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Leptin Signaling Deficiency Impairs Humoral and Cellular Immune Responses and Attenuates Experimental Arthritis

Nathalie Busso,* Alexander So,* Véronique Chobaz-Péclat,* Carole Morard,* Eduardo Martínez-Soria,† Dominique Talabot-Ayer,§ and Cem Gabay2§

Leptin is produced almost exclusively by adipocytes and regulates body weight at the hypothalamic level. In addition, recent studies showed that leptin plays an important role in T lymphocyte responses. To examine the role of leptin in Ag-induced arthritis, the development of joint inflammation was assessed in immunized leptin-deficient mice (ob/ob), +/+ , and wild-type mice (+/+ ) following the administration of methylated BSA into the knees. The results showed that ob/ob mice developed less severe arthritis compared with control mice. The levels of IL-1β and TNF-α mRNA in the synovium of arthritic knees were lower in ob/ob than in +/+ mice. In vitro Ag-specific T cell proliferative responses were significantly decreased in ob/ob mice with lower IFN-γ and higher IL-10 production, suggesting a shift toward a Th2-type response in ob/ob mice. The serum levels of anti-methylated BSA Abs of any isotype were significantly decreased in arthritic ob/ob mice compared with controls. Essentially identical results were obtained in db/db mice, which lack the expression of the long isoform of leptin receptor. By RT-PCR, we observed that B lymphocytes express leptin receptor mRNA, indicating that in addition to its effect on the cellular response, leptin may exert a direct effect on B cell function. In conclusion, leptin contributes to the mechanisms of joint inflammation in Ag-induced arthritis by regulating both humoral and cell-mediated immune responses. The Journal of Immunology, 2002, 168: 875–882.

Leptin, the product of the ob gene, is a 16-kDa nonglycosylated peptide hormone synthesized almost exclusively by adipocytes that regulates appetite and energy expenditure at the hypothalamic level (1). However, it has become increasingly apparent that leptin has other direct effects on nonneuronal cells (2, 3). Recently, the effect of leptin on the immune system has received particular attention (reviewed in Refs. 4 and 5). Both CD4+ and CD8+ T cells express the long isoform of leptin receptor (Ob-Rb)3 (2), which is considered of prime importance for leptin signaling (6). In vitro, leptin stimulates the proliferative response of CD4+ T cells in the context of mixed lymphocyte reaction and enhances the effect of lectins on the expression of cell surface activation markers. Leptin-deficient (ob/ob) mice exhibit defective cell-mediated immunity and a shift toward Th2 cytokine production by stimulated CD4+ T cells (7). In addition, these mice have a marked reduction of size and cellularity of the thymus due to increased thymocyte apoptosis (8).

The role of leptin in vivo has been examined in different experimental models using either ob/ob mice or mice with a deletion in the Ob-Rb (db/db mice). The results of these studies demonstrated that leptin possesses both anti- and pro-inflammatory effects according to the disease model. Leptin deficiency enhanced the sensitivity to LPS- and TNF-α-induced mortality, with a protective effect of exogenously administered recombinant leptin in ob/ob mice, suggesting that leptin plays an important role in modulating the host response to inflammation (9, 10). In contrast, ob/ob and db/db mice were protected from T cell-mediated hepatotoxicity induced by the injection of Con A, and replacement of leptin was able to restore the responsiveness of ob/ob mice to Con A (11).

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease characterized by synovial hyperplasia, inflammatory cell recruitment, and, in its later stages, cartilage and bone destruction. Several studies have demonstrated that the Th1/Th2 balance plays an important role in RA, with Th1 and Th2 cytokines exerting pro- and anti-inflammatory effects, respectively (12). Fasting patients with RA exhibit an improvement in different clinical and biological parameters of disease activity associated with a marked decrease in serum levels of leptin and a shift toward Th2 cytokine production (13). These features closely resemble those observed in ob/ob mice, suggesting that leptin may play a role in the inflammatory mechanisms of arthritis through the induction of Th1 responses.

The potential role of leptin in RA needs to be assessed in vivo. Using a murine model (Ag-induced arthritis (AIA)) that is T and B cell dependent and that recapitulates some of the histologic and functional alterations of RA, we have explored the phenotype of leptin-deficient mice. Moreover, mice deficient for the long isoform of the leptin receptor (db/db mice) provided an opportunity to evaluate the role of leptin signaling during AIA.

Materials and Methods

Mice

Leptin-deficient mice (ob/ob), control (+/+) littermates, and wild-type (+/+) mice, all in a C57BL/6 background, were purchased from Janvier (Le Genest St. Isle, France). Leptin receptor-deficient mice (db/db) and +/+ littermates in C57BL/Ks were purchased from Harlan Nederland (Horst, The Netherlands).

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Induction of arthritis

AAIA was established as previously described (14). Briefly, mice were immunized on days 0 and 7 with 100 μg methylated BSA (mBSA; Sigma, Buchs, Switzerland) emulsified in 0.1 ml CFA containing 200 μg mycobacterial strain H37RA (Difco, Basel, Switzerland) by intradermal injection at the base of the tail. On the same days 0.2 × 10^7 heat-killed Bordetella pertussis organisms (Berna, Bern, Switzerland) were injected i.p. as an additional adjuvant. Arthritis was induced on day 21 by intra-articular injection of 100 μg mBSA in 10 μl sterile PBS into the right knee; the left knee was injected with sterile PBS alone. Institutional approval was obtained for these experiments.

Isotopic quantification of joint inflammation

Joint inflammation was measured by 99mTc-technetium (99mTc) uptake in the knee joint, as previously described (14). Briefly, mice were first anesthetized by methoxyflurane and then injected s.c. in the neck region with 10 μCi 99mTc. The accumulation of the isotope in the knee was determined by external gamma counting after 15 min. The ratio of 99mTc uptake in the inflamed articular knee vs 99mTc uptake in the contralateral control knee was calculated. A ratio of >1.1 indicated joint inflammation.

Histological grading of arthritis

At least six mice per group were sacrificed, and the knees were dissected and fixed in 10% buffered formalin for 7 days. Fixed tissues were decalcified for 3 wk in 15% EDTA, dehydrated, and embedded in paraffin. Sagittal sections (5 μm) of the whole knee joint were stained with Safranin-O and counterstained with Fast Green/iron hematoxylin. Histological sections were graded independently by two observers who were unaware of the animal genotype using the following parameters. Synovial membrane thickness, which reflects the degree of synovial inflammation and hyperplasia, was scored on a scale of 0 to 6 (0 = normal thickness; 6 = maximum thickness). Cartilage proteoglycan depletion, reflected by loss of Safranin-O staining intensity, was scored on a scale of 0 (fully stained cartilage) to 6 (totally unstained cartilage) in proportion to severity. For each histopathologic parameter, the score (mean ± SEM) of all slides was calculated.

Synovial IL-1β and TNF-α mRNA levels

The right knee joints of arthritic ob/ob and +/+ mice (day 8 of arthritis) were dissected, and the synovial membranes were prepared and snap-frozen. Total RNA was extracted with TRIzol (Life Technologies, Paisley, U.K.). RNA was quantitated using OD260 determination, and the integrity of RNA was verified by an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). Total mRNA was extracted with TRIzol from the RNA samples and reverse transcribed to cDNA using SuperScript II (Invitrogen, Carlsbad, CA). Real-time quantitative PCR was performed with the SYBR Green PCR Core kit (Applied Biosystems, Foster City, CA). The primers for TNF-α and GAPDH mRNA were created as recently described (15). The riboprobe for TNF-α mRNA was created using T7 RNA polymerase and 32P-labeled CTP. RNase protection assays were performed as recently described (16), and quantification of cytokine and GAPDH mRNA fragments was performed by PhosphorImager (ImageQuant, Molecular Dynamics, Sunnyvale, CA).

T cell proliferation assay

Mice were sacrificed according to the experimental protocol. Inguinal lymph nodes were removed, and single-cell suspensions were incubated in RPMI supplemented with β-ME, penicillin, streptomycin, and 1% autologous serum. Lymph node cells (LN) (4 × 10^5/200 μl/well) were plated in 96-well flat-bottom plates and stimulated with 50 μg/ml mBSA (Sigma). The cells were incubated at 37°C in 5% CO2 for 48 h, then 1 μCi/well 3H-thymidine was added to the cultures for 18 h. The cells were harvested, and [3H]thymidine uptake was measured using a beta scintillation counter.

Determination of cytokine production in vitro

LN were isolated and cultured with or without 50 μg/ml mBSA. The culture supernatants were harvested after 72 h for determination of IFN-γ, IL-4, and IL-10 levels. Quantification of cytokine production was performed by ELISA kits specific for murine IFN-γ (Amersham Pharmacia, Buchendon, Switzerland), IL-4, and IL-10 (Quantikine, R&D Systems, Abingdon, U.K.).

Measurement of serum levels of anti-mBSA Abs

For determination of anti-mBSA IgG, 96-wells plates (Maxisorp-Nunc, Life Technologies, Basel, Switzerland) were coated overnight at 4°C with 1% BSA in PBS. After four washings with TTBS (50 mM Tris (pH 7.4) and 140 mM NaCl containing 0.05% Tween 20), 100 μl serum, serially diluted in 1% gelatin/PBS (final dilutions, 1/100, 1/200, and 1/400), were incubated for 2 h at room temperature. Wells were washed for 5 min. Then, 100 μl/well alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma) diluted 1/500 in TTBS was added for 30 min. After four washings with TTBS, color was developed with 100 μl/well p-nitrophenylphosphate (Sigma), and the reaction was stopped by adding 25 μl/well 3 M NaOH. Plate reading was performed at 405 nm, and results were calculated according to a standard curve with a reference serum. For determination of murine IgG1-specific IgG, 50 μl/well of sera, diluted 1/200 in 1% gelatin/PBS was incubated for 2 h at room temperature. Alkaline phosphatase-conjugated secondary Ab (100 μl/well; BD Pharmingen) was added, and color was developed as described above. Plate reading was performed at 405 nm, and results were calculated according to a standard curve constructed with purified murine IgG1, IgG2a, IgG2b, or IgM or with a reference mouse serum for IgG3 and IgA.

Determination of serum levels of total Ig isotypes

Ninety-six-well plates (Maxisorp-Nunc) were coated overnight at 4°C with 50 μl of one of each isotype-specific rat anti-mouse Ig (as described above) diluted 1/5 in PBS. After four washings in TTBS, each well was incubated with 200 μl blocking solution (1% BSA in PBS) for 30 min at room temperature. After four washings, 100 μl of the appropriate dilution of serum to be tested (1/2,000 for IgG3 and IgA, 1/10,000 for IgG1 and IgM, 1/50,000 for IgG2a, and 1/100,000 for IgG2b) were incubated for 2 h at room temperature. Then, alkaline phosphatase-labeled rat anti-mouse Ig mAb was added, and wells were processed as described above. Results were calculated according to a standard curve constructed with purified murine IgG1, IgG2a, IgG2b, or IgM or with a reference serum for IgG3 and IgA.

B lymphocyte purification and Ob-Rb identification by RT-PCR

Spleens from C57BL/6 mice were removed and disrupted. Erythrocytes from spleens were lysed, and the remaining cells were washed three times and counted. Spleen cells (5 × 10^7) were incubated for 30 min on ice with PE-conjugated B-220 (RA3-6B2) Ab (BD Pharmingen) in a final volume of 1 ml. After three washes, spleen cells were resuspended in serum-free DMEM at a concentration of 10^7 cells/ml. Sorting was performed on a FACSAirCell sorter (Becton Dickinson, San Jose, CA). Sorted B220-positive cells as well as B220-stained but unsorted spleen cells were stained with either FITC-conjugated anti-Mac-1 (M1/70) or anti-Thy-1.2 (TIB107) Abs, which specifically recognize monocytes and T lymphocytes, respectively. Double-stained cells were then analyzed using a FACSCalibur flow cytometer (BD Biosciences).

Total RNA from purified spleen B lymphocytes was extracted using TRIzol (Life Technologies). One microgram of total RNA was treated with Ampgrade DNase I (Life Technologies) and reverse transcribed, and PCR was performed for 40 cycles at 94°C for 1 s, 57°C for 30 s, and 72°C for 30 s, using specific primers for Ob-Rb (long isoform): 5'–ATT ATT TCC TCT TGT GTC CTA and 3’–GTC TGA TAA AAG GAA AAA TGT (17). The integrity of the cDNA was determined by assessing the relative levels of murine GAPDH as previously described (18). Total spleen RNA was used as a control. The amplified products were electrophoresed within a 1% agarose gel. The amplified products of murine Ob-Rb and GAPDH were 587 and 211 bp in size, respectively. The purity of B cell preparation was confirmed by the absence of markers of T cells (IL-18R mRNA) or monocytes/macrophages (Fcε receptor chain mRNA) as assessed by RT-PCR.

Statistical analysis

The Wilcoxon/Kruskal-Wallis (rank-sum test) for unpaired variables was used to compare differences between groups with non-Gaussian distribution. The unpaired Student t test was used to compare the groups with normally distributed values. A level of p < 0.05 was considered statistically significant.
Results

Effect of leptin deficiency on the severity of arthritis

To explore whether leptin deficiency had an effect on the course of AIA, we measured the levels of knee joint inflammation according to the ratio of $^{99m}$Tc uptake in the arthritic joint over that in the noninflamed knee joint. For each time point the mean ± SEM ratio is shown. C57BL/6 mice (+/+) on days 1, 3, and 7 ($n = 14, 13$, and 8, respectively), lean littermates (+/?; $n = 8, 8$, and 5, respectively), and ob/ob mice ($n = 18, 14$, and 10, respectively) were studied. Joint inflammation was significantly reduced on days 1 and 3 in ob/ob mice vs +/+ and +/+ mice.

Decreased synovial levels of IL-1β and TNF-α in leptin-deficient (ob/ob) mice

Several studies in experimental models of arthritis suggested that TNF-α and IL-1 exert different effects. TNF-α plays an important...
role in the induction of synovial inflammation, whereas IL-1 could be predominantly involved in the mechanisms leading to cartilage destruction (19). Thus, we examined whether a difference in IL-1β and TNF-α levels can contribute to the discrepancy between synovial thickness and cartilage damage in ob/ob mice. As shown in Fig. 4, synovial levels of IL-1β and TNF-α mRNA were significantly decreased in the arthritic knees of ob/ob compared with +/+ mice on day 8 of arthritis. Results in wild-type C57BL/6 mice were not different from those in +/+ mice (data not shown).

Effect of leptin deficiency on humoral and cellular responses
The humoral response was examined by measuring the serum levels of total IgG and of specific anti-mBSA Abs by ELISA in naive mice and in immunized mice on day 8 after the onset of arthritis.

Anti-mBSA Abs were undetectable in nonimmunized mice (data not shown). In arthritic mice, the circulating levels of anti-mBSA Abs were significantly lower in ob/ob mice than in +/+ and ob/ob mice (p < 0.01; Fig. 5). The serum levels of IgG isotypes, IgM, and IgA were not significantly different in naive ob/ob compared with +/+ mice (Fig. 6A). In contrast, the levels of anti-mBSA IgG1, IgG2a, IgG2b, IgG3, IgM, and IgA were significantly lower in ob/ob than in control mice (Fig. 6B).

The role of leptin in cell-mediated immune responses was examined by isolating inguinal LNC from mice with AIA. The T cell proliferation rate induced by mBSA, as assessed by [3H]thymidine uptake, was significantly lower in cells isolated from ob/ob mice than in cells from +/+ and +/+ mice, respectively (Fig. 7). We also measured the levels of IFN-γ and IL-10 in LNC supernatants, as typical Th1 and Th2 cytokines, respectively. The mBSA-induced production of IFN-γ was significantly lower in ob/ob mice than in controls, whereas opposite results were observed for IL-10 (Fig. 8). The levels of IL-4 were not detectable by ELISA in any of the tested conditions. This finding was not unexpected, since other investigators observed that IL-4 production was very weak using the same experimental system (20).

The role of the long isoform of leptin receptor, Ob-Rb, in AIA
In db/db mice a point mutation in the Ob-Rb gene creates a new splice donor site that inserts a premature stop into the Ob-Rb 3’ end, resulting in the replacement of the long Ob-Rb by a short intracytoplasmic tail of Ob-Ra (21). This mutation results in the inability to respond appropriately to leptin and the presence of an obese phenotype. Arthritis was induced in mice deficient in Ob-Rb (db/db) and their lean littermates (+/+) as described above. The results recapitulate the arthritic phenotype of ob/ob mice. The severity of articular inflammation as assessed by 99mTc uptake was lower in db/db than in +/+ mice on days 1, 3, and 7 of AIA, although only the decrease on day 1 was statistically significant (Fig. 9A). The levels of Abs against mBSA measured after 8 days of arthritis were lower in db/db than in +/+ mice (Fig. 9B). The proliferation rate of LNC in response to mBSA in culture was also significantly lower in db/db than in control mice (Fig. 9C). In addition, production of IFN-γ in culture was lower in db/db mice than in lean +/+ littermates, whereas opposite results were observed for IL-10 (Fig. 9, D and E, respectively).

Ob-Rb mRNA is expressed by B lymphocytes
Our results showed that circulating levels of anti-mBSA Abs of any isotype were strongly reduced in arthritic ob/ob and db/db mice as opposed to arthritic controls. We hypothesized that leptin

FIGURE 3. Histological grading of arthritic knee joints. Mice were sacrificed on day 4 of AIA. Synovial thickness and cartilage damage were scored histologically using an arbitrary scale from 0 to 6. Results are expressed as the mean ± SEM (at least six mice per group were examined). Induction of arthritis led to a significantly decreased synovial thickness in ob/ob as compared with control mice (p < 0.04). In contrast, the severity of cartilage damage was similar in these mice.

FIGURE 4. Quantification of IL-1β and TNF-α mRNA levels in the inflamed synovium of mice with AIA. Dissected synovial membranes were obtained from the right (arthritic) knee of ob/ob and +/+ mice on day 8 of AIA. Total RNA was prepared, and levels of IL-1β, TNF-α, and GAPDH mRNA were determined by RNase protection assay (see Materials and Methods). The gels were dried, and autoradiography was immediately performed (A). Qualitative analysis was performed by PhosphorImager (B). The results are expressed as IL-1β/GAPDH and TNF-α/GAPDH mRNA ratios. The values represent the mean ± SEM. n is the number of mice per group. * p < 0.01 compared with ob/ob mice.

FIGURE 5. Determination of anti-mBSA Abs levels in mouse sera. Sera were collected on day 8 of AIA. Anti-mBSA Abs were measured by specific ELISA, and the results are expressed as a percentage of the concentration in +/+ sera. ob/ob mice had significantly lower levels of Abs against mBSA than +/+ mice. (+/+, n = 8; ob/ob, n = 14; p < 0.003).
The results of several studies indicate that leptin plays an important role in the regulation of immune responses. Leptin levels are regulated by nutritional status and fall rapidly during starvation. Thymic and lymphoid atrophy are well-recognized consequences of food deprivation in humans and animals (22–24). A reduced delayed-type hypersensitivity reaction to Ags observed during fasting can be restored with leptin replacement (25). Consistent with these findings, mice deficient in leptin exhibit evidence of defective cell-mediated immunity and lymphoid atrophy, analogous to those observed in chronic undernutrition in humans (7). Ob/ob mice have a marked reduction in the size and cellularity of thymus with a high level of thymocyte apoptosis, which can be rescued by the exogenous administration of leptin (8). Leptin stimulates the proliferation of CD4+ T cells, with a stronger effect on naive CD4+CD45RA+ than on memory CD4+CD45RA– T cells, thus playing an important role in primary T cell immune responses (7). In addition, leptin has been shown to promote Th1 responses (7). Consistent with these studies we observed that T cell proliferation and production of IFN-γ by isolated LNC were significantly decreased in ob/ob mice.

Circulating levels of anti-mBSA IgG, IgM, and IgA were significantly lower in arthritic ob/ob than in control mice. This impaired humoral response can be secondary to a direct effect of leptin signaling on B cells and/or to an indirect effect of leptin on CD4+ T cell function. By RT-PCR we showed that Ob-Rb mRNA is expressed by isolated murine B cells, suggesting that leptin can act directly on B cells to regulate the production of Ig. A direct effect of leptin on B cells is also supported by previous studies showing that the number of peripheral and bone marrow B cells is decreased in db/db mice (26). Cognate interactions between B cells and Ag-specific CD4+ T cells play an important role in the activation and maturation of the humoral response. Thus, the presence of impaired T cell responses is also likely to contribute to the decreased Ig production in ob/ob and db/db mice. In addition to these direct cell-cell interactions between B and T lymphocytes, the Ig production is modulated by CD4+ T cell-derived lymphokines. Indeed, Th1 cytokines enhance the production of IgG2a and IgG3, whereas Th2 cytokines promote the synthesis of IgG1 (27–29). However, the presence of decreased anti-mBSA IgG levels in ob/ob mice cannot be solely explained by a switch toward Th2
responses, as the levels of all IgG subtypes were lower in leptin-deficient mice.

The presence of hyperglycemia and high cortisol levels in ob/ob animals can also decrease the immune responses. However, previous experiments with food restriction showed that reduced plasma levels of cortisol and glucose did not reverse the immune defects in ob/ob mice, whereas leptin replacement restored a normal immune response in ob/ob mice (7, 8). In addition, humans with congenital leptin deficiencies secondary to mutations in the ob gene exhibit low total T cell counts, impaired cell-mediated immune responses, and an increased rate of infections in the absence of hypercortisolemia and hyperglycemia (30, 31). Taken together, these findings in rodents and humans suggest that leptin deficiency is directly responsible for the immune abnormalities in ob/ob mice.

The experimental model of arthritis used in this study required prior immunization with mBSA. As leptin-deficient and leptin receptor-deficient mice have impaired immune responses, we were not able to analyze separately the effect of leptin signaling on the inflammatory response. This question is of interest, since the results of some in vitro and in vivo studies suggested that leptin may exert anti-inflammatory actions (9, 10, 32). Studies are currently in progress in our laboratory using a model in which active immunization is not required for the development of arthritis.

FIGURE 8. IFN-γ and IL-10 production by LNC in culture. IFN-γ and IL-10 production by LNC were measured by specific ELISAs. The results represent the fold increase in mBSA-stimulated cells over unstimulated cells. In ob/ob mice the fold increase in IFN-γ expression was significantly lower compared with that in +/+ mice (p < 0.04). Conversely, the fold increase in IL-10 expression was significantly higher in ob/ob than in +/+ and +/+ mice (p < 0.02 and p < 0.01, respectively). +/+, n = 12; +/?, n = 9; ob/ob, n = 3.

FIGURE 9. Phenotype of leptin receptor-deficient mice during AIA. A, Joint inflammation was measured by external gamma counting of 99mTc uptake on days 1, 3, and 7 after Ag challenge in the right knee in +/+ and db/db mice (days 1 and 3, 16–18 mice/group; day 7, 7–9 mice/group). Results are expressed as the ratio of 99mTc uptake in the right (R) arthritic knee joint over that in the left (L) noninflamed knee joint. Mean ± SEM ratios are shown for each time point. Joint inflammation was significantly reduced on day 1 in db/db mice vs the control group (p < 0.05). B, Circulating levels of anti-mBSA Abs were determined by specific ELISA, and results were expressed as a percentage of the concentration obtained in +/+ sera. Levels of IgG against mBSA were significantly lower in db/db than in +/+ mice (+/+ mice, n = 17; db/db mice, n = 16; p < 0.04). C, LNC proliferation in culture was assessed by [3H]thymidine incorporation. The cell proliferation rate induced by mBSA was lower in db/db compared with +/+ (p < 0.0001; +/+, n = 18; db/db, n = 9). The results represent the fold increase in mBSA-stimulated over unstimulated cells. D and E, Determination of IFN-γ and IL-10 production by isolated LNC in response to mBSA was performed by ELISA. In db/db mice the fold increase in IFN-γ production was lower than that in +/+ mice (p < 0.04). Conversely, the fold increase in IL-10 production was higher in db/db than in +/+ mice (p < 0.002). +/+, n = 9; db/db, n = 3.
The results of histology studies showed that ob/ob mice had a lower score for synovial thickness, indicating the presence of decreased synovial inflammation compared with control mice. In contrast, there was no statistical difference between ob/ob and control mice regarding cartilage degradation. This uncoupling between synovial inflammation and cartilage destruction can be related to the relative contribution of cytokines such as TNF-α and IL-1β (19). However, the results of RNase protection assays showed that the mRNA levels of both TNF-α and IL-1β were decreased in ob/ob mice, indicating that our results cannot be explained by a predominant contribution of one of these cytokines. Although other cytokines can also be involved, it is likely that the discrepancy between synovial inflammation and tissue damage may be due to a lack of sensitivity of Safranin-O staining to detect subtle changes in cartilage degradation.

Several different isoforms of leptin receptor (Ob-Ra to Ob-Rf) have been cloned. They are produced from the same gene by mRNA splicing and contain a common extracellular domain (29). Ob-Rb has a long cytoplasmic tail and is considered of prime importance for leptin signaling. Ob-Ra has a shorter cytoplasmic domain and is the predominant Ob-R isoform found in most tissues and cells (33). Using transfection experiments, Ob-Ra was able to transduce intracellular signals upon binding with its ligand (34). However, no definitive demonstration of the signaling role of this short Ob-R isoform has been reported to date. The milder form of AIA in mice deficient in Ob-Rb is in accordance with the presence of defective T cell immune responses in db/db mice (35) and reproduces the results in ob/ob mice, thus indicating that the effect of leptin in AIA is mediated through its interaction with Ob-Rb. LNC isolated from ob/ob and db/db mice produced high levels of IL-10 in the presence of mBSA. IL-10 is a Th2 cytokine that has been demonstrated to possess anti-inflammatory properties (36). Similar findings, including milder AIA, decreased LNC proliferation, and enhanced IL-10 production in vitro, were observed in IL-6 knockout mice (20), suggesting that IL-6 and leptin may have common effects. Indeed, leptin augments IL-1-induced corticosterone and IL-6 production, two effects typically observed after administration of IL-6-related cytokines (37, 38). In addition, both leptin and IL-6 are able to stimulate the production of IL-1Ra by monocytes in culture (32, 39). Finally, leptin and its receptor have structural similarities with IL-6 family members. Ob-Rb is related to class I cytokine receptors, which include gp-130, the common signal-transducing component for the IL-6-related receptor family (33). Ob-Rb has signaling activities similar to those of IL-6-type cytokine receptors, including activation of the STAT3 and ERK pathways (40, 41). Taken together, these structural and functional homologies may explain the presence of common findings in mice deficient in IL-6 and in ob/ob and db/db mice.

In conclusion, our results in leptin-deficient and leptin receptor-deficient mice indicate that leptin plays an important role in the regulation of cellular and humoral responses. Future studies should be performed to determine whether therapies interacting with leptin signaling would be of value in the treatment of patients with arthritis and other immune-mediated inflammatory disorders.

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References


