids produced by lipolysis were extracted and measured by the method of Vaughan and Belfrage (28). The LPL activity was linear for duration of the assay. Lipolytic activity measured in the assay was due to lipoprotein lipase since activity was inhibited by more than 80% by the inclusion of 15 M NaCl or omission of fasted rat serum. One milliunit of lipolytic activity represents release of 1 nmol of fatty acid/min.

3T3-L1 Cell Culture. 3T3-L1 mouse embryo fibroblasts were obtained from American Type Culture Collection (Rockville, MD) and cultured as described previously (29, 30). Briefly, cells were seeded at a density of 2 to 4 × 10^5 cells/60-mm dish (Costar) and cultured in 4 ml of Dulbecco’s modified Eagle’s medium-25 mm glucose (GIBCO or INOVAR), supplemented with 10% fetal bovine serum (GIBCO or INOVAR) and 8.0 µg of pantothentic acid per ml, referred to as complete medium. Cultures were maintained at 37°C in an atmosphere of 5% CO2—95% air. Fifty- and 100-µl aliquots of the media were removed and immediately assayed for LPL activity.

Heparin-releasable LPL activity in the culture medium was assayed as described above with the following exceptions. The preactivated stock mixture consisted of 1 volume of the stock emulsion, 19 volumes of 3% bovine serum albumin in 0.2 M Tris-HCl (pH 8.1), and 5 volumes of heat-inactivated fasted rat serum (heated at 60°C for 10 min); this mixture was incubated at 37°C for 15 to 30 min. For assay, 100 µl of the activated substrate mixture were added to an aliquot of culture medium, brought to a total volume of 200 µl with 0.9% NaCl, and incubated at 37°C for 60 min. Fatty acids were recovered and enzyme activity was defined as noted above.

Statistics. Differences between groups were assessed using Student’s unpaired t test. The calculations were performed using CLINFO software (BBN Software Products, Cambridge, MA). All data are given as means ± SE.

RESULTS

IL-6 Serum Levels. Previous studies demonstrated that i.p. administration of IL-6 results in a more persistent elevation of serum IL-6 concentration than i.v. administration (32). Since animals with tumors have chronically elevated levels of circulating IL-6, the i.p. route of administration was used for the studies in this paper. Circulating IL-6 concentrations (Table 1) 4 h after administration of either 10 µg of rHTNF or 100 µg of rHL6 were elevated to levels similar to those found in mice with large tumor burdens (13, 19). The injection of HBSS vehicle in the control animals produced no detectable serum IL-6 activity. Representative sera from mice that received IL-6 were analyzed for TNF activity by the L929 cytolytic assay, and none was detected (data not shown). Thus, under our experimental conditions, TNF injection increased circulating IL-6.

Adipose Tissue LPL Activity. Injection of rHL6 into mice reduced adipose tissue LPL activity by 53% (Table 2), while in mice treated with rHTNF, adipose tissue LPL activity was lowered by 70%. The entire periovarian and peritoneal fat pads were carefully removed and used for the LPL assay. Since there was no obvious difference between the fat pad weights of the control and cytokine-treated groups, cytokine treatment did not appear to reduce the triglyceride content of the tissues during the brief time period examined here.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (h)</th>
<th>Serum IL-6 activity (HGF units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>&lt;20</td>
</tr>
<tr>
<td>IL-6</td>
<td>4</td>
<td>422 ± 113</td>
</tr>
<tr>
<td>TNF</td>
<td>4</td>
<td>543 ± 204</td>
</tr>
</tbody>
</table>

Table 1 Effect of TNF or IL-6 on serum IL-6 activity in mice

Mice were given i.p. injections in the morning of HBSS vehicle (control) or cytokines rHL6 and rHTNF, at 100 and 10 µg, respectively. Tailbleeds were performed 4 h later and the serum IL-6 levels were determined using the B9/HGF assay. Values represent the mean ± SE of six animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>(milliunits/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>492 ± 64</td>
</tr>
<tr>
<td>IL-6</td>
<td>12</td>
<td>230 ± 26^a</td>
</tr>
<tr>
<td>TNF</td>
<td>12</td>
<td>150 ± 15^b</td>
</tr>
</tbody>
</table>

Table 2 Effects of IL-6 and TNF in vivo on LPL activity in murine adipose tissue

rHTNF (10 µg) or rHL6 (100 µg) in HBSS was administered i.p. in the morning to mice. From 4.5 to 5.5 h later, the entire periovarian and peritoneal fat pads were removed, homogenized with a Polytron, and assayed for lipoprotein lipase activity. Values are means ± SE of two experiments with six animals/ experiment.
3T3-L1 Adipocyte LPL Activity. Previous studies have indicated that the heparin-releasable fraction of LPL activity in 3T3-L1 adipocytes reflects whole cell LPL activity (8). Treatment of the 3T3-L1 adipocytes with IL-6 reduced LPL activity in a dose-dependent manner (Fig. 1). Maximal reduction of heparin-releasable LPL activity was seen with 50,000 HGF units/ml of murine IL-6 reduced LPL activity by 43% and 50,000 units/ml of murine IL-6 reduced LPL activity by 50% (Fig. 1). Recombinant human IL-6 gave similar results; 5000 HGF units/ml reduced heparin-releasable LPL activity by 43% and 50,000 HGF units/ml of rHIL-6 by 70%. As previously reported (31), a maximally effective rHTNF concentration of 50 ng/ml lowered LPL activity by 96%.

DISCUSSION

Lipoprotein lipase plays an important role in adipocyte metabolism by hydrolyzing circulating triglycerides to fatty acids for subsequent storage as fat in the adipocyte. Thus, a reduction in the activity of LPL would contribute to a decrease in total body fat stores. Reduced lipoprotein lipase activity has been documented in patients and animals with cachetic syndrome (4–6). An intensive search for mediators of cachexia has been conducted in a number of laboratories, with special emphasis on the role of TNF/cachectin (12). However, elevated TNF concentrations have generally not been found in cachexia patients with cachexia (33, 34). In addition, animals with tumors of different etiologies have circulating TNF concentrations that were undetectable or minimal (13, 35), but serum IL-6 concentrations were elevated in parallel with tumor burden (13, 19). Similarly, other illnesses which can result in the cachectic syndrome, such as infectious disease, have been associated with elevated serum concentrations of IL-6 (15, 16, 36).

In this study, IL-6 was shown to reduce LPL activity in adipose tissue in vivo when injected i.p. in mice. Thus, this cytokine may be important in promoting the depletion of fat stores observed in the cachetic syndrome associated with disease states. Further support for this hypothesis stems from a recent study in which the introduction of a retroviral expression vector containing the IL-6 coding sequence into the bone marrow of mice resulted in high serum concentrations of IL-6 and mice with markedly decreased amounts of s.c. fat (37).

The evidence presented in this paper indicates that the 3T3-L1 adipocyte is a direct target for IL-6 action. IL-6, like TNF, reduces LPL activity when added to the culture medium. Although TNF decreases the rate of LPL gene transcription (31, 38, 39), the mechanism of action for IL-6 is unknown. As with other actions attributed to IL-6 (14), both murine and recombinant human IL-6 were equipotent in their effects on the adipocyte, indicating no species specificity in the murine response.

Many cytokines, including TNF, interleukin 1, interleukin 2, and α-interferon elevate serum IL-6 concentrations when injected into animals (12). Moreover, many types of cells, including fibroblasts, monocytes, and endothelial cells, elaborate IL-6, and the production of this cytokine increases in response to TNF as well as other cytokines (40). The 3T3-L1 preadipocyte cell line is a clonal population of cells derived from mouse fibroblasts (41), raising the possibility that the TNF effects on different differentiated adipocytes observed in this study might be mediated by the increased production of IL-6. Alternatively, the presence of IL-6 might potentiate the direct actions of TNF on differentiated adipocytes. Whether alone or in conjunction with TNF, the data clearly suggest a role for IL-6 in the development of the cachectic syndrome.

ACKNOWLEDGMENTS

The authors thank Drs. C. Londos and A. Kimmel as well as Dr. M. Lotze for many constructive conversations, Dr. S. Roberts for valuable advice during preparation of the manuscript as well as assistance with statistical analysis, and also Dr. S. Steinberg for assistance with the statistical analysis.

Note Added in Proof

Further support for the role of IL-6 in cancer cachexia is found in the recent paper by Strassman et al. (Evidence for the involvement of interleukin 6 in experimental cancer cachexia. J. Clin. Invest., K9: 1681–1684. 1992), who found that injection of anti-IL-6 antibody in tumor-bearing mice significantly inhibited the appearance of various parameters of cachexia.

REFERENCES

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