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Impairment of Dendritic Cell Functionality and Steady-State Number in Obese Mice

Laurence Macia,²* Myriam Delacre,²* Georges Abboud, † Tan-Sothée Ouk,§ Anne Delanoye,²* Claudie Verwaerde,²* Pasquine Saule,‡ and Isabelle Wolowczuk²,3*  

There is a finely tuned interplay between immune and neuroendocrine systems. Metabolic disturbances like obesity will have serious consequences on immunity both at the cellular and at the cytokine expression levels. Our in vivo results confirm the immune deficiency of ob/ob mice, leptin deficient and massively obese, characterized by a reduced Ag-specific T cell proliferation after keyhole limpet hemocyanin immunization. In this report, we show that dendritic cells (DCs), major APCs involved in T lymphocyte priming, are affected in obese mice. Both their function and their steady-state number are disturbed. We demonstrate that DCs from ob/ob mice are less potent in stimulation of allogenic T cells in vitro. This impaired functionality is not associated with altered expression of phenotypic markers but with the secretion of immunosuppressive cytokines such as TGF-β. Moreover, we show increased in vivo steady-state number of epidermal DCs in ob/ob mice, which is not due to a migratory defect. The ob/ob mice are characterized by the absence of functional leptin, a key adipokine linking nutrition, metabolism, and immune functions. Interestingly, intradermal injection of leptin is able to restore epidermal DC number in obese mice. Thus, DCs might be directly sensitive to metabolic disturbances, providing a partial explanation of the immunodeficiency associated with obesity. The Journal of Immunology, 2006, 177: 5997–6006.

The incidence of obesity and associated comorbidities is reaching worldwide epidemic proportions. This pathology is the result of an imbalance between caloric intake and energy expenditure, resulting in excess energy storage, mostly due to environmental and genetic factors (1). Several metabolic disturbances are closely linked to obesity, including type 2 diabetes and cardiovascular diseases, producing a complex medical condition referred to as metabolic syndrome X (2).

In addition to this metabolic imbalance, obesity dramatically influences other important physiological functions, including the immune response (3, 4). Several epidemiological studies report that obese individuals have increased susceptibility to systemic infections. The obese patients are more prone to develop infectious complications after surgery (5), and a positive correlation between body mass index (weight in kilograms divided by height in square meters) and nosocomial diseases has been reported (6). Moreover, up to 50% of obese persons develop cutaneous infections and display reduced wound-healing capabilities (7–9). Impairment of the immune system has also been reported in obese animals. The obese Zucker rats have an increased susceptibility to Candida albicans infections (10), whereas obese leptin-deficient ob/ob and leptin-resistant db/db mice display an impaired response to Listeria monocytogenes (11).

The immune system comprises innate and acquired immunity, both affected by obesity. Indeed, it is reported that macrophages accumulate within the white adipose tissue of obese mice, proportionally to adipocyte size and number (12). Macrophage functionality is also impaired with a reduced phagocytic capacity and a defective oxidative burst (13, 14). As in human obesity, obese animals present a delayed wound healing associated with increased polymorphonuclear leukocyte infiltration (15). Acquired immunity is also affected by obesity, because T cell- and B cell-mediated immune responses are impaired in obese ob/ob (16) and diabetic db/db mice (17). Obesity is further characterized by an imbalance of the cytokine network, resulting in a low-grade systemic inflammatory status (18). The inflammatory cytokines IL-6, IL-1, and TNF-α, abnormally elevated in obesity, mostly originate from the activated macrophages infiltrating the white adipose tissue (12, 19). However, macrophages are not the only secreting cells within this tissue, because adipocytes also produce and secrete a wide variety of proteins, collectively termed adipokines, with some having inflammatory functions. Of these molecules, the best characterized are leptin, adiponectin, resistin, adipin, and visfatin (20). Altered adipokine levels have been reported in obesity; leptin expression is increased in both human and animal obese states and is positively correlated with adipose tissue mass (20–23). Additionally, adipocytes also secrete classical “immune” cytokines (TNF-α, IL-6, IL-1 receptor antagonist, and TGF-β), whose levels are significantly increased in obesity, that contribute to the overall inflammatory status of obese persons (24–27). Thus, obesity is presently viewed as an inflammatory disease, referred to as “obesitas” (28), affecting both innate and acquired immune systems. Although leptin was originally characterized for its capacity to finely regulate body weight (29), subsequent studies have demonstrated...

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MHCII, MHC class II.

cyanin; LC, Langerhans cell; BM-dDC, bone marrow-derived DC; CT, control;

Abbreviations used in this paper: DC, dendritic cell; KLH, keyhole limpet

Animals

Mice (five ob/ob and five controls) were s.c. injected at the tail base with 50 μg of KLH emulsified 1:1 in CFA. Mice were sacrificed 10 days after immunization by cervical dislocation. All experiments were done in accordance with the guidelines of laboratory animal care published by the French Ethical Committee. Splenic cell suspensions were prepared from KLH-immunized mice, depleted of RBC, and cultured in 96-well plates (BD Labware) at a concentration of 10^5 cells per well in complete medium with either Con A (1 μg/ml) or KLH (10, 25, and 50 μg/ml). Each condition was run in triplicate and each experiment was done twice. Proliferation was measured by adding 0.5 μCi of [methyl-^3H]thymidine to the medium 48 h before harvest, and harvested on a 12-h light/dark schedule. Animals had ad libitum access to water and food (standard chow diet; UAR).

Abs, medium, and reagents

Abs were purchased from BD PharMingen unless otherwise stated: FITC-anti-I-A^d, PE-conjugated (PE-) anti-I-A^d, FITC-anti-CD80, FITC-anti-CD86, biotinylated anti-CD40, and biotinylated anti-CD11c Abs were used for flow cytometry. Biotinylated Abs were revealed by PE-streptavidin or FITC-streptavidin. Purified rat anti-mouse IgG (Sigma-Aldrich).

For ELISA, we used purified anti-murine IL-4, IFN-γ, IL-10, and TGF-β as primary Abs and the corresponding biotinylated Abs according to manufacturer’s recommendations. Leptin concentration was determined using the ELISA kit DuoSet mouse leptin (R&D Systems).

All components of the Complete Culture medium were purchased from Invitrogen Life Technologies unless those otherwise indicated: RPMI 1640 medium complemented with heat-inactivated FCS (10%), pyruvate (1 mM), gentamicin (50 μg/ml), penicillin (25 U/ml), and streptomycin (25 μg/ml), and 2-ME (5 × 10^-3 M) (Merck). Dynabeads mouse Pan T (Dynal Biotech) were used for negative selection of splenic T lymphocytes. The following reagents and factors were also used: 3-amino-9-ethylcarbazol (Sigma-Aldrich), AB-Complex HRP (DakoCytomation), murine recombinant GM-CSF (PeproTech), murine recombinant leptin (PeproTech), murine recombinant TNF-α (R&D Systems), FITC-dextran (Sigma-Aldrich) (m.w., 40,000), KLH (Pierce, PerbioScience), CFA (Sigma-Aldrich), Con A (Sigma-Aldrich), LPS purified from Salmonella typhimurium (Sigma-Aldrich), [methyl-^3H]thymidine (Amersham Biosciences), scintillation fluid (Betaplate Scint; PerkinElmer), ECL reagent (Amersham Biosciences), TRizol (Invitrogen Life Technologies), and ammonium thiocyanate (Sigma-Aldrich). The lysis buffer used for protein extraction consisted of Tris-HCl (20 mM; pH 7.4), NaCl (50 mM), EDTA (5 mM), Triton X-100 (1%), to which PMSF (1 mM), leupeptin (1 μM), and apropin (5 IU/μl) were added before use.

Materials and Methods

Animals

We purchased female C57BL/6J@Rj, C57BL/6J@Rj-ob (ob/ob), and BALB/c mice (8–12 wk old) from Janvier Laboratory. Mice were housed in a pathogen-free area in our animal facilities and maintained in a temperature- and humidity-controlled room on a 12-h light/dark schedule. Animals had ad libitum access to water and food (standard chow diet; UAR).

4 Abbreviations used in this paper: DC, dendritic cell; KLH, keyhole limpet hemocyanin; LC, Langerhans cell; BM-dDC, bone marrow-derived DC; CT, control; MHCII, MHC class II.
Cell-bound radioactivity was counted in a beta counter (MicroBeta TriLux Wallac; PerkinElmer). Results were expressed as mean ± SEM.

**Immunostaining and flow cytometry**

Cell immunostaining was performed on at least $10^5$ cells, using FITC- or PE-conjugated mouse mAbs. Appropriate mouse IgG were used as isotypic controls. Samples were analyzed on an EPIC XL-MCL (Beckman Coulter) on a total of 10,000 total events.

**Preparation and analysis of epidermal sheets**

The epidermis was separated from the dermis and epidermal sheets for further processing, as previously described (35, 36). Briefly, epidermal

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**FIGURE 1.** Immune responsiveness to KLH of ob/ob mice. A, Spleen cells from KLH-immunized control (CT, □) and ob/ob (■) mice were cultured with 0–50 μg/ml KLH (five mice per group). Each stimulatory condition was done in triplicate. Results are expressed as mean of [methyl-3H]thymidine incorporation ± SEM. †, p < 0.05 by a two-way ANOVA. B, Spleen cells from KLH-immunized control (□) and ob/ob (■) mice were cultured with 0 or 1 μg/ml Con A (five mice per group). Each stimulatory condition was done in triplicate. Results are expressed as mean of [methyl-3H]thymidine incorporation ± SEM. C, Determination by ELISA of IL-10 and IFN-γ concentrations in culture supernatants of KLH-immunized control (□) and ob/ob (■) mice. All tests were done in duplicate. D, Determination by ELISA of total IgG, IgG1, and IgG2a anti-KLH Ab levels in individual sera of nonimmunized and KLH-immunized control (□) and ob/ob (■) mice. All tests were done in duplicate. Student’s t test: *, p < 0.05. E, Determination by FACS of percentages of DCs in the spleen of nonimmunized control (left panel) and ob/ob mice (right panel). Cells were double-stained using anti-FITC-coupled MHCII and PE-coupled anti-CD11c Abs. The experiment was performed on five animals, and the difference between both groups was significant using the Student t test; †, p < 0.05.
sheets were isolated and LCs were visualized by immunohistochemical staining using rat purified anti-I-F-A/E as primary Ab and biotinylated conjugated goat anti-rat IgG as secondary Ab. Staining intensity was amplified using streptavidin-conjugated HRP. In the final step, sheets were developed with 3-amino-9-ethyl carbazol and mounted onto glass slides in glycerol gelatin mounting medium (Sigma-Aldrich). Epidermal sheets were prepared from each mouse of each experimental group, and 12 random fields per sheet were examined. Cell frequency was converted to LC/ mm², and results were expressed as mean ± SEM. A minimum of five sheets was analyzed per experiment, and experiments were done twice.

**TNF-α- and leptin-induced LC migration assay**

Mice were injected intradermally with 30 μl of either TNF-α (50 ng, in sterile PBS) or leptin (3 μg, in sterile PBS) into both ear pinnae. Epidermal sheets were analyzed 1 h after TNF-α, or 16–18 h after leptin administration. Enumeration of LCs was performed as described above.

**Skin explant assay**

Ears were rinsed with 70% ethanol and split into ventral and dorsal halves with forceps (37). Four sheets per animal (n = 5) were then floated, dermal side down, on 2 ml of complete culture medium in 24-well plates, for 48 h in a 5% CO2 humidified atmosphere. LCs remaining in epidermal sheets were enumerated as described above.

**Preparation and culture of bone marrow-derived DCs** (BM-dDCs)

BM-dDCs were generated from bone marrow cultures obtained from ob/ob and control mice (38). Bone marrow cells were isolated from tibias and femurs and seeded at 2 × 10⁵/ml in bacterial-grade petri dishes (Radient Nord). Cells were cultured for 11 days in complete medium with an intermediate reseeding at 2.5 × 10⁵/ml at day 7. Recombinant murine GM-CSF (10 ng/ml) was added on days 0, 3, 7, and 10. Efficiency of DC differentiation was assessed by analyzing CD11c-expressing cells using flow cytometry. For DC activation, LPS (100 ng/ml) was added during the last 24 h of culture. PBS-treated DCs were considered as immature DCs and LPS-treated cells as mature DCs.

**Table II. Expression of costimulatory surface markers on ob/ob BM-dDCs**

<table>
<thead>
<tr>
<th>Groups</th>
<th>CD11c (%)</th>
<th>MHCII (Strong) (%)</th>
<th>CD40 (%)</th>
<th>CD80 (%)</th>
<th>CD86 (%)</th>
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<tr>
<td>CT - LPS</td>
<td>96.2 ± 1.9</td>
<td>7.1 ± 1</td>
<td>47.8 ± 5</td>
<td>65.8 ± 1.7</td>
<td>68.9 ± 2.3</td>
</tr>
<tr>
<td>CT + LPS</td>
<td>94.9 ± 1.4</td>
<td>16.8 ± 0.7</td>
<td>77.2 ± 2.6</td>
<td>82.9 ± 1.2</td>
<td>74.1 ± 1</td>
</tr>
<tr>
<td>ob/ob - LPS</td>
<td>94.5 ± 1.9</td>
<td>5.3 ± 1.7</td>
<td>46.23 ± 6</td>
<td>63.93 ± 5</td>
<td>64.1 ± 0.1</td>
</tr>
<tr>
<td>ob/ob + LPS</td>
<td>95.7 ± 0.5</td>
<td>17.23 ± 1</td>
<td>78.6 ± 5</td>
<td>85.6 ± 1.6</td>
<td>75.6 ± 0.5</td>
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</table>

*Surface markers expression, MHCII, CD80, CD86, CD40 (FITC-positive cells) and CD11c (PE-positive cells), was revealed by flow cytometry. −LPS represents for each group immature BM-dDCs, and +LPS represents mature BM-dDCs. The analysis was based on the acquisition of 10,000 events. The experiment was performed from cells differentiated from five mice per group and was done twice. Results show mean ± SEM from one representative experiment.

**Endocytosis**

To investigate endocytic capacity of BM-dDCs, 1 × 10⁵ cells were incubated at 37 or 4°C for 1 h with 0.25 mg/ml FITC-dextran. The experiment was done twice with five animals per group.

**Mixed lymphocyte reaction**

Irradiated (15 Gy) immature or mature BM-dDCs were used to stimulate 2 × 10⁵ allogeneic T cells. The MLR was conducted in U-bottom 96-well culture plates (BD Labware). Graded doses of irradiated BM-dDCs were added to responder allogenic splenic T lymphocytes from BALB/c mice, as indicated in Results. DCs were cultured for 4 days at 37°C, in a 5% CO2 humidified atmosphere. Cultures were pulsed with [methyl-3H]Tdr during the last 18 h of the culture period. The amount of incorporated [methyl-3H]Tdr was measured as described above. As negative controls, splenic T cells or BM-dDCs were cultured alone. Results are expressed as mean cpm of triplicate cultures ± SEM.

**Figure 2. Cytokine expression and endocytic capacities of BM-dDCs from ob/ob mice.** A. Determination by real-time PCR of IL-10 expression levels in immature (−LPS, □) and mature (+LPS, ■) BM-dDCs generated from CT and ob/ob mice. Results are expressed as fold increase using the expression levels of immature BM-dDCs from either strain as reference. Analyses were done from cells differentiated from three to five animals. *, p < 0.05 with Mann-Whitney U test. B. Determination by FACS of endocytic capacity of BM-dDCs generated from CT and ob/ob mice. Mature (gray line) or immature (black line) BM-dDCs were cultured with FITC-dextran at 37°C. The negative control used was the same experiment performed at 4°C. The experiment was done twice with five animals per group. We used the Student t test for statistical analysis.

**Table III. Expression of chemokine receptors and cytokines by ob/ob BM-dDCs**

<table>
<thead>
<tr>
<th>mRNA</th>
<th>CT+/CT- fold increase</th>
<th>OB+/OB- fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR7</td>
<td>11.3*</td>
<td>12.3*</td>
</tr>
<tr>
<td>CCR5</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>CCR1</td>
<td>0.77</td>
<td>0.5</td>
</tr>
<tr>
<td>IL-6</td>
<td>185*</td>
<td>270*</td>
</tr>
<tr>
<td>IL-12</td>
<td>1327.9*</td>
<td>719.9*</td>
</tr>
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</table>

*Total RNA was extracted from immature and mature ob/ob BM-dDCs (OB+ and OB-, respectively), and from immature and mature control BM-dDCs (CT- and CT+). Results are expressed as fold increases over the expression levels in immature cells as reference for both groups. Results were normalized to β-actin expression level. Experiment was done from BM-dDCs differentiated from n = 3 to n = 6 animals per group. Statistical analysis used was Mann-Whitney U*, p < 0.05 represents a significant change in expression between immature and mature BM-DCs.
Cytokine, leptin, and Ig ELISAs
IL-4, IL-10, and TGF-β in culture supernatants were determined by sandwich ELISAs using purified specific Abs against IL-4, IL-10, and TGF-β, respectively, and the corresponding biotinylated Abs, according to the recommended protocol. Leptin concentration in serum was determined by a specific ELISA, according to the manufacturer’s instructions. Blood was harvested at the following time points: before leptin administration, 15, 30, 60, 120, 180, 240, 480 min, and 18 h after leptin injection.

KLH-specific Abs were measured in the serum from each immunized mouse, obtained by retro-orbital puncture. KLH (1 µg/well) was used for coating. ELISA was performed using biotinylated anti-IgG, -IgG1, and -IgG2a mAbs. Data from the colorimetric assay are expressed as optical densities at 492 nm.

RNA extraction and real-time PCR
RNAs were extracted from a minimum of 5 × 10⁶ BM-dDCs, or from skin homogenized in liquid nitrogen, and stored at −20°C in TRizol (Invitrogen Life Technologies). One microgram of total RNA was reverse-transcribed using primers for DNA synthesis (Roche) and SuperScript (Invitrogen Life Technologies), and used as template for real-time PCR using a LightCycler (Roche). The primer sequences are indicated in Table I and the PCR conditions were as follows: 8 min at 95°C, and 40 cycles of 10 s at 95°C, 7 s at 62°C, and 1 s per 25 bp of product at 72°C. A supplementary elongation for 30 s at 72°C was added to generate the amplification curve. Results were normalized to those for the housekeeping gene CT; cycle threshold (CT, cycle threshold) described previously (39).

Statistics
All results are expressed as mean ± SEM. Statistical significance was calculated using either Student’s t test or Mann-Whitney U for the comparison of two groups and two-way ANOVA when more than two parameters were studied. The software SigmaStat 3.0 was used for all of the analyses.

Results
Impairment of T cell responsiveness in ob/ob mice
The ob/ob mice are leptin-deficient and massively obese (33). Indeed, at 8 wk, their average weight is 42.8 ± 1.28 g, whereas the control mice (CT) weigh 19.28 ± 0.3 g (p < 0.05). The basal splenic cellularity of ob/ob and of CT mice is comparable, with 85 ± 10.4 × 10⁶ and 98.3 ± 26.5 × 10⁶ cells, respectively. To study their T cell responsiveness, we immunized both groups of mice with KHL. After immunization, control mice showed the expected increase in the number of splenocytes (to 139.05 ± 13.5 × 10⁶), whereas ob/ob mice displayed only a slight increase of the splenic cellularity (to 88.5 ± 12.03 × 10⁶). In contrast, the abundance of T (CD4+), CD8+ and B cells were similar between ob/ob and control mice, whether immunized or not. The proportions of cells staining positive for CD4+, CD8+, and B220+ cells in nonimmunized animals were 24.7 ± 22%, 17.3 ± 18.4%; and 47.5 ± 46.9% for control vs ob/ob mice, respectively. In immunized animals, percentages were 21.1 ± 18% for CD4+, 18.4 ± 17.4% for CD8+, and 36.9 ± 33.1% for B220+ for control vs ob/ob mice (data not shown). Assessment of in vitro KLH-specific T cell responsiveness (Fig. 1A) showed that splenic cells from immunized ob/ob mice displayed a reduced proliferation level when compared with control animals. This lower T cell reactivity in ob/ob mice was not

FIGURE 3. MLR with mature BM-dDCs from ob/ob mice. A, MLR was performed using irradiated mature BM-dDCs from either ob/ob mice (□) or control mice (■) cocultured with splenic T cells isolated from BALB/c mice. The T:DC ratio ranged from 5 to 100. Each stimulatory condition was run in triplicate. Means of [methyl-³H]thymidine incorporation ± SEM are represented for cells obtained from five animals. *, p < 0.05 by two-way ANOVA. B, Determination by ELISA of IL-10 and IL-4 levels in the MLR supernatants of ob/ob BM-dDCs (■) and control BM-dDCs (□). All conditions were done in triplicate. Means ± SEM are represented. A two-way ANOVA was performed; *, p < 0.05. ND, Not detectable. C, Determination by ELISA of TGF-β concentration in MLR supernatants. Means ± SEM are represented. *, p < 0.05 by a two-way ANOVA. ND, Not detectable. D, TGF-β concentration determined by ELISA in supernatants of BM-dDCs derived from ob/ob (■) and control (□) mice. All conditions were done in duplicate with the following conditions: 2000, 4000, 8000, or 40,000 BM-dDCs. *, p < 0.05 by a two-way ANOVA. ND, Not detectable.
observed after nonspecific mitogenic (i.e., Con A) stimulation (Fig. 1B).

To further characterize the immune response of immunized animals, we measured cytokine secretions in culture supernatants. Higher levels of IFN-γ and of IL-10 were detected in the supernatants from ob/ob mice cultures (Fig. 1C).

Finally, we measured KHL-specific Ab levels in the sera of both ob/ob and CT immunized-mice (Fig. 1D). Upon immunization, both groups significantly increased total specific IgG levels, this increase being more prominent in control mice. The IgG1 isotype levels were lower in ob/ob mice, whereas IgG2a levels were equivalent in both groups.

Because T lymphocytes from ob/ob mice had normal mitogen proliferative response, we hypothesized that the reduced Ag-specific proliferation might result from an impairment of the major APCs, i.e., the DCs. Interestingly, we found more DCs, identified as CD11c MHC class II (MHCII)-expressing cells, in the spleen of naive ob/ob animals compared with control mice (13 vs 5%; p < 0.05) (Fig. 1E). This prompted us to further investigate this population at two levels: 1) to study their functionality and 2) to analyze their steady-state number, apparently altered.

Normal phenotypic and functional characteristics of immature and mature ob/ob BM-dDCs

The DCs were differentiated from bone marrow precursors cultured in the presence of GM-CSF. We first measured the abundance of CD11c+ cells in the fresh bone marrow of control vs obese mice and found no significant difference between the two groups (11.5 and 14.3%, respectively). After inducing differentiation of bone marrow precursor cells, we did not observe differences in the morphology or the yield of immature DCs obtained from control and ob/ob groups (data not shown). Mature DCs were generated from BM-dDCs of ob/ob or control animals by means of LPS activation. It has been documented that immature DCs exposed to LPS in this fashion up-regulate surface expression of MHCII molecules, adhesion molecules, and costimulatory molecules, rendering them fully competent Ag-presenting and costimulatory cells (34). As expected, we observed that when immature DCs were incubated with LPS, the surface expression of MHCII, CD40, CD80, and CD86 was readily increased. Nevertheless, there was no difference between ob/ob and control, immature and LPS-activated mature DCs (Table II). Moreover, maturation of DCs also includes acquisition of high cellular motility resulting from modification in the expression of several surface chemokine receptors. Among these, CCR7 has been shown to be up-regulated on mature DCs to ensure their proper migration, whereas CCR1 and CCR5 are down-regulated during the maturation process (34). Using real-time PCR, we found in both groups a significant increase of CCR7 mRNA expression after LPS-induced DCs activation, with no difference between the two groups (Table III). As expected, CCR1 and CCR5 were down-regulated in both groups with no difference between ob/ob and control animals (Table III). We then determined the pattern of cytokine expression in immature and mature BM-dDCs. After activation, both control and ob/ob BM-dDCs displayed a significant increase in IL-6 mRNA expression and in IL-12 mRNA expression. This increase was slightly lower in ob/ob mice (Table III). Interestingly, whereas the expression of IL-10 mRNA was significantly increased in both groups after maturation, we showed that mature DCs generated from ob/ob mice expressed slightly, but significantly, less IL-10 than mature DCs derived from control animals (Fig. 2A).

Immature DCs are very efficient in protein capture whereas mature DCs have switched from a role in Ag uptake to that of an Ag-presenting cell (34). By flow cytometry, we thus investigated the capacity of immature DCs from ob/ob and control mice to uptake FITC-dextran. As shown by Fig. 2B, immature BM-dDCs from both origins have similar endocytic capacities, equivalently decreasing after LPS-induced maturation.

FIGURE 4. Epidermal LC enumeration and migration in ob/ob mice. A, LCs were enumerated from epidermal sheets by anti-MHCII staining. LCs from 12 fields per ear sheets were counted and the results converted in number of LCs per square millimeter. The picture shown is representative of the results obtained from 10 epidermal sheets per group, and the mean ± SEM is indicated below the corresponding picture. *, p < 0.05 with Student’s t test. B, Isolated explant assay: epidermal sheets were isolated and LCs were enumerated, as described above (A). □ represents the basal number of LCs per square millimeter, and ■ represents the remaining LCs. n = 10 epidermal sheets per group were enumerated. The statistical analysis was done using the Student t test; *, p < 0.05. C, TNF-α induced migration assay: Ears were intradermally injected with either TNF-α (■) or with the equivalent volume of sterile 0.9% NaCl solution (vehicle) (□), and LCs were enumerated, as described in A, from 10 epidermal sheets per group, *, p < 0.05 by the Student t test. D, IL-4 mRNA quantification by real-time PCR from ventral ear sheet from three animals per group. Results are expressed as fold increase with control epidermis as reference (fold increase = 1). Control epidermis is represented by □, whereas ob/ob epidermis is represented by ■.
Decreased capacity of mature BM-dDCs from ob/ob mice to induce allogenic T lymphocyte proliferation

Stimulatory effects of ob/ob DCs on T cells were further tested in a heterologous MLR between T cells from BALB/c origin, and allogenic (e.g., C57BL/6 background) immature and mature BM-dDCs from either ob/ob or control animals. Immature DCs from both origins induced a weak and comparable allogenic T cell stimulation. As expected, after LPS maturation, the T cell-stimulatory abilities of mature DCs of both strains were significantly higher compared with the immature DCs (two-way ANOVA, p < 0.001 for both groups) (data not shown). Interestingly, mature BM-dDCs from ob/ob mice were less efficient to stimulate heterologous T lymphocytes than those from control animals (Fig. 3A).

To explore this phenomenon in greater depth, we measured cytokine secretions in supernatants of allogenic cultures with mature DCs. IL-12 (p40) and IFN-γ were detected with no difference regarding the origin of the DCs (data not shown). On the contrary, IL-10 and IL-4 secretion levels were significantly lower in cultures with ob/ob DCs (Fig. 3B). Of note, we detected low levels of IL-10 in control BM-dDCs cultured alone, whereas no IL-10 was detectable in obese BM-dDCs indicating that, in this culture system, IL-10 was mostly secreted by allogenic T lymphocytes (data not shown). Finally, and interestingly enough, TGF-β was exclusively found in allogenic cultures with ob/ob DCs; its concentration increasing with DC numbers (Fig. 3C). Furthermore, we showed that TGF-β was mainly secreted by DCs and not T lymphocytes (Fig. 3D).

Higher numbers, increased spontaneous migration, and normal TNF-α-induced migratory capacities of epidermal LCs in ob/ob mice

Because murine epidermal DCs (e.g., LCs) have been described to require TGF-β to differentiate (40), we evaluated the LC network in the epidermis of ob/ob and control mice (Fig. 4A). Despite no morphological differences, LCs were higher in numbers in the epidermis of ob/ob mice compared with control animals (602.3 ± 12.93 and 457.8 ± 18.08 LCs/mm², respectively; p < 0.001).

To thoroughly characterize this observation, we used two complementary approaches. First, we assessed the spontaneous migration of LCs from skin explants cultured in vitro for 48 h (Fig. 4B). In control mice, compared with a freshly isolated epidermis, the number of LCs remaining in the epidermis after 48-h culture dramatically decreased from 457.8 ± 18.08 to 282.4 ± 15.46 LCs/mm², representing a reduction of 39%. The percentage of spontaneously migrating LCs in ob/ob mice reached 50% with a decrease in LCs numbers from 602.3 ± 12.93 to 301.2 ± 16.26 LCs/mm². This analysis shows that spontaneous migration of those cells was not impaired.

As a second step, we investigated LC migration in a system known to strongly and actively promote LC departure to skin draining lymph nodes (41). We injected TNF-α intradermally into ear pinnae of ob/ob or control mice and assessed the departure of LCs from the skin by enumerating the remaining epidermal LCs (Fig. 4C). One hour after TNF-α injection, 332.2 ± 6.5 LCs/mm² remained in control epidermis vs 449.4 ± 17.5 LCs/mm² in ob/ob mice. Despite the fact that the number of LCs in ob/ob mice was again higher than in the control epidermis (449.4 ± 17.5 vs 332.2 ± 6.5), the percentage of TNF-α-induced LCs departure was comparable between experimental groups (27.4% for control mice vs 28.8% for ob/ob mice).

TNF-α and IL-1β favor LCs departure, whereas IL-4 and IL-10 are proposed to be negative regulators of LCs migration (42). In addition, CCR7 was recently identified as a key regulator of cutaneous DCs network under both inflammatory and steady-state conditions (43). We therefore used real-time PCR to determine the relative expression levels of TNF-α, IL-1-β, IL-4, IL-10, TGF-β, and CCR7 in the ear skin of ob/ob or control mice. No differences were observed except in the case of IL-4, for which expression was dramatically increased in ob/ob mice (Fig. 4D), and CCR7, for which expression levels were 20-fold lower in ob/ob mice skin (data not shown).

Leptin restores LC steady-state number in ob/ob mice epidermis

The obese phenotype of ob/ob mice results from leptin deficiency (33). However, leptin has also been implicated in wound healing (15). Therefore, we strove to link our immunological observations in ob/ob mice to the absence of leptin. As shown in Fig. 5A, injection of leptin into ears of control mice had no effect on LC

![FIGURE 5](http://www.jimmunol.org/)

A. Ears of CT (□) or ob/ob (■) were intradermally injected with leptin or with the equivalent volume of sterile 0.9% NaCl solution (vehicle). Enumeration of LCs within epidermal sheets was done as described in Fig. 4A. The basal condition represents the number of LCs in epidermal sheets prepared from noninjected ears. The experiment was performed from at least six epidermal sheets per group. * p < 0.05 with the Student t test. B. Serum leptin dosage (in picograms per millilitre) before leptin intradermal injection (t = 0) or 15, 30, 60, 120, 240 min, and 18 h after leptin injection. The experiment was performed on n = 3 ob/ob mice and n = 2 control mice. Represented are the means ± SEM.
migration. However, administration of leptin to leptin-deficient ob/ob mice induced LC migration. Interestingly, after leptin injection, the number of LCs was comparable between ob/ob and control animals (Fig. 5A). It should be noted that, at the time of the experiment, i.e., 18 h after the injection, leptin was no longer detectable in obese animals (Fig. 5B).

Discussion

In this work, we demonstrate for the first time that the immune deficiency observed in leptin-deficient obese mice is associated with an impairment of DC function. The ob/ob mice display reduced cellular responses, a decreased humoral response, and an altered cytokine secretion profile following KLH immunization. We have shown that Ag-specific T cell proliferation, but not Con A mitogen response, was significantly diminished in obese animals, providing a plausible explanation for their diminished increase in splenic cellularity after immunization, compared with control animals. The humoral response was also lower in ob/ob mice after immunization. The inefficient stimulation of specific T and B lymphocytes by APC in ob/ob mice may account for the above findings, because APC interact with both lymphocyte subsets, according to the process of “ménage à trois” (44).

We have also uncovered important differences in the cytokine profile secretion in both the in vivo and in the in vitro experiments. On one hand, more IL-10 and IFN-γ were secreted by splenic cells from obese animals in an Ag-specific response (anti-KLH; Fig. 1C). In contrast, higher amounts of IL-10 and of IL-4 were detectable in control supernatant in a protocol of MLR (Fig. 3A). It should be noted that, at the time of the experiment, i.e., 18 h after the injection, leptin was no longer detectable in obese animals (Fig. 5B).

To study more precisely DC functionality, we performed an MLR using either ob/ob mice BM-dDCs or control mice BM-dDCs cultured with allogenic T lymphocytes. DCs differentiated from obese animals were less potent in stimulating allogenic T lymphocytes, further reinforcing the reduced functionality of DCs in obesity. This feature is neither due to an altered expression of key surface markers nor to the decrease secretion of IL-12. However, this reduced capacity of obese mice BM-dDCs to activate T lymphocytes may be directly due to their TGF-β secretion (45). Moreover, a recent work demonstrates the capacity of myeloid DCs to secrete TGF-β and thus exert immunosuppressive effects (46).

TGF-β is positively correlated with obesity and up-regulated both in human and in ob/ob mice white adipose tissue (27, 47); this cytokine also plays a critical role in LC differentiation (40). After analysis of obese mice epidermal sheets, we found that like in spleen, the number of DCs was higher in obese mice compared with control mice. This accumulation of LCs within the skin of obese mice did not result from an imbalance between the proinflammatory and the anti-inflammatory cytokine environment except for IL-4, highly expressed in obese mice and previously described to be associated with high LC number in transgenic mice (48). To test whether those cells had migratory defect, we used two distinct approaches. First, using the explant assay, we demonstrated that spontaneous migration of LCs was similar in both groups, invalidating the hypothesis of LC sequestration in the epidermis. Of interest is the finding that LCs from obese mice were, 48 h after floating, at the same final proportion as for control mice. Thus, ob/ob mice LCs have either an increased capacity to migrate and/or LCs isolated from their “metabolically disturbed environment” recover the capacity to migrate. Moreover, we document a decrease of CCR7 (20 times less) in obese murine skin, which is a key regulator involved in DC migration under steady-state conditions (43). Our histological analysis of ob/ob mice skin revealed a striking hypertrophy of the hypodermis, mostly constituted of adipocytes, implying that the environment of LCs is markedly different in ob/ob and control mice (data not shown). Thus, the fat itself could play a critical role in our observations, because the white adipose tissue of ob/ob mice display increased proportion of n-6 fatty acids (49) that have been reported to increase DC recruitment (50). In addition to being involved in the alteration of DC steady-state number we presently describe in ob/ob mice, white adipose tissue could also participate to their impaired functionality because membrane fatty acid composition of DCs highly depends on surrounding adipocytes and any alteration in this composition affects their functionality (51, 52), like the decreased KLH response we observed. Finally, to go further with “the altered environment hypothesis,” we confirmed through the TNF-α-induced LC migration test, that those cells had no intrinsic migratory defect. Like DCs, macrophages, another kind of major APCs, were also found in excess in obesity, specifically in adipose tissue (12).

![FIGURE 6. A tentative model of DC resistance to leptin in obesity. Under physiological conditions, the equilibrium between metabolism and immune system ensures both immune system homeostasis with an efficient immune responsiveness and metabolism homeostasis with finely controlled cytokine and adipokine levels. In obesity, this balance is disturbed; the immune response is impaired and a state of leptin resistance gradually developed. We propose that, in addition to T cells (57), leptin resistance also affects DCs, partly explaining the immune deficiency occurring in obesity.](http://www.jimmunol.org/)

**Legend:**
- Lymphocyte
- Dendritic cell
- Metabolic parameters like glycemia or insulinemia
Among the environmental molecules that could affect LCs, the adipokines are good candidates because they are highly altered in obesity (20). Among those, leptin is considered as an adipostat (29) but also as a key cytokine able to act on the immune system (30). We thus wanted to study leptin effects on LC network and migration. However, evaluating the in vivo effect of leptin in this model of leptin deficiency (the ob/ob mice) is a particularly delicate task because usual protocol of chronic injections of leptin leads to general metabolic improvement (32) rendering difficult to study the effect of leptin per se. Along those lines, the consequences of leptin administration on the thymic cellularity of ob/ob mice were reported to be coupled with corticosteronemia decrease (53). Therefore, compared with the usual protocol (32), we chose to inject intradermally very low doses of leptin (3 vs 400 μg) during a short time (18 h vs 10 days). We noticed that leptin injection had no effects on LC number in the epidermis of control mice. However, leptin not only induced LC migration from ob/ob skin but also permitted the apparent return to steady-state number. This effect could be direct, because we have detected the presence of the long form of the leptin receptor (Ob-Rb) on murine DCs both by real-time PCR and Western blotting (data not shown). However, leptin’s effect may also be indirect. We injected it in a localized area (i.e., intradermally), but its blood concentration increased significantly (10-fold), suggesting a possible systemic effect. Thus, leptin could target peripheral cells like white adipose tissue cells able to secrete inflammatory cytokines (24–26) and/or cells located within skin like keratinocytes that also express Ob-Rb (54) and that are in close contact to LCs. This is the first demonstration of the in vivo effects of leptin on DCs and apparently on LC steady-state number. Recently, in vitro results demonstrated that leptin was also a powerful factor promoting DC functionality. This means that the role of leptin on DCs may be 2-fold, acting both on their basal number and on their participation in T cell activation (55).

To conclude, in this report, we explored an aspect of the close relationship between the immune system and metabolism (56). Obviously, the model of leptin deficiency is not representative of most obesity yet shares several features with more physiological obesity, such as the inflammatory status defining obesity as “obesities” (28). However, similar analyses should be performed in diet-induced obese (DIO) mice, an experimental model closer to human obesity. This more “classical obesity,” induced by excess high-fat food, is characterized by high leptin levels and an inability to reduce body weight, a phenomenon referred to as leptin resistance. We propose that leptin absence may not be so far from extreme leptin resistance, and thus immune impairments that we observed in ob/ob mice, touching notably DCs, could be extrapolated to classical obesities (Fig. 6). The very recent description of a reduced intracellular leptin signaling in splenic T lymphocytes isolated from diet-induced obese mice (57) is perfectly in line with our proposal of a model of leptin resistance affecting immune cells (T cells (Ref. 57) and DCs (our present work)). Thus, we propose that our demonstration of a functional deficiency of DCs and of an alteration of their steady-state number in ob/ob mice could be generalized to more frequent forms of obesity and could partly explain the immune deficiency commonly associated with the obese state.

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on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunc-
32. Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz,
35. Angeli, V., C. Faveeuw, O. Roye, J. Fontaine, E. Teissier, A. Capron,
I. Wolowczuk, M. Capron, and F. Trottein. 2001. Role of the parasite-derived
prostaglandin D2 in the inhibition of epidermal Langerhans cell migration during
36. Shelley, W. B., and L. Juhlin. 1977. Selective uptake of contact allergens by the
J. M. Austyn. 1990. Migration and maturation of Langerhans cells in skin trans-
38. Lutz, M. B., N. Kukutsch, A. L. Ogilvie, S. Rossner, F. Koch, N. Romani, and
G. Schuler. 1999. An advanced culture method for generating large quantities of
highly pure dendritic cells from mouse bone marrow. J. Immunol. Methods 223:
77–92.
data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25:
402–408.
endogenous transforming growth factor β1 in Langerhans cell biology: the skin
of transforming growth factor β1 null mice is devoid of epidermal Langerhans
43. Ohl, L., M. Mohaupt, N. Czeloth, G. Hintzen, Z. Kifaird, J. Zwirner,
T. Blankenstein, G. Henning, and R. Forster. 2004. CCR7 governs skin dendritic
cell migration under inflammatory and steady-state conditions. Immunity 21:
279–288.
44. Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu,
Transforming growth factor-β regulation of immune responses. Annu. Rev. Im-
munol. 24: 99–146.
46. Ghiringhelli, F., P. E. Puig, S. Roux, A. Parcellier, E. Schmitt, E. Solary,
immature myeloid dendritic cells into TGF-β-secreting cells inducing
β1 release by human adipose tissue is enhanced in obesity. Metabolism 54:
1546–1551.
A. Rot, and G. Stingl. 2002. Overexpression of IL-4 alters the homeostasis in the
49. Enser, M., and M. Ashwell. 1983. Fatty acid composition of triglycerides from
dendritic cells in perinodal adipose tissue during chronic mild inflammation.
fatty acid composition of dendritic cells and associated adipose tissue in popliteal
depot, mesentery, and omentum and their modulation by chronic inflammation
52. Pond, C. M. 2005. Adipose tissue and the immune system. Prostaglandins Leu-
53. Howard J. K., G. M. Lord, G. Matarese, S. Vendetti, M. A. Ghatei, M. A. Ritter,
54. Stallmeyer, B., H. Kamps, F. Podda, R. Kaufmann, J. Pfleischer, and
S. Frank. 2001. A novel keratinocyte mitogenic regulation of leptin and its func-
Leptin promotes differentiation and survival of human dendritic cells and licenses
56. Macia, L., C. Verwaerde, O. Viltart, N. Salome, J. Mairesse, M. Delacre,
multidirectional communication network controlling energy homeostasis. Curr.
Mantzoros. 2006. Leptin receptor expression and signaling in lymphocytes: ki-
tenes in lymphocyte activation, role in lymphocyte survival, and response to
Invest. 83: 1641–1643.
and dendritic cells express distinct MHC class I molecules in vivo. J. Exp.