Requirement for Leptin in the Induction and Progression of Autoimmune Encephalomyelitis


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Requirement for Leptin in the Induction and Progression of Autoimmune Encephalomyelitis


Recent evidence indicates that leptin modifies T cell immunity, and may provide a key link between nutritional deficiency and immune dysfunction. To study the influence of leptin on autoimmunity, susceptibility to experimental autoimmune encephalomyelitis induced by immunization with a myelin-derived peptide was examined in leptin-deficient, C57BL/6J- ob/ob mice, with or without leptin replacement, and in wild-type controls. Leptin replacement converted disease resistance to susceptibility in the C57BL/6J- ob/ob mice; this was accompanied by a switch from a Th2 to Th1 pattern of cytokine release and consequent reversal of Ig subclass production. Our findings suggest that leptin is required for the induction and maintenance of an effective proinflammatory immune response in the CNS. The Journal of Immunology, 2001, 166: 5909–5916.

The obese gene product, leptin, is a hormone belonging to the helical cytokine family derived primarily from adipocytes, described as a central mediator of the neuroendocrine pathways involved in the control of food intake, basal metabolism, and reproductive function (1, 2). Recently, leptin has also been shown to modulate the cognate T cell-mediated immune response by signaling through the long isoform of the leptin receptor (ObRb) expressed on the cell surface of mature CD4+ T lymphocytes (3). Specifically, leptin reverses the immunodeficiency and lymphoid organ atrophy, induced by acute starvation in rodents (3, 4). Leptin-deficient obese mice (C57BL/6J- ob/ob) display immune dysfunction similar to that observed in starved animals and malnourished humans, both conditions associated with low circulating leptin concentrations (4–6). C57BL/6J- ob/ob mice exhibit impaired cell-mediated immunity and thymic atrophy (3, 4, 7). Furthermore, chronic leptin deficiency in these animals leads to reduced in vitro secretion, upon Ag stimulation, of the classical Th1-type proinflammatory cytokines such as IL-2 and IFN-γ and an increased production of IL-4, typical of the Th2 regulatory phenotype (3).

Experimental autoimmune encephalomyelitis (EAE) is an animal model of human multiple sclerosis, which can be induced in susceptible strains of mice by immunization with self Ags, derived from CNS myelin (8). The disease is characterized by the generation of autoreactive T cells that traffic to the brain and the spinal cord and initiate injury to CNS myelin, resulting in a chronic or relapsing-remitting paralysis (9). Direct evidence for the role of CD4+ T cells in EAE induction has come from adoptive transfer studies, in which myelin Ag-reactive Th1 CD4+ cell lines or clones induce encephalomyelitis and demyelination leading to paralysis following transfer (10). Th1 cytokines are present in inflammatory EAE lesions in the CNS, whereas Th2 cytokines are absent, suggesting that Th1 cytokines play a role in the pathogenesis of the disease (8, 11). Recovery from EAE in mice is associated with an increase in the presence of Th2 cytokines in the CNS (12). Furthermore, IL-4 administration during EAE reduces both the intensity and the progression of the disease (13).

To determine the contribution of leptin to the pathogenesis of the multiple sclerosis-like disease provoked by the immunodominant myelin oligodendrocyte glycoprotein (MOG) peptide, MOG35–55 (14), we tested disease susceptibility in naturally leptin-deficient C57BL/6J- ob/ob mice before and after chronic leptin administration, comparing it with wild-type controls under the same experimental conditions. Both leptin-deficient and control mice are on the C57BL/6J-susceptible genetic background (H-2b) for MOG35–55 peptide-induced EAE (14). We report in this work that, upon immunization with MOG35–55 peptide or after adoptive transfer of pathogenic MOG35–55 specific CD4+ Th1 cells, mice lacking circulating leptin do not develop any neurological impairment. Conversely, only after chronic leptin administration C57BL/6J- ob/ob mice become susceptible to the antigenic peptide or to adoptive transfer of pathogenic T cells. In wild-type controls, leptin supplementation causes a more severe and chronic disease than in the untreated group. These findings show for the first time that EAE may not be induced in the absence of leptin, and that chronic leptin administration enables leptin-deficient mice to develop EAE actively induced by
MOG<sub>35–55</sub> peptide or adoptively transferred by MOG<sub>35–55</sub>-specific T cells.

Materials and Methods

**Mice**

Female C57BL/6J wild-type and C57BL/6J-ob/ob leptin-deficient obese mice 8–10 wk old were obtained from Charles River Italy (Calco, Italy) and from Harlan Italy (Corezzana, Italy). Experiments were performed under an approved protocol in accordance with the animal use guidelines of the Istituto Superiore di Sanita (Rome, Italy). Wild-type and leptin-deficient mice were age matched for individual experiments and were group-housed two to six mice per standard cage according to the different experimental protocol, with a 12-h light/dark cycle. Paralyzed mice were afforded easier access to food and water to prevent dehydration.

**Antigens**

The peptide used in this study is the immunodominant MOG<sub>35–55</sub> peptide (MEVGWYRSPFSRVHLYRNGK) (14). It was synthesized by PRIMM s. r. l. (Milan, Italy); purity was assessed by HPLC (>97% pure), and amino acid composition was verified by mass spectrometry. MOG<sub>35–55</sub> peptide batches for in vivo and in vitro assays were all from one preparation, initially solubilized in LPS-free saline solution at 4 mg/ml concentration, and stored at −80°C.

**Leptin administration**

Mouse recombinant leptin (releptin) was purchased from R&D Systems Europe (Oxon, U.K.); purity was >97%, as determined by SDS-PAGE and visualized by silver staining analysis. The endotoxin level was <0.1 ng/µg of leptin, as determined by the Limulus amebocyte lysate method. Mice comprised three groups (n = 6–11 per group) for C57BL/6J-ob/ob leptin-deficient obese mice (all housed in pairs) and two groups (n = 6–10 per group) for C57BL/6J normal age- and sex-matched control mice (housed two to six mice/cage). For the active disease induction, mice were injected i.p. with releptin or PBS starting 10 days before the immunization and continuing over a period of 40 days; for adoptively induced disease, mice were treated starting 3 days before the transfer of T cells and continuing over a period of 30 days. Of the groups of leptin-deficient mice, one was injected with 200 µl of PBS twice daily (at 0:00 a.m. and 6:00 p.m.) and allowed to feed ad libitum; the second group was injected with murine releptin (0.5 µg/g initial body weight twice daily in 200 µl volume i.p., for a total of 1 µg/g/day of releptin); and the third group was pair fed to the food intake of the releptin-treated mice and received twice daily injections of PBS according to the same schedule (4). Of the two groups of C57BL/6J wild-type mice, one was injected with PBS twice daily and allowed to feed ad libitum; the second was injected twice daily with releptin according to the same schedule of obese mice. All mice were weighed and their food intake was recorded daily.

**Induction of active and adoptive disease**

For actively induced EAE, mice were immunized s.c. in the flank with 100 µl of CFA (Difco Laboratories, Detroit, MI) emulsified with 200 µg of MOG<sub>35–55</sub> peptide on days 0 and 7, and with 200 ng of pertussis toxin (Sigma, St. Louis, MO) i.p. on days 0, 1, 7, and 8. Control mice (n = 5 mice per group) were injected with CFA emulsified with PBS plus pertussis toxin, according to the same schedule (14). For adoptively transferred EAE (14), 9–10 female donor C57BL/6J mice (6–8 wk old) were primed s.c. with 300 µg of MOG<sub>35–55</sub> peptide in CFA distributed over four sites. After 9–10 days, draining lymph nodes (axillary and inguinal) and spleens were harvested, homogenized into a single cell suspension, and cultured separately in vitro in 24-well plates (Falcon; Becton Dickinson, Franklin Lakes, NJ) (8 × 10<sup>6</sup> cells/well) with RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS (Life Technologies), 2 mM L-glutamine (Life Technologies), 0.1 mM nonessential amino acids (Life Technologies), 1 mM sodium pyruvate (Life Technologies), 50 µM 2-mercaptoethanol (Sigma), 100 µg/ml penicillin, 100 µg/ml streptomycin (Life Technologies), and 25 µg/ml of MOG<sub>35–55</sub> peptide. After 4 days in culture and addition to medium of 2 U/ml of rIL-2 (Roche Biochemicals, Monza, Italy), the cells were harvested and centrifuged over a Ficoll gradient (Pharmacia Biotech, Uppsala, Sweden) to remove debris. Recipient syngeneic naïve female leptin-deficient or wild-type control mice in the different conditions of treatment after 500 rad irradiation were i.v. injected with 2.5 × 10<sup>7</sup> T cells in a final volume of 500 µl PBS. Mice were weighed and scored daily according to the clinical severity of symptoms on a scale of 0 to 6 (14) by a “blinded” to mice identity experimenter (A.D.G.), with 0.5 points for intermediate clinical findings: grade 0, no abnormality; grade 0.5, partial loss of tail tonicity, assessed by inability to curl the distal end of the tail; grade 1, reduced tail tone or slightly clumsy gait; grade 2, tail atony, moderately clumsy gait, impaired righting ability, or any combination of these signs: grade 3, hind limb weakness or partial paralysis; grade 4, complete hind limb paralysis or fore limb weakness; grade 5, tetraplegia or moribund state; grade 6, death. The data were plotted as daily mean clinical score for all animals in a particular treatment group. Scores of asymptomatic mice (score = 0) were included in the calculation of the daily mean clinical score for each group. The brains and spinal cords were dissected between 1 and 5 days after immunization and fixed in 10% Formalin. Paraffin-embedded sections of 5 µm thickness were cut from optic nerve, forebrain, cerebellum, hind brain, cervical, thoracic, lumbar, and sacral spinal cord regions and stained with hematoxylin-eosin and Luxol fast blue (Sigma) for evidence of inflammation and demyelination. Sections from 4–10 segments per mouse were examined blindly by one investigator (A.D.T.) using a published scoring system for inflammation and demyelination, respectively (15).

**Induction of delayed-type hypersensitivity (DTH) (footpad-swelling assay)**

DTH responses to MOG<sub>35–55</sub> peptide during induction of disease were also quantitated using a time-dependent (12–72 h) footpad-swelling assay (16). Briefly, mice previously sensitized with MOG<sub>35–55</sub> in CFA were challenged by s.c. injection of 25 µg of MOG<sub>35–55</sub> (in 50 µl PBS) into the right hind footpad. PBS alone was injected into the left footpad to serve as control for measurements. As negative control, we used unimmunized mice (sensitized with CFA alone). Footpad thickness was measured 12, 24, 48, and 72 h after challenge by a “blinded” to sample identity experimenter.
mice showed classical disease progression in PBS-injected mice and an increase in disease severity and progression in rleptin-treated mice (n per group). In the adoptive transfer of disease, mice were injected with rleptin starting 3 days before the transfer until day 25. Doses of rleptin used were by extrapolation from the appropriate standard curve. The lower limits of the concentration of the cytokines in the cell supernatants was determined cytokine assay. IFN-γ of MOG 35–55 peptide (from 0 to 50 ng/ml peptide). As control for prolif -eration, anti-CD3 Ab stimulation (2C11 hybridoma supernatant, diluted 1/100) was also performed 48 – 60 h after initiation of culture cell super-natants (100 l) were removed from single well and frozen at 80°C for cytokine assay. IFN-γ and IL-4 were measured by ELISA developed in our laboratory using cytokine-specific capture and detection Abs (PharMingen), and g/ml in PBS- Tween/10% FCS were added for 45 min. After six washes, 1/100 diluted ExtrAvidin-peroxidase (Sigma) was added for 30 min. The reaction was developed with Sigma-Fast OPD (-phenylenediaminedihydrochloride, peroxidase substrate) (Sigma) and read after 30 min at 450 or 492 nm after detection for each assay were: <2 pg/ml for IFN-γ; <0.6 pg/ml for IL-4. The remaining cells were incubated for an additional 16 h, pulsed with 0.5 μCi/well of [3H]thymidine (Amersham Pharmacia Biotech, Piscataway, NJ), harvested on glass-fiber filters using a Tomtec (Orange, CT) 96-well cell harvester, and counted in a 1205 Betaplate liquid scintillation counter (Wallac, Gaithersburg, MD). Results are expressed as mean cpm ± SD from duplicate cultures. ELISA for MOG35–55-specific Abs Serum samples obtained from tail veins during the period of observation of the animals (1 day before the immunization, and then 7, 25, and 35 days after the immunization). All samples were tested for MOG35–55-specific total IgG (1/100 dilution), IgG1 (1/100 dilution), and IgG2a (1/100 dilu-tion) on MOG35–55 peptide-coated 96-well ELISA plates (Corning Glass, Corning, NY). For total IgG measurements, Mouse ExtrAvidin Staining Kit (Sigma) and for IgG1/IgG2a subclasses biotin anti-IgG1 (clone A85-1) and anti-IgG2a (clone Igh-1b) mouse Abs (PharMingen) were used (17). Briefly, 100 μl of MOG35–55 peptide was added to 96-well ELISA plates at final concentration of 10 μg/ml in carbonate buffer, pH 8.2. After 16 h at 4°C, the plates were washed in PBS, blocked with 200 μl of PBS/10% FCS for 2 h, and repeatedly washed. Diluted sera in PBS-Tween/10% FCS were added at 100 μl/well for 2 h at room temperature. After five washes, anti-mouse subclasses-specific biotin-conjugated Abs at 2 μg/ml in PBS- Tween/10% FCS were added for 45 min. After six washes, 1/100 diluted ExtrAvidin-peroxidase (Sigma) was added for 30 min. The reaction was developed with Sigma-Fast OPD (o-phenylenediaminedihydrochloride, peroxidase substrate) (Sigma) and read after 30 min at 450 or 492 nm after...
stopped with 1 M HCl in an ELISA plates reader (Bio-Rad Laboratories, Hercules, CA).

**Statistical analyses**

Analyses were performed using Mann-Whitney U test (for unpaired two group analyses) and Kruskal-Wallis ANOVA test (for three or more group analyses). Results are expressed as mean ± SD; p values < 0.05 were considered to be statistically significant.

**Results**

*Leptin replacement converts C57BL/6J-ob/ob mice from resistance to susceptibility to actively or passively transferred EAE*

We first tested the ability of MOG_{35-55} peptide to induce EAE in leptin-deficient C57BL/6J-ob/ob and wild-type age- and sex-matched C57BL/6J controls with and without leptin treatment. None of the PBS-treated leptin-deficient mice ad libitum fed or pair fed to the leptin-treated group developed any sign of clinically evident disease after active immunization with MOG_{35-55} peptide (Table I; Fig. 1a). Leptin administration, starting 10 days before the immunization and continuing over a period of 40 days, restored disease susceptibility in C57BL/6J-ob/ob mice, comparable with that of the PBS-treated C57BL/6J wild-type controls (Table I; Fig. 1, a and b). Furthermore, C57BL/6J mice treated with leptin exhibited a significantly more severe and chronic disease than PBS-treated animals, as indicated by a more severe clinical score and mortality (Table I; Fig. 1b). None of the animals that survived EAE showed signs of recovery at the termination of the experiments (40 days). The possibility that the adjuvants used for the immunization were pathogenic in these mice was ruled out by the fact that none of the C57BL/6J-ob/ob or C57BL/6J mice (n = 5 for each group) injected with CFA, alone and with pertussis toxin, developed disease (data not shown). Consistent with these results, upon histological examination of the CNS tissues from both the leptin-deficient PBS- or PBS-pair fed-treated mice, no perivascular infiltrates or signs of demyelination were found in the brain and spinal cord (Fig. 2, a-c). The absence of inflammatory foci in the C57BL/6J-ob/ob animals eliminated the occurrence of silent disease. In contrast, the leptin C57BL/6J-ob/ob-treated group showed extensive mononuclear cell infiltration throughout the brain and spinal cord with signs of demyelination (Fig. 2, a-c). The frequency and the degree of inflammation and demyelination were comparable between the C57BL/6J-ob/ob treated with leptin and the wild-type PBS-treated control group (Fig. 2c). As for the clinical score, administration of leptin to C57BL/6J wild-type controls significantly increased the numbers of inflammatory foci and demyelination when compared with that of PBS-treated mice (Fig. 2c). No cellular infiltration or demyelination was observed in control animals receiving adjuvants and pertussis toxin only (data not shown).

In separate experiments, we also found that leptin was able to affect the adoptive transfer of disease by Ag-specific T lymphocytes, derived from susceptible C57BL/6J mice previously sensitized to MOG_{35-55} and injected into the tail vein of naive mice irradiated with 500 rad. As for actively induced disease, leptin-deficient mice treated with PBS or PBS-pair fed mice showed complete resistance to EAE induction when adoptively transferred with 2.5 × 10^7 MOG_{35-55}-specific encephalitogenic T cells (Table II; Fig. 1c). Treatment of recipient mice with leptin, starting 3 days before the transfer and continuing until day 25, converted leptin-deficient mice to a state of susceptibility for the adoptively induced disease (Table II; Fig. 1c). Wild-type C57BL/6J PBS-treated mice showed a frequency and disease scores similar to that of C57BL/6J-ob/ob treated with leptin. As with the actively induced disease, there was a statistically significant increase in clinical score and cellular infiltrates in the C57BL/6J mice injected with leptin, when compared with C57BL/6J PBS-treated mice (Table II; Fig. 1d). Histological examination of brain and spinal cord showed that adoptive transfer of encephalitogenic T cells produced perivascular and parenchymal inflammation in the CNS tissues of the C57BL/6J-ob/ob mice treated with leptin, whereas no infiltration was seen in either C57BL/6J-ob/ob PBS or PBS-pair fed animals (data not shown), confirming resistance to the adoptively transferred disease. The possibility that the passive
transfers were pathogenic in these mice was ruled out by the fact that none of the C57BL/6J-ob/ob or C57BL/6J mice (n = 5 for each group) injected with PBS or T cells from unprimed animals and with pertussis toxin developed disease (data not shown).

**Acquisition of susceptibility to EAE is accompanied by the induction of DTH to MOG<sub>35–55</sub> peptide**

To determine the nature of the in vivo T cell response against MOG<sub>35–55</sub> peptide in leptin-deficient mice, DTH reactions (16) were performed in all groups of mutant and wild-type control mice treated or not with leptin. Seven days after priming with the MOG<sub>35–55</sub> peptide emulsified in CFA, mice were challenged with 25 μg of MOG<sub>35–55</sub> peptide injected intradermally in the footpad. The degree of local footpad swelling was measured as a readout for the DTH reaction. Typical DTH kinetics were observed with footpad swelling peaking between 24 and 48 h and subsiding after 72–96 h. DTH responses to the MOG<sub>35–55</sub> priming epitope were absent in both leptin-deficient C57BL/6J-ob/ob PBS- and PBS-pair fed-treated mice, whereas the ones treated with leptin exhibited a significant DTH response (Fig. 3a), similar to that observed in PBS-treated wild-type mice (Fig. 3b). C57BL/6J mice injected with leptin showed a statistically significant increase in the DTH response when compared with the PBS-treated group (Fig. 3b). None of the control mice injected with CFA alone developed any significant anti-MOG<sub>35–55</sub> peptide DTH reaction (not shown).

**Leptin replacement reverses the pattern of Th cell polarization in response to MOG<sub>35–55</sub> peptide**

We examined whether the absence of leptin in C57BL/6J-ob/ob mice affected the activation and cytokine secretion of MOG<sub>35–55</sub>-specific T cells in vitro (18). The T cell response to MOG<sub>35–55</sub> peptide was tested on draining lymph node and spleen cells, taken from all groups of mice 35 days after immunization and cultured in the presence or absence of different concentrations of Ag. As shown in Fig. 4, a–c, lymph node cells derived from leptin-deficient PBS- or PBS-pair fed-treated mice showed very low levels of proliferation and IFN-γ production, but consistent amounts of IL-4 when exposed to MOG<sub>35–55</sub> peptide. rLeptin administration in these mice increased the proliferative response to a level 11-fold higher than untreated obese control mice (Fig. 4, a and d, and b and e, respectively), whereas IFN-γ secretion was increased 21-fold and IL-4 was inhibited 11-fold when compared with obese PBS or PBS-pair fed groups of mice (Fig. 4, b and c). Proliferation, IFN-γ, and IL-4 levels in rleptin-treated obese mice were comparable with those observed in C57BL/6J PBS-treated mice. Viability and capacity of T cells to respond to a polyclonal TCR-mediated stimulation were also assessed by anti-CD3-induced activation (Fig. 4, a, b, and c, inset graphs). In wild-type C57BL/6J mice, leptin did not alter proliferative responses during either MOG<sub>35–55</sub>- or anti-CD3-induced proliferation (Fig. 4d, and inset graph filled symbol), while leptin did increase IFN-γ and reduce IL-4 secretion (Fig. 4, e and f, and inset graph filled symbol). Similar results were also observed using spleen cells as the responder population (not shown).

**Reversal of the pattern of cytokine production by MOG<sub>35–55</sub>-specific T cells correlates with the subclass of anti-MOG<sub>35–55</sub> IgG production**

Serum levels of MOG<sub>35–55</sub> peptide-specific Ab were also tested 7, 25, and 35 days after immunization using an ELISA. We measured MOG<sub>35–55</sub>-specific total IgG and IgG1/IgG2a subclasses typical of an in vivo switch toward a Th2 or a Th1 response, respectively (19, 20). Significant levels of anti-MOG<sub>35–55</sub> IgG were present in all injected mice, regardless of their leptin phenotype (Fig. 5, a and b).

![FIGURE 3. Effect of leptin treatment on anti-MOG<sub>35–55</sub> DTH reaction in leptin-deficient and wild-type control mice measured as footpad swelling.](http://www.jimmunol.org/)

<table>
<thead>
<tr>
<th>Mice</th>
<th>Incidence</th>
<th>Onset (range) (days)</th>
<th>Mortality</th>
<th>Duration (days)</th>
<th>Clinical Score</th>
<th>Initial Body Weight (g)</th>
<th>Final Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J-ob/ob PBS</td>
<td>0/6 (0.0%)</td>
<td>0.0 (0–0)</td>
<td>0/6 (0.0%)</td>
<td>0.0</td>
<td>0.0</td>
<td>50.7 ± 1.9</td>
<td>53.0 ± 0.7</td>
</tr>
<tr>
<td>C57BL/6J-ob/ob PBS-pair fed</td>
<td>0/6 (0.0%)</td>
<td>0.0 (0–0)</td>
<td>0/6 (0.0%)</td>
<td>0.0</td>
<td>0.0</td>
<td>51.5 ± 1.7</td>
<td>37.9 ± 0.5</td>
</tr>
<tr>
<td>C57BL/6J-ob/ob leptin</td>
<td>6/6 (100.0%)</td>
<td>7.1 ± 1.8 (5–10)</td>
<td>0/6 (0.0%)</td>
<td>18.0&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.9 ± 0.3&lt;sup&gt;2&lt;/sup&gt;</td>
<td>50.4 ± 2.0</td>
<td>24.8 ± 1.2&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>C57BL/6J PBS</td>
<td>6/6 (100.0%)</td>
<td>8.3 ± 2.0 (5–10)</td>
<td>0/6 (0.0%)</td>
<td>18.0&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.8 ± 0.4&lt;sup&gt;2&lt;/sup&gt;</td>
<td>21.2 ± 1.0</td>
<td>21.6 ± 2.0</td>
</tr>
<tr>
<td>C57BL/6J leptin</td>
<td>6/6 (100.0%)</td>
<td>5.2 ± 0.9 (4–7)</td>
<td>1/6 (16.7%)</td>
<td>22.0&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.8 ± 0.4&lt;sup&gt;2&lt;/sup&gt;</td>
<td>21.5 ± 1.5</td>
<td>18.9 ± 0.8&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Leptin treatment significantly increased total IgG titers on days 7 and 25 for leptin-deficient mice, and on days 7, 25, and 35 in wild-type animals. More specifically, IgG1 levels were higher in C57BL/6J-\textit{ob/ob} PBS or PBS-pair fed animals compared with those found after rleptin administration (Fig. 5c). In wild-type animals, IgG1 levels were slightly but significantly reduced by rleptin treatment (Fig. 5d). In contrast, peptide-specific IgG2a levels were absent at all the time points in leptin-deficient mice, but were markedly increased after rleptin administration (Fig. 5e). In wild-type control mice, IgG2a levels were also increased by rleptin injection, although to a lesser degree (Fig. 5f).

**Discussion**

This study examines the role of leptin in the activation of autoreactive T cells in vivo, using leptin-deficient C57BL/6\textit{j}-\textit{ob/ob} mice before and after rleptin administration. The data presented in this work provide evidence that, under the experimental conditions used, leptin is required for the induction and progression of autoimmune-mediated demyelination induced by MOG35–55 in C57BL/6\textit{j} strain of mice. Leptin deficiency had a protective role in that C57BL/6\textit{j}-\textit{ob/ob} animals were resistant to both the MOG 35–55-induced and adoptively transferred disease; conversely, systemic administration of leptin rendered these mice susceptible to EAE induction. In wild-type mice, leptin treatment increased disease severity and inflammatory cell infiltration in the brain in both the MOG 35–55-induced and adoptively transferred EAE. The conversion of resistant leptin-deficient mice to a state of susceptibility by rleptin administration implies that this molecule is influential in MOG35–55-induced EAE. The absence of disease in the leptin-deficient mice was accompanied by the almost complete absence of a measurable DTH response to the MOG35–55 peptide; the DTH reaction was fully restored by rleptin replacement. The presence or absence of leptin also determined different patterns of in vitro T cell responses in both leptin-deficient and wild-type rleptin-treated mice. In fact, in C57BL/6\textit{j}-\textit{ob/ob} mice injected with rleptin, the proliferative response and IFN-\gamma levels were induced, whereas IL-4 was suppressed when compared with obese PBS or PBS-pair fed controls. In wild-type animals, we observed mainly an increase in IFN-\gamma production after rleptin administration. Taken together, these findings indicate that activation and differentiation toward a Th1 phenotype of MOG35–55-specific T cells were hindered in leptin-deficient mice, whereas the switch to a Th2-type response was promoted. The presence of leptin restored the capacity of MOG35–55 to induce a classical Th1 pathogenic response in leptin-deficient mice and enhanced the inflammatory response in wild-type animals. Specific Ab titers reflected the nature of the T cell response in C57BL/6\textit{j}-\textit{ob/ob} mice after rleptin administration. Despite an apparent normal
MOG$_{35-55}$-specific IgG response, the pattern of IgG subclass production was markedly different in the presence or absence of leptin. The response was dominated by IgG1 in the absence of leptin, with almost no IgG2a production. This pattern was reversed by leptin administration, consistent with the reversal of Th cell polarization.

Active immunization of leptin-deficient mice with MOG$_{35-55}$ leads to the generation of IL-4-secreting T cells and IgG1 Abs that are not able to induce a clinically evident disease and brain infiltration (21, 22). Furthermore, the resistance of C57BL/6j-ob/ob mice to adoptively induced disease and the absence of DTH reaction after transfer of CD4$^+$ T cells (data not shown) suggest a level of resistance downstream of the generation of encephalitogenic T cells. Because leptin has been shown to affect endothelial cell function (23), expression of adhesion molecules such as ICAM-1 and VLA-2 on CD4$^+$ T cells (3, 24), and survival of thymocytes (4), the interplay between one or more of these mechanisms may be responsible for resistance of leptin-deficient mice to adoptive transfers. Furthermore, the adoptive transfer experiments show for the first time that the presence of leptin is required for the expansion, differentiation, and maintenance of activated Th1 pathogenic T cells in the peripheral immune compartment to mediate tissue injury and disease progression.

It is clear that leptin is a pleiotropic molecule with effects on multiple biological systems, of which the immune system is but one. Leptin influences the neuroendocrine system at several levels, including the hypothalamic-pituitary-adrenal, thyroid, gonadal, and growth hormone axes (2). Therefore, it is possible that the interplay between these endocrine systems and the immune response may have influenced, indirectly, the pattern of disease susceptibility and evolution observed in this study. The data described in this work indicate that the influence of leptin on T cell immunity is sufficiently profound to control susceptibility to autoimmune disease. These findings suggest that the immune effects of leptin deficiency in the context of nutritional deficiency may be far reaching, and conversely that antagonism of the leptin axis may have potential in the field of immunotherapy.

**Acknowledgments**

This work is dedicated to the memory of Dr. Antonino Di Tuoro, as a tribute to his unique human and scientific qualities. We are particularly indebted to A. Coppola for histological analysis. We also thank G. Sequino for technical advice in the animal facility; G. Ruggiero for ELISA reader support; P. Reynolds for reading the manuscript; and F. Perna for photographic assistance.

**References**


