Adipose Tissue Is a Major Source of Interleukin-1 Receptor Antagonist

Upregulation in Obesity and Inflammation

Cristiana E. Juge-Aubry,[1] Emmanuel Somm,[1] Vittorio Giusti,[2] Agnès Pernin,[1] Rachel Chicheportiche,[3] Chantal Verdumo,[2] Françoise Rohner-Jeanrenaud,[1] Danielle Burger,[3] Jean-Michel Dayer,[3] and Christoph A. Meier[1]

The secreted form of the interleukin-1 receptor antagonist (IL-1Ra) is an acute-phase protein intervening in the counterregulation of inflammatory processes. We previously showed that this cytokine antagonist is upregulated in the serum of obese patients, correlating with BMI and insulin resistance. In this study, we examined the expression pattern of IL-1Ra and showed that it is highly expressed not only in liver and spleen, but also in white adipose tissue (WAT), where it is upregulated in obesity. In WAT of obese humans, IL-1Ra was also markedly increased. Moreover, human WAT explants secreted IL-1Ra into the medium, a process that could be stimulated fivefold by interferon-β. Finally, lipopolysaccharide administration induced a long-lasting expression of IL-1Ra in mouse WAT, suggesting that adipose tissue is an important source of IL-1Ra in both obesity and inflammation. In summary, we demonstrated that WAT is one of the most important sources of IL-1Ra quantitatively, suggesting that this tissue could represent a novel target for anti-inflammatory treatment. Moreover, it can be speculated that IL-1Ra, whose production is markedly increased in WAT in obese individuals, contributes further to weight gain because of its endocrine and paracrine effects on the hypothalamus and adipocytes, respectively. *Diabetes* 52:1104–1110, 2003

From the [1]Endocrine Unit, Division of Endocrinology, Diabetes and Nutrition, Department of Internal Medicine, University Hospital, Geneva, Switzerland; the [2]Division of Endocrinology, Diabetology, and Metabolism, Department of Medicine, University Hospital, Lausanne, Switzerland; and the [3]Hans Wilsdorf Laboratory, Division of Immunology and Allergy, Department of Internal Medicine, University Hospital, Geneva, Switzerland.

Address correspondence and reprint requests to Dr. Christoph A. Meier, Endocrine Unit, University Hospital Geneva, 24, rue Micheli-du-Crest, CH-1211 Geneva 14, Switzerland. E-mail: christoph.meier@medecine.unige.ch.

Received for publication 6 January 2003 and accepted in revised form 12 February 2003.

C.E.J.-A. and E.S. contributed equally to this work.

BAT, brown adipose tissue; FBS, fetal bovine serum; HT, hypothalamus; icIL-1Ra, intracellular IL-1Ra; IFN-β, interferon-β; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; LPS, lipopolysaccharide; PMA, phorbol myristate acetate; PPAR-γ, peroxisome proliferator–activated receptor-γ; sIL-1Ra, secreted IL-1Ra; TNF-α, tumor necrosis factor-α; WAT; white adipose tissue; WATe, epididymal WAT; WATi, inguinal WAT.

The interleukin-1 receptor antagonist (IL-1Ra) is a member of the interleukin (IL)-1 family that binds to IL-1 receptors without inducing a cellular response, thereby antagonizing the effects of IL-1α and -1β (1). Three forms of IL-1Ra have been described, two of which are expressed as intracellular proteins (icIL-1RaI and icIL-1RaII) and one of which is secreted (sIL-1Ra) (2). An understanding of the function(s) of the intracellular forms of IL-1Ra is still elusive (3), whereas it is clear that sIL-1Ra inhibits IL-1α and -1β actions on target cells. Because of its beneficial effects in many models of disease, sIL-1Ra has been used as a therapeutic agent in human patients and was recently approved by the U.S. Food and Drug Administration for treating rheumatoid arthritis (4,5). Furthermore, IL-1Ra knockout mice have an enhanced susceptibility to septic shock and demonstrate an increased predisposition to the spontaneous development of inflammatory disorders, an observation that illustrates the functional relevance of this anti-inflammatory factor (6–8).

The production of sIL-1Ra is induced by many of the stimuli that usually induce IL-1 in the same cell (rev. in 2). However, we and others have recently demonstrated that the anti-inflammatory cytokine interferon-β (IFN-β) and leptin can induce sIL-1Ra production without the simultaneous increase in IL-1 in human monocytes and monocytic cell lines (9,10). This finding can be explained by the observation that factors involved in the signaling pathways of these stimuli, such as serine/threonine phosphatases, differentially regulate IL-1β and IL-1Ra production in monocytes-macrophages (11). The premise that leptin is able to induce sIL-1Ra has been further extended by the finding that serum sIL-1Ra levels are sevenfold higher in subjects with hyperleptinemic obesity, as compared to nonobese subjects (12). Furthermore, sIL-1Ra levels dropped after weight loss was induced by intestinal bypass surgery. Interestingly, in these patients, the serum sIL-1Ra concentrations correlated better with BMI and the degree of insulin resistance than with serum leptin levels, suggesting that metabolic regulators other than leptin may control the production of sIL-1Ra. Based on these observations, the present study was undertaken to investigate *1*) whether tissues other than liver and spleen are able to express and produce sIL-1Ra, *2*) whether IL-1Ra produc-

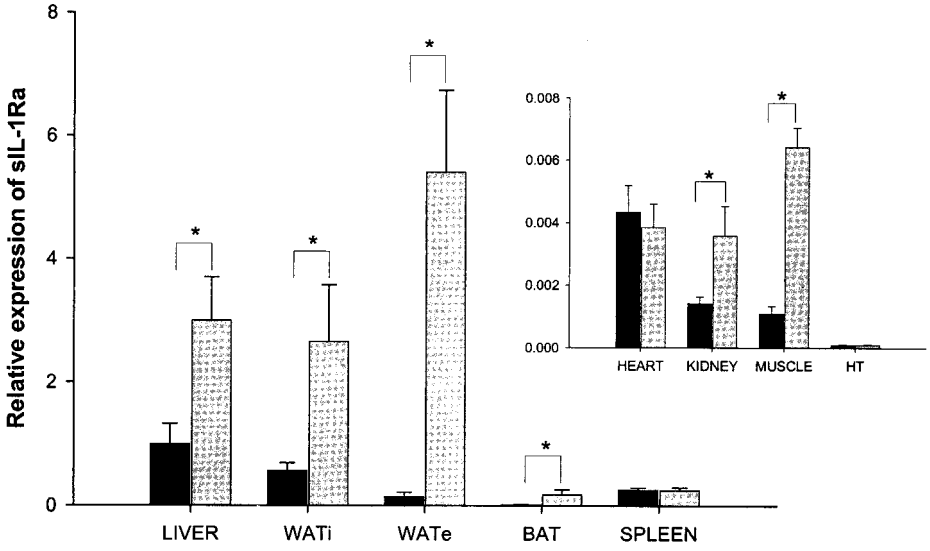

FIG. 1. sIL-1Ra is expressed in WAT of lean mice and upregulated in *ob/ob* mice. Levels of sIL1-Ra mRNA were determined by quantitative real-time RT-PCR from tissues of lean (■) and obese (▨) animals. The results are expressed relative to liver after normalization for the levels of 36B4 RNA. The columns represent means ± SE of four to five animals. Each sample is the average of three separate amplification reactions. Expression levels were lower in heart, kidney, skeletal muscle, and HT (inset; note the different scale of the ordinate axis). Statistical differences between the tissues from normal and *ob/ob* mice were assessed by the Mann-Whitney *U* test (*$P < 0.05$).

tion is increased in these tissues in obesity and after an inflammatory stimulus, and *3*) what are the putative metabolic factors regulating the production of IL-1Ra. Our results demonstrate that white adipose tissue (WAT) is a major source of sIL-1Ra and that its expression in this tissue is markedly increased in obesity and inflammation.

RESEARCH DESIGN AND METHODS

Animals. C57BL/6 wild type and *ob/ob* mice age 12 weeks were obtained from the Center d'Elevage Janvier (Le Genest-Saint-Isle, France) and killed 10 days after arrival. The animals were perfused with sterile isotonic saline through an intracardiac puncture before the tissues were removed and immediately frozen in liquid nitrogen for RNA preparation. Where appropriate, the animals were injected with lipopolysaccharide (LPS) at 5 μg/g body wt or vehicle and killed after 6 and 18 h.

Human adipose tissue. After authorization by the ethical commission of the Center Hospitalier Universitaire Vaudois and informed consent from the patients were received, subcutaneous and visceral adipose tissue samples were obtained from 28 morbidly obese women with a median BMI of 45 kg/m^2 (range 37–65 kg/m^2) and with no comorbidity who were undergoing gastric banding or gastric bypass surgery. Subcutaneous adipose tissue samples were also obtained from 10 patients (9 women, 1 man) with a BMI <30 kg/m^2 undergoing abdominal plastic surgery. Tissues were immediately frozen in liquid nitrogen.

RNA preparation and mRNA quantification. Total RNA was prepared from 500 mg of human WAT, 250 mg of mouse WAT, or 50 mg of rodent liver, heart, spleen, muscle, brown adipose tissue (BAT), or hypothalamus (HT) by using the TRIzol reagent (Invitrogen, Basel, Switzerland), according to manufacturer's instructions. Total RNA (5 μg) was reverse transcribed using 800 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen), in the presence of 0.3 units/μl RNAsin (Promega, Madison, WI), 7.5 μmol/l of random primers (oligo(dN)6), 1.2 mmol/l dNTP, and 12 μmol/l of dithiothreitol.

The expression of RNAs for human sIL-1Ra, icIL-1Ra, and 28S, as well as for mouse sIL-1Ra, IL-1β, tumor necrosis factor-α (TNF-α), and 36B4 RNA, was determined by quantitative real-time PCR using a LightCycler (Roche Diagnostics, Rotkreuz, Switzerland) with the DNA Master SYBR Green I or Fast Start DNA Master SYBR Green I (Roche Molecular Biochemicals, Rotkreuz, Switzerland) kits as appropriate. Each of the transcript levels was quantitated in at least two independent LightCycler runs in each individual sample. The PCR products of sIL-1Ra and icIL-1Ra were verified by automated sequencing (ABI PRISM 3100; Perkin-Elmer/Cetus, Norwalk, CT) and cloned into the T/A cloning vector pCR3.1 according to the manufacturer's instructions (Invitrogen, Groningen, the Netherlands).

Preparation of adipose tissue homogenates and cytokine determination. Human WAT (50 μg) was treated in a glass/glass homogenizer (Dounce) in 500 μl of lysis buffer containing 50 mmol/l Tris base, 150 mmol/l NaCl, 2 mmol/l EDTA, 2 mmol/l EGTA, 40 mmol/l β-glycerophosphate, 50 mmol/l NaF, 10 mmol/l sodium pyrophosphate, 10% glycerol, 1% Triton X-100 (pH 7.4), and a protease inhibitor cocktail (Complete Mini EDTA-Free; Roche Diagnostics). After being centrifuged at 700*g* for 30 min, the supernatants were used for the determination of IL-1Ra, IL-6, and leptin. IL-1Ra and IL-6 concentrations in the serum, WAT homogenates, and supernatants of WAT explants were determined with the Quantikine enzyme-linked immunosorbent assay kits (R&D Systems Europe Ltd, Abingdon, UK), measuring the total amounts of these cytokines. IL-1α and IL-1β were determined with kits from Immunotech (Marseille, France), and TNF-α was determined using antibodies from Biosource International (Lucernachem, Lucerne, Switzerland). Leptin levels were determined using a radioimmunometric assay kit from Linco Research (St. Louis, MO). The performance of these kits in quantifying cytokines in protein extracts from tissues was validated by the addition of known amounts of recombinant cytokines.

The measurement of serum sIL-1Ra levels in rodents is influenced by the interference from serum proteins, and no adequately validated kit is currently available.

Explant preparation and culture. Human subcutaneous WAT, obtained from patients undergoing abdominoplasty, was prepared as previously described (13). Briefly, tissue was placed in PBS (prewarmed at 37°C and containing penicillin [100 units/ml] and streptomycin [100 mg/ml]), and connective tissue and blood vessels were removed by dissection before mincing the tissue into 5- to 10-mg pieces. Minced tissue fragments were filtered through a nylon mesh (350 μm) and washed with medium (M199, supplemented with 5% fetal bovine serum [FBS] and P+S; Invitrogen). Then 0.5–1 g of minced tissue was placed into 10 ml M199 (5% FBS, penicillin, and streptomycin) for 18 h before being stimulated for 48 h with the appropriate compounds. At the end of the stimulation period, the supernatants were collected for cytokine determination and the explants were recovered and placed directly into TRIzol reagent (Invitrogen) for immediate RNA preparation.

Statistics. Results are expressed as means ± SE. The nonparametric Mann-Whitney *U* test was used for comparison between two groups of mice ($n = 4$–5), and the paired Student's *t* test was used for the human visceral and subcutaneous adipose tissues ($n = 28$). These tests and the linear regression analysis were performed with SYSTAT 10.01 (SPSS, Chicago, IL).

RESULTS

sIL-1Ra is expressed in WAT of lean and obese mice. To determine whether sIL-1Ra was differentially expressed in lean and *ob/ob* mice, we first assessed the tissue-specific expression pattern of sIL-1Ra and the ribosomal housekeeping gene 36B4 by means of real-time quantitative PCR. In both wild-type C57BL/6 and *ob/ob* mice, sIL-1Ra mRNA was mainly expressed in liver and spleen, as well as in epididymal and inguinal WAT (WATe and WATi, respectively) (Fig. 1), whereas sIL-1Ra mRNA levels were low in BAT, kidney, skeletal muscle, and heart (Fig. 1, inset). In contrast, the HT did not express detectable amounts of sIL-1Ra transcript. When compared to lean mice, most tissues from *ob/ob* animals expressed

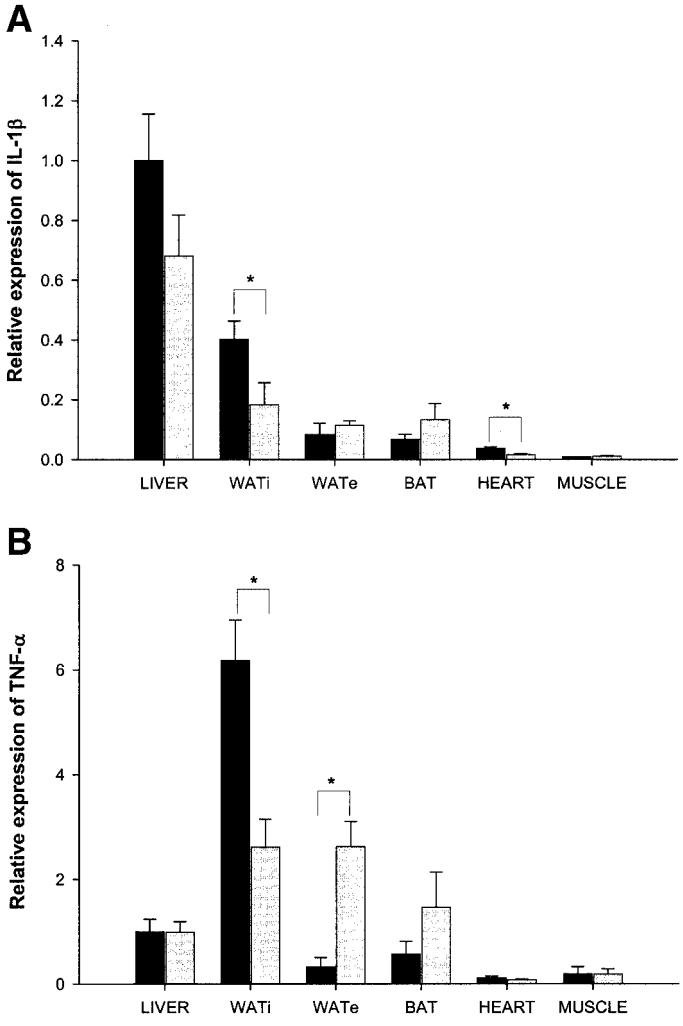

FIG. 2. Expression of IL-1β and TNF-α in WAT of lean and *ob/ob* mice. IL-1β (*A*) and TNF-α (*B*) are downregulated in WATi of obese animals (▨) as compared to lean animals (■), whereas TNF-α expression is increased in WATe of *ob/ob* mice. Levels of sIL-1β and TNF-α mRNA were determined by quantitative real-time PCR. The results are expressed relative to liver after normalization for the levels of 36B4 RNA. The columns represent means ± SE of four to five animals. Each sample is the average of three separate amplification reactions. Statistical differences between the tissues from normal and *ob/ob* mice were assessed by the Mann-Whitney *U* test (*$P < 0.05$).

higher amounts of sIL-1Ra, with a 37- and 5-fold increase in WATe and WATi from obese mice, respectively. BAT, skeletal muscle (M. gastrocnemius), and liver from *ob/ob* animals expressed 11-, 6-, and 3-fold higher sIL-1Ra transcript levels than lean control mice.

Differential regulation of IL-1β and TNF-α in *ob/ob* mice. Because the induction of sIL-1Ra is often accompanied by a parallel increase in IL-1β, the presence of IL-1β mRNA was measured in the tissues of lean and *ob/ob* mice. IL-1β transcript was expressed in liver, WATe, WATi, BAT, and, to a lower extent, in skeletal muscle and heart (Fig. 2*A*). In contrast to sIL-1Ra, IL-1β mRNA was significantly decreased in WATi and heart of *ob/ob* as compared to lean mice (Fig. 2*A*), but was expressed in similar amounts in skeletal muscle, BAT, and WATe.

Because TNF-α was previously shown to be upregulated in WATe of obese rodents (14), we also assessed the expression levels of this cytokine in various tissues from lean and *ob/ob* mice. With the exception of WATe, in which it was significantly induced, TNF-α mRNA was decreased in WATi of *ob/ob* mice as compared to controls (Fig. 2*B*), which paralleled the reduced expression of IL-1β in WATi. No significant differences were observed in the other tissues examined.

Expression of sIL-1Ra mRNA and protein in human adipose tissue. To test whether sIL-1Ra was also upregulated in human adipose tissue, samples of subcutaneous WAT were obtained from 10 patients with a BMI <30 kg/m^2. In addition, visceral and subcutaneous WAT was obtained from 28 obese patients undergoing gastric banding or bypass surgery. Protein extracts and total RNA were prepared for assessing the presence of IL-1Ra and other cytokines at mRNA and protein level.

Adipose tissue homogenates were assayed for their protein contents in IL-1Ra, Il-1α, Il-1β, TNF-α, IL-6, and leptin using validated immunoassays. IL-1Ra protein was present in all subcutaneous tissue samples, with an average content of 2.8 ± 0.5 pg/mg of tissue in nonobese patients and a significantly increased content (3.1-fold) in the obese group (Fig. 3*A*). Using quantitative real-time PCR, we also determined the respective amounts of mRNA for sIL-1Ra and icIL-1Ra using a standard curve with known amounts of the cloned cDNA for either form. As was seen in the rodents, sIL-1Ra mRNA was significantly increased in WAT from obese patients (6.6-fold) (Fig. 3*B*), whereas the intracellular form remained unchanged. Moreover, the expression of sIL-1Ra was 8- and 98-fold higher than that of icIL-1Ra in nonobese and obese subcutaneous WAT, respectively (Fig. 3*B*). Therefore, the increased concentrations of IL-1Ra protein present in the tissues from obese patients most likely represented the secreted form of the protein. sIL-1Ra mRNA was equally expressed in visceral and subcutaneous adipose tissue, with a significant correlation between the two within patients ($r^2 = 0.42$, $P < 0.001$; data not shown). There was no significant difference in the protein content for IL-1Ra or TNF-α between subcutaneous and visceral WAT; however, IL-1β was slightly increased in visceral WAT and IL-1α was undetectable (Fig. 3*C*, left panel). In contrast, IL-6 protein was nearly fivefold more abundant in visceral than in subcutaneous WAT (Fig. 3*C*, left panel), whereas the leptin content was slightly lower in visceral fat (Fig. 3*C*, right panel). The IL-1Ra protein content of subcutaneous WAT correlated with serum IL-1Ra levels ($r^2 = 0.45$; $P < 0.001$) and, to a lesser extent, with BMI ($r^2 = 0.2$; $P < 0.02$). However, serum leptin levels did not correlate with its abundance in WAT (data not shown). For comparison, the IL-6 protein content per weight unit of WAT from the obese patients was 5- to 10-fold lower than for IL-1Ra, but was 3- to 4-fold higher for leptin (Fig. 3*C*). The ratio of the protein content of IL-1Ra to IL-1β was increased 4.8-fold in WAT from obese patients (Fig. 3*D*), suggesting that the metabolic effects of locally produced IL-1 might be inhibited by IL-1Ra in adipose tissue.

Before discarding the possibility of sIL-1Ra being expressed mainly by blood vessels of adipose tissue, the presence of mRNA for the vascular endothelial marker, cadherin (VE-cadherin), was assessed. Although VE-cadherin mRNA was detectable in adipose tissue, the amounts did not correlate with sIL-1Ra transcript expression, sug-

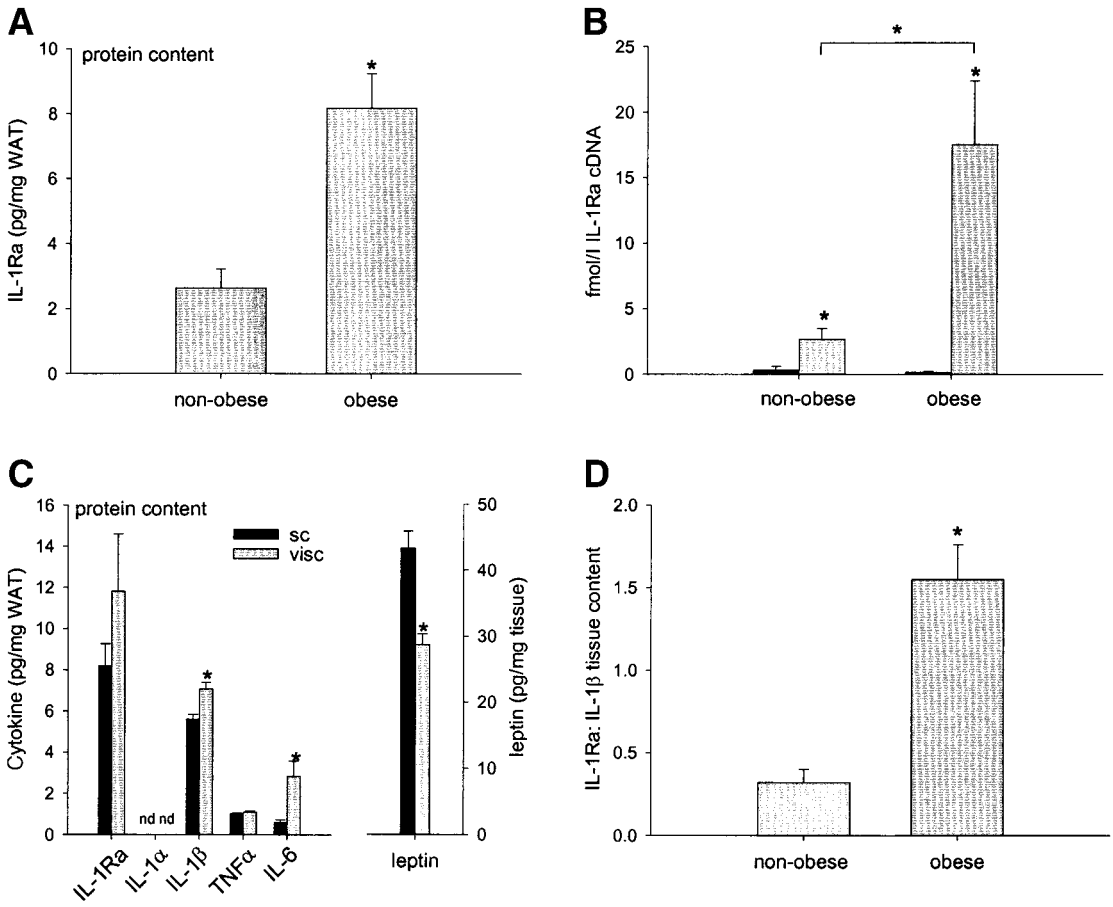

FIG. 3. IL-1Ra protein and mRNA are present in human WAT and are increased in obesity. *A*: Protein content in IL-1Ra was significantly higher in samples of subcutaneous adipose tissue of 28 morbidly obese patients as compared to 10 nonobese control subjects. *$P < 0.05$. *B*: Amount of icIL-1Ra and sIL-1Ra mRNA in obese and nonobese tissues was determined by quantitative real-time PCR using standard curves derived from the cloned cDNAs for sIL-1Ra (▨) and icIL-1Ra (■). Similar to the IL-1Ra protein, its mRNA was significantly higher expressed in WAT from obese compared to nonobese patients, whereas icIL-1Ra remained unchanged. In both obese and nonobese tissues, icIL-1Ra was significantly less expressed than the secrete form. *$P < 0.05$. *C*: Protein contents in IL-1Ra, IL-1α, IL-1β, TNF-α IL-6, and leptin were determined by validated immunometric assays in samples of subcutaneous (■) and visceral (▨) WAT from 28 morbidly obese patients. IL-1α was not detectable in either tissue. IL-1β and IL-6 were significantly upregulated in visceral WAT, whereas leptin content was decreased. No statistically significant differences were found for IL-1Ra. *$P < 0.05$. *D*: Ratio of the tissue content in IL-1Ra to IL-1β was increased in obese WAT as compared to nonobese WAT. *$P < 0.05$.

gesting that vascular endothelium was not the major source of IL-1Ra in our samples (data not shown).

Taken together, our results suggest that adipose tissue is a quantitatively relevant source of serum sIL-1Ra.

Human adipose tissue in explants secrete IL-1Ra. Because WAT expresses and produces IL-1Ra, we wished to test whether explants from human WAT were capable of secreting IL-1Ra. To this end, we incubated human adipose explants from subcutaneous WAT for 48 h with various stimulators known to induce IL-1Ra secretion in monocytes, such as phorbol myristate acetate (PMA), IFN-β, and the peroxisome proliferator–activated receptor-γ (PPAR-γ) ligand rosiglitazone, which, as we have recently shown, regulates IL-1Ra in human monocytes (15). WAT explants secreted 14 ± 3.6 ng · g explant^{-1} · 48 h^{-1} of IL-1Ra in the basal state; this secretion was significantly enhanced by either PMA or IFN-β (to 39 ± 8.4 or 73 ± 21.2 ng · g explant^{-1} · 48 h^{-1}, respectively) (Fig. 4*A*). In contrast to what was seen in monocytes, rosiglitazone treatment resulted in an inhibition of PMA-stimulated secretion. With regard to the steady-state levels of IL-1Ra mRNA, the regulation of expression was similar to that of its secretion, with PMA increasing the basal level 4.6-fold and a strong upregulation being obtained with IFN-β (29-fold) (Fig. 4*B*). Taken together, these results show that human WAT is a quantitatively important and regulated source of sIL-1Ra.

Lipopolysaccharide induces the expression of IL-1Ra in WAT. LPS administration to mice is known to increase the serum levels of sIL-1Ra. To determine whether WAT is also a source of sIL-1Ra in inflammation, normal lean mice were injected with 5 μg/g body wt LPS. sIL-1Ra mRNA expression increased >100-fold in liver and spleen 6 h after LPS administration. Moreover, a 120- and 30-fold augmentation in IL-1Ra transcript levels was found in WATi and WATe, respectively (Fig. 5). Interestingly, the highest effect was observed in WATe only at 18 h after injection of LPS, whereas the increase in sIL-1Ra mRNA reached its peak 6 h after the injection of LPS in liver, spleen, and WATi. This suggests a site-specific regulation of sIL-1Ra expression in response to an inflammatory stimulus.

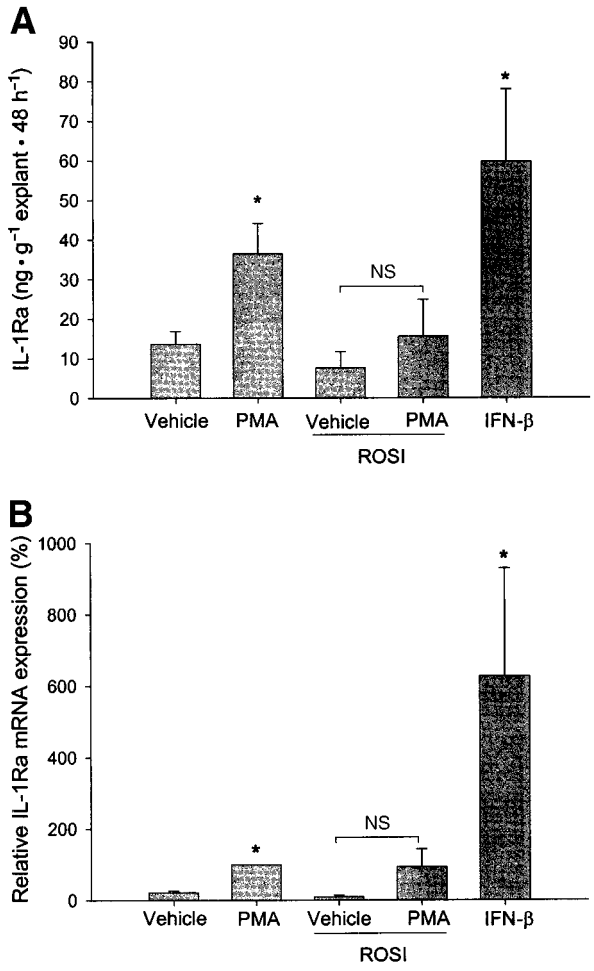

FIG. 4. Human WAT explants were incubated for 48 h with 25 nmol/l PMA, 1 μmol/l rosiglitazone (ROSI), or 10^4 unit/ml of IFN-β. *A*: IL-1Ra was then measured in supernatants. PMA and IFN-β significantly induced IL-1Ra secretion, whereas rosiglitazone inhibited the PMA-induced secretion of IL-1Ra. *$P < 0.05$. *B*: Expression of IL-1Ra in WAT explants was stimulated by PMA and IFN-β, whereas rosiglitazone inhibited the PMA-induced expression. The results are expressed relative to the expression level of IL-1Ra after stimulation with PMA, which was defined as 100%.

DISCUSSION

Obesity has previously been shown to be associated with the increased secretion of various proteins by WAT, including IL-6, TNF-α, plasminogen activator inhibitor-1, angiotensinogen, as well as adipsin, adiponectin, and leptin (rev. in 16,17). Indeed, the increased production of the proinflammatory cytokines IL-6 and TNF-α has been implicated in the pathogenesis of cardiovascular complications and insulin resistance, respectively (18–20). However, although all of these factors can be detected in obese adipose tissue at the mRNA and protein levels, the elevation in circulating serum levels is more equivocal. Hence, it was surprising to note that serum levels of the anti-inflammatory cytokine antagonist IL-1Ra were elevated more than sevenfold in human obesity (12), corresponding to levels that occur in inflammatory autoimmune diseases and sepsis. We have now shown that even in the basal state, WAT is an important source of IL-1Ra, with a further dramatic increase in obese conditions. Subcutaneous and visceral human WAT of obese patients contained 0.4 and 0.7 ng/mg protein of IL-1Ra, respectively, as compared to 7 ng of IL-1Ra in unstimulated white blood cells (data not shown). The protein detected in human WAT is likely to have been secreted in significant quantities, as cultures of explants from human subcutaneous WAT secreted 14 ng of IL-1Ra per gram of cultured tissue per 48 h, equaling five times the quantity of IL-1Ra contained in the homogenates of these explants (2.6 ng/g tissue). Given the important quantitative contribution of WAT to whole-body composition in lean individuals, and even more so in obese subjects, it can be inferred that adipose tissue is one of the main sources of IL-1Ra production in humans. Taking as an example an obese person weighing 120 kg with 50% body fat, the total WAT is estimated to contain 0.6 mg of IL-1Ra protein, corresponding to 200 times the amount of IL-1Ra present in the total serum. In comparison, the protein content of leptin and IL-6 in WAT of this obese subject is estimated at 2 mg and 0.1 mg, respectively.

The expression of IL-1Ra increases nearly 40-fold in WAT of obese rodents; the question then arises as to which factors mediate this marked augmentation. The fact that the increased expression of IL-1Ra is accompanied to a similar extent by the augmentation of TNF-α mRNA, but not IL-1β mRNA, argues against a general inflammatory state associated with obesity. This suggestion is also substantiated by human studies in which IL-6 serum levels were not increased beyond the upper limit of the normal range (12,21). Hence, it appears that metabolic factors selectively enhance the expression of the IL-1Ra gene in adipose tissue. Indeed, we have recently shown that PPAR-γ ligands enhance the expression and secretion of IL-1Ra by stimulated human monocytes. However, our experiments with human WAT explants have shown a strong stimulatory effect of PMA and IFN-β, whereas the PPAR-γ ligand inhibited the PMA-induced secretion of IL-1Ra, indicating a tissue-specific regulation. This finding was in keeping with the observation that in morbidly obese humans, the serum sIL-1Ra levels are less well correlated with BMI than with the degree of insulin resistance (12), the latter being mainly attributable to an altered insulin sensitivity of skeletal muscle (17). Furthermore, we found the expression of sIL-1Ra to be markedly increased in skeletal muscle from obese mice, suggesting that this tissue may also contribute to circulating IL-1Ra levels in obesity. Our experiments with human WAT explants show, however, that PPAR-γ ligands are unable to induce the secretion of IL-1Ra, whereas PMA and IFN-β are potent inducers. Because IFN-β not only is a fibroblast-derived interferon, but also is produced by other cell types such as macrophages, epithelial, endothelial, and lymphoid cells (22), it is tempting to speculate that stromal cells and preadipocytes might be involved in regulating IL-Ra secretion through a paracrine mechanism. Based on our data obtained from rodents, it appears that the regulation of cytokine expression in WAT is multifactorial, although *1*) marked site-specific differences in the basal and obesity-related expression of IL-1Ra exist (this is also true for IL-1β and TNF-α, the latter being markedly upregulated in epididymal fat of *ob/ob* mice, as previously described [14)], but with an opposite effect in inguinal WAT) and *2*) in response to LPS, both the maximal induction of sIL-1Ra in WAT as well as the time course are markedly different in WATe and WATi, with the former

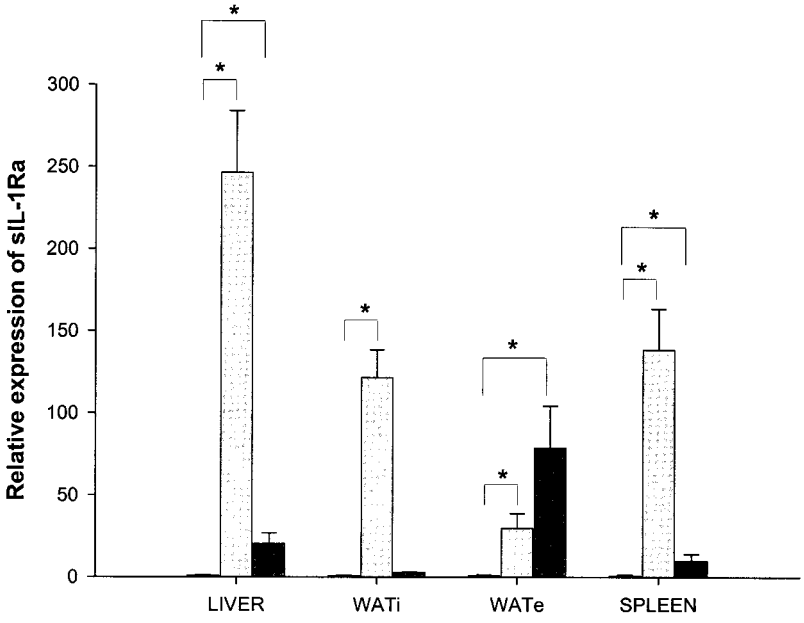

FIG. 5. LPS increased the expression of IL-1Ra in WAT of normal mice. Animals were injected intraperitoneally with vehicle (■) or 5 μg/g body wt LPS and sacrificed 6 h (▨) or 18 h (■) later. Expression of sIL-1Ra was analyzed as described above.

showing a markedly prolonged enhancement of sIL-1Ra expression compared to any of the other tissues examined. However, the factors responsible for this difference are unknown.

The functional consequences of the increased production of IL-1Ra in obesity remain speculative. However, beside the anti-inflammatory, and hence potentially antiatherogenic, effects of IL-1Ra (7,23), the question arises whether this cytokine antagonist also has metabolic activities. Although this issue remains largely unexplored, data from Luheshi et al. (24) indicate that IL-1Ra induces an acquired resistance to leptin in rodents; it has been hypothesized that this mechanism is also operative in humans (12). Perhaps even more important, IL-1β was shown to stimulate lipolysis, glucose transport, and adipocyte maturation in adipose tissue, as well as inhibit lipogenesis (25), an effect that is likely mediated through the IL-1 receptor type 1 and the IL-1 receptor accessory protein (26–30), both of which we have shown to be expressed in human WAT (unpublished data). Hence, it can be speculated that the auto- and paracrine effects of the increased IL-1Ra:IL-1β ratio may inhibit these effects, contributing to an increase in body fat and weight gain. Whether IL-1Ra has any additional metabolic effects and/or an antiatherogenic protective effect in human obesity remains to be demonstrated.

Taken together, these findings imply that adipose tissue is an important source of sIL-1Ra, whose secretion can be induced by inflammatory stimuli as well as obesity-associated factors. This, in turn, implies that adipose tissue might serve as a novel target for developing therapeutic strategies for inflammatory autoimmune diseases. Moreover, because IL-1 appears to mediate, at least in part, the central effects of leptin on appetite regulation and thermogenesis, it can be speculated that the upregulation of IL-1Ra in obesity results in a partial blockade of the hypothalamic actions of leptin, resulting in an acquired resistance to leptin (12,24).

ACKNOWLEDGMENTS

This work was supported by Swiss National Science Foundation Grants 3200–064078.01 to C.A.M. and 32–68286.02 to J.-M.D., as well as a grant from the Hans Wilsdorf Foundation to D.B. and C.A.M.

We are grateful to Philippe Cettour-Rose for his expertise in performing the animal experiments, as well as to Dr. Pascale Roux-Lombard and the technician of the Laboratory of Clinical Immunology and Allergology, Immunology Division of our hospital for cytokine determination in human serum. We also gratefully acknowledge Drs. Brigitte Pittet and Navid Alizadeh for providing some of the human samples of adipose tissue and Roswitha Rem for her critical reading and corrections of the manuscript.

REFERENCES

1. Seckinger P, Lowenthal JW, Williamson K, Dayer JM, MacDonald HW: A urine inhibitor of interleukin 1 activity that blocks ligand binding. *J Immunol* 139:1546–1549, 1987
2. Burger D, Dayer JM: IL-1Ra. In *Cytokine Reference*. Oppenheim JJ, Feldmann M, Eds. New York, London, Academic Press, 2000, p. 319–336
3. Arend WP, Guthridge CJ: Biological role of interleukin 1 receptor antagonist isoforms. *Ann Rheum Dis* 59 (Suppl. 1):i60–i64, 2000
4. Dayer JM, Feige U, Edwards CK III, Burger D: Anti-interleukin-1 therapy in rheumatic diseases. *Curr Opin Rheumatol* 13:170–176, 2001
5. Dayer JM, Bresnihan B: Targeting interleukin-1 in the treatment of rheumatoid arthritis. *Arthritis Rheum* 46:574–578, 2002
6. Horai R, Saijo S, Tanioka M, Nakae S, Sudo K, Okahara A, Ikuse T, Asano M, Iwakura Y: Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J Exp Med* 191:313–320, 2000
7. Nicklin MJH, Hughes DE, Barton JL, Ure JM, Duff GW: Arterial inflammation in mice lacking the interleukin 1 receptor antagonist gene. *J Exp Med* 191:303–311, 2000
8. Hirsch E, Irikura VM, Paul SM, Hirsh D: Functions of interleukin 1 receptor antagonist in gene knockout and overproducing mice. *Proc Natl Acad Sci U S A* 93:11008–11013, 1996
9. Gabay C, Dreyer MG, Pellegrinelli N, Chicheportiche R, Meier CA: Leptin directly induces the secretion of interleukin 1 receptor antagonist in human monocytes. *J Clin Endocrinol Metab* 86:783–791, 2001
10. Jungo F, Dayer JM, Modoux C, Hyka N, Burger D: IFN-beta inhibits the ability of T lymphocytes to induce TNF-alpha and IL-1beta production in monocytes upon direct cell-cell contact. *Cytokine* 14:272–282, 2001
11. Vey E, Dayer JM, Burger D: Direct contact with stimulated T cells induces

the expression of IL-1beta and IL-1 receptor antagonist in human monocytes: involvement of serine/threonine phosphatases in differential regulation. *Cytokine* 9:480–487, 1997
12. Meier CA, Bobbioni E, Gabay C, Assimacopoulos-Jeannet F, Golay A, Dayer JM: IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin? *J Clin Endocrinol Metab* 87:1184–1188, 2002
13. Hauner H, Skurk T, Wabitsch M: Cultures of adipose tissue and precursor cells. In *Adipose Tissue Protocols*. Ailhaud G, Ed. Totowa, NJ, Humana Press, 2001, p. 197–212
14. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259:87–91, 1993
15. Meier CA, Chicheportiche R, Juge-Aubry CE, Dreyer MG, Dayer JM: Regulation of the interleukin-1 receptor antagonist in THP-1 cells by ligands of the peroxisome proliferator-activated receptor γ. *Cytokine* 18:320–328, 2002
16. Mohamed-Ali V, Pinkney JH, Coppack SW: Adipose tissue as an endocrine and paracrine organ. *Int J Obes Relat Metab Disord* 22:1145–1158, 1998
17. Kahn BB, Flier JS: Obesity and insulin resistance. *J Clin Invest* 106:473–481, 2000
18. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V: Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 148:209–214, 2000
19. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor α: a key component of the obesity-diabetes link. *Diabetes* 43:1271–1278, 1994
20. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, Yamashita S, Miura M, Fukuda Y, Takemura K, Tokunaga K, Matsuzawa Y: Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med* 2:800–803, 1996
21. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppack SW: Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab* 82:4196–4200, 1997
22. Kunzi MS, Rowe PP: IFNα, IFNβ, and IFNω ligands. In *Cytokine Reference*. Oppenheim JJ, Feldmann M, Eds. New York, London, Academic Press, 2000, p. 627–638
23. Francis SE, Camp NJ, Dewberry RM, Gunn J, Syrris P, Carter ND, Jeffery S, Kaski JC, Cumberland DC, Duff GW, Crossman DC: Interleukin-1 receptor antagonist gene polymorphism and coronary artery disease. *Circulation* 99:861–866, 1999
24. Luheshi GN, Gardner JD, Rushforth DA, Loudon AS, Rothwell NJ: Leptin actions on food intake and body temperature are mediated by IL-1. *Proc Natl Acad Sci U S A* 96:7047–7052, 1999
25. Beutler BA, Cerami A: Recombinant interleukin 1 suppresses lipoprotein lipase activity in 3T3–L1 cells. *J Immunol* 135:3969–3971, 1985
26. Hardardottir I, Doerrler W, Feingold KR, Grunfeld C: Cytokines stimulate lipolysis and decrease lipoprotein lipase activity in cultured fat cells by a prostaglandin independent mechanism. *Biochem Biophys Res Commun* 186:237–243, 1992
27. Fried SK, Appel B, Zechner R: Interleukin 1α decreases the synthesis and activity of lipoprotein lipase in human adipose tissue. *Horm Metab Res* 25:129–130, 1993
28. Garcia-Welsh A, Schneiderman JS, Baly DL: Interleukin-1 stimulates glucose transport in rat adipose cells: evidence for receptor discrimination between IL-1 beta and IL-1 alpha. *FEBS Lett* 269:421–424, 1990
29. Delikat SE, Galvani DW, Zuzel M: The metabolic effects of interleukin 1 beta on human bone marrow adipocytes. *Cytokine* 7:338–343, 1995
30. Delikat S, Harris RJ, Galvani DW: IL-1 beta inhibits adipocyte formation in human long-term bone marrow culture. *Exp Hematol* 21:31–37, 1993