INT J TUBERC LUNG DIS 18(8):981–987

http://dx.doi.org/10.5588/ijtld.13.0780

Leptin does not enhance cell-mediated immune responses following mycobacterial antigen stimulation

N. Santucci,* A. Díaz,* E. Bianchi,* S. Spinelli,* L. D'Attilio,* B. Bongiovanni,* G. Dídoli,* N. Brandan,† L. Nannini,† M. L. Bay,* O. Bottasso*

***Institute of Immunology, School of Medical Sciences, National University of Rosario, Santa Fe, Argentina; †Respiratory Diseases, Hospital Escuela Eva Perón, Granadero Baigorria Santa Fe, Argentina**

SUMMARY

BACKGROUND: Tuberculosis (TB) is a infectious disease characterised by a profound immune-endocrine metabolic imbalance, including a diminution in leptin plasma levels. Leptin appears to be the link between nutritional status and the development of a protective immune response.

OBJECTIVE: To examine the effects of leptin on the proliferation and production of interferon-gamma (IFN-γ) by peripheral blood mononuclear cells (PBMC) in TB patients and healthy controls stimulated with mycobacterial antigens with or without leptin. As macrophages are key cells in mycobacterial containment, the effect of leptin on the production of interleukin (IL) 1β and IL-1Ra by the monocytic cell line THP-1 was also studied.

RESULTS: Leptin diminished the proliferative capacity of PBMC on mycobacterial stimulation, and had no effect on IFN-γ production in terms of measurements in culture supernatants or intracytoplasmic analysis using flow cytometry. Real-time polymerase chain reaction studies of PBMC from TB patients revealed a preserved expression of leptin receptor. Furthermore, IL-1β and IL-1Ra secretion by THP-1 cells was not modified by leptin treatment.

CONCLUSION: The study results do not support the utility of treatment with leptin to correct immune imbalances due to TB.

KEY WORDS: pulmonary tuberculosis; immunomodulation; cytokines; adipocytokines

TUBERCULOSIS (TB) is a global health problem that mainly affects low-income countries. It is one of the most widely prevalent infectious diseases and the main cause of death due to an infectious agent.[1,2] The development rate of TB is considerably increased in situations of malnutrition or immune disorders.[3,4] TB is a multifaceted disease in which the infection coexists with an adverse neuro-immune-endocrine (NIE) response. Studies have demonstrated that such a response may be detrimental for patients, including metabolic-related manifestations, which may account for the extreme weight loss associated with the disease. TB presents a profound diminution in the levels of circulating leptin together with an increase in ghrelin and adiponectin concentrations, compatible with a paradoxically orexigenic pattern.[5]

Leptin is produced mainly by adipocytes working as a satiation factor.[6] Leptin also exerts immunomodulatory properties; in the presence of suboptimal concentrations of phytohaemagglutinin (PHA), leptin stimulates the proliferation and production of interferon-gamma (IFN-γ) by peripheral blood mononuclear cells (PBMC) and lymphocytes.[7] Leptin resulted in some improvement of patients with common variable immunodeficiency,[8] while worsening the course of multiple sclerosis.[9] Leptin has been considered as a pro-Th1 adipocytokine, signalling through its receptor Ob-R.[10] Hypoleptinaemia states are associated with an increased risk of infection. Individuals with a congenital deficiency in leptin production exhibit a decreased proliferation of $CD4^+$ cells, normalised by the exogenous administration of the hormone.[11] However, most of these studies have been conducted with pharmacological doses of leptin and/or PHA as co-adjuvant. In the light of our previous findings of hypoleptinaemia in TB patients together with an impaired immune response,[5] we assessed whether leptin was able to modify some immunological responses of relevance in TB pathophysiology. We tested the in vitro effects of treatment with leptin on PBMC functionality in patients with pulmonary TB and human monocytic THP-1 cells.

Correspondence to: Natalia Santucci, Institute of Immunology, School of Medical Sciences, National University of Rosario, Santa Fe 3100, CUAS IV, 2nd Floor, S2000CKG Rosario, Argentina. Tel: (+54) 341 355 8742; (+54) 341 483 3854. Fax: (+54) 341 480 4569. e-mail: nesantucci@gmail.com; nataliasantucci@yahoo.com.ar

Article submitted 22 October 2013. Final version accepted 20 April 2014.

MATERIALS AND METHODS

Study groups

The study was approved by the Ethical Committee of the Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Santa Fe, Argentina. Participants were enrolled after providing written informed consent. Eight human immunodeficiency virus (HIV) negative patients with newly diagnosed pulmonary TB were included. Diagnosis of TB was based on clinical and radiological data, together with the identification of TB bacilli in sputum. Disease severity as assessed by X-ray pattern revealed that the cases had moderate disease, i.e., unilateral involvement of two or more lobes; cavities, if present, had a total diameter <4 cm. The control group was composed of seven healthy individuals without any known prior contact with a TB patient. Both groups had a similar age and sex distribution. All subjects were bacille Calmette-Guérin (BCG) vaccinated and had purified protein derivative positive reactions. Blood samples were obtained from TB patients before initiating antituberculosis treatment. Height and weight were measured at the time of diagnosis; the body mass index was calculated by dividing weight by height squared (kg/m^2).

Leptin preparation for in vitro stimulation

Lyophilised human recombinant leptin (PeproTech, Mexico City, Mexico) was dissolved in sterile water as recommended by the manufacturer. Aliquots were prepared and stored at –70°C. A dose-response titration curve was created to assess the optimal leptin concentration.

Mononuclear cell isolation and in vitro stimulation

PBMC were isolated from fresh blood treated with ethylenediaminetetraäcetic acid disodium, as previously described.[5] Cells were cultured in quadruplicate in flat-bottomed microtitre plates (2x10^5 cells/well in 200 μl) with or without (basal cultures) addition of antigen obtained by the sonication of heat-killed H37Rv *Mycobacterium tuberculosis* (Tso; 8 μg/ml), leptin 10 nM, PHA 2 μg/ml or concanavalin A 2.5 μg/ml (ConA) (both from Sigma-Aldrich Laboratories, St Louis, MO, USA). PBMC cultures were incubated for 5 days at 37°C in 5% carbon dioxide (CO_2) humidified atmosphere and pulsed with [^{3}H]thymidine (1 μCi/well, Amersham Biosciences, Piscataway, NJ, USA) for 18 h before cell harvesting. Results of proliferation were expressed as mean counts per minute (cpm) of the quadruplicate cultures. In addition, another set of PBMC was cultured in 24-well plates (1×10^6 cells/well in 1.0 ml) (Greiner Bio-One GmbH, Frickenhausen, Germany) and treated with different doses of leptin (0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, and 50 nM) or left untreated (0 nM) and Tso. Supernatants were obtained at 18 h and 4 days for IFN-γ determination.

THP-1 cell culture

Human monocytic THP-1 cells were preserved in RPMI 1640 medium (PAA Laboratories GmbH, Cölbe, Germany) supplemented with 10% heat-inactivated foetal bovine serum (FBS) (GIBCO® by Life Technologies, Gaithersburg, MD, USA), 100 units/ml penicillin (GIBCO), 100 μg/ml streptomycin (GIBCO) and 2 mM L-glutamine (mega caps [MC]) in a 5% CO_2-air humidified atmosphere at 37°C. THP-1 cells were seeded at 5×10^5 cells per well in 12-well plates (Greiner) and the volume of each well was made up to 3 ml with the same MC. Cells were challenged with 2, 5, 10, and 50 nM of leptin, and cells and supernatants were collected immediately or at 0.5, 5, 10, and 24 h. Supernatants and cells were preserved at –70°C until use. Preliminary experiments were also carried out to show that THP-1 cells expressed the ObR receptor by real-time polymerase chain reaction (RT-qPCR).[10]

Quantification of cytokines

Plasma leptin levels (R&D Systems, Inc., Minneapolis, MN, USA, along with IFN-γ (BD Biosciences, San Jose, CA, USA), IL-1β and IL-1Ra (Life Technologies) in culture supernatant were assessed using enzyme immunoassay according to the manufacturers' instructions.

Assessment of intracytoplasmic interferon-gamma by flow cytometry

PBMC were cultured in 25 cm^2 flasks (Greiner), at 10^6 cells/ml, over 6 days in the presence or absence of leptin 10 nM. At day 7, cells cultured without leptin were transferred to 24-well plates (Greiner, Germany) and cultured for 24 h again at 10^6 cells/ml, with the addition of Tso, leptin 10 nM, or both. PBMC cultured only with culture medium (basal, negative control) were also included. Cells from the cultures to which leptin was added were also transferred to 24-well plates and cultured with or without Tso, maintaining the concentration of leptin at 10 nM. Four hours before PBMC collection, 10 μl per well of Brefeldin A (BD Biosciences) was added. The content from each well was subsequently harvested and centrifuged. The cells were stained with the following monoclonal antibodies: anti-CD3 (PerCP-Cy5.5), anti-CD4 (fluorescein isothiocyanate [FITC]), anti-CD45RO (Pe-Cy7), fixed and permeabilised using the Cytofix/Cytoperm™ commercial system, and labelled with anti-IFN-γ (PE) (all BD Biosciences). Measurements and analysis were performed using a flow cytometer with FACSAria™ and FACSDiva™ software (BD Biosciences).

Statistical analysis

Comparisons between groups were performed by non-parametric methods such as the Mann-Whitney *U*-test. Paired comparisons used the Friedman analysis of variance and Wilcoxon test. $P < 0.05$ was considered statistically significant.

RESULTS

As shown in Table 1, TB patients had a lower body mass index (BMI) and lower levels of circulating leptin than controls ($P < 0.05$ and $P < 0.01$, respectively). As leptin and BMI are positively correlated,[9] the leptin/BMI ratio was calculated, which remained significantly lower in TB patients.

Effects of leptin on blastogenesis mediated by Tso

Leptin was shown to improve cell proliferation and IFN-γ production from cultured PBMCs exposed to suboptimal concentrations of PHA and leptin.[7] We investigated whether leptin exerted a similar effect when PBMCs from the healthy controls and the TB patients were treated with 10 nM leptin, Tso, or both. The working leptin concentration was selected according to the titration dose-response curve. PBMC exposed to leptin plus Tso had a significantly lower stimulation index (SI = cpm treated/cpm basal) than cultures treated with Tso alone, regardless of study group (Figure 1A, 1B). Further comparison of the leptin-induced relative diminution of proliferation between controls and TB patients revealed no differences (Figure 1C).

Effect of leptin on in vitro interferon-gamma synthesis

IFN-γ is a key cytokine for *M. tuberculosis* infection control.[12] Leptin was shown to promote T-cell activation, inducing a Th1 profile.[13] We therefore quantified IFN-γ production in 18-h or 4-day culture supernatant from PBMC challenged with Tso and different leptin doses. PBMCs from TB patients produced less IFN-γ than those of controls in all combinations, in some cases statistically significantly different (Table 2). While Tso stimulation induced an increased IFN-γ synthesis, treatment with leptin did not modify cytokine production. Intragroup comparisons of IFN-γ production (Tso-stimulated cultures vs. those exposed to Tso plus different leptin doses) revealed no significant differences in controls or TB patients.

Intracytoplasmic interferon-gamma production

Experiments next focused on the detection of intracytoplasmic and cell-specific surface markers, partly because some studies have described a differential effect of leptin on naïve ($CD4^+CD45RA^+$) or memory ($CD4^+CD45RO^+$) T-cells.[14] PBMC from TB patients and controls were cultured over 6 days in the presence or absence of 10 nM leptin. The cells were then transferred to 24-well plates and stimulated with Tso, 10 nM leptin (alone or combined), or no stimulus for 24 h. While Tso caused a significant increase of $CD4^+$ IFN-γ^+ cells in controls, no synergistic effect between leptin and Tso was seen among the different culture conditions in either TB patients or controls (Figure 2).

Stimulation of the secretion of IL-1Ra and IL-1β in the THP-1 cell line in response to leptin

Macrophage activation is fundamental for a proper response to *M. tuberculosis* infection.[12] The THP-1 cell line is widely used as a model for studying monocyte/macrophage activity.[15] Gabay et al. reported IL-1Ra production by THP-1 cells stimulated with high leptin doses.[10] We thus investigated the effects of leptin in THP-1 cells on IL-1β and IL-1Ra production. IL-1Ra and IL-1β levels in the supernatant of cultured THP-1 cells treated with leptin showed a time-related increase of both cytokines, irrespective of leptin treatment (Figure 3). The levels of IL1β and IL-1Ra transcripts and proteins at the leptin doses assayed were similar to baseline values; the IL-1β/IL-1Ra ratio was similar in all treatment conditions.

DISCUSSION

TB patients present a sustained and substantial pro-inflammatory response, accompanied by a neuroendocrine-metabolic imbalance with decreased leptin

Table 1 Characteristics of study groups*

	TB ($n = 8$) median (25–75 percentiles)	Healthy controls ($n = 7$) median (25–75 percentiles)	*P* value
Sex, F:M	1:7	2:5	NS
Age, years	30 (22–38)	29 (25–38)	NS
BMI, kg/m^2	21.83 (19.5–25.0)	25.6 (19.2–31.9)	<0.05
Leptin, pg/ml	1857 (1390–3637)	7005 (5650–9507)	<0.01
Leptin/BMI ratio	83.1 (59.9–163.8)	245.1 (145.9–420.9)	<0.05

* Controls and TB patients did not differ in sex and age distribution. TB patients showed a decreased BMI and lower amounts of plasma leptin than controls ($P < 0.05$ and $P < 0.01$, respectively). The leptin/BMI ratio in TB patients was statistically significantly lower ($P < 0.05$).
TB = tuberculosis; NS = non-significant; BMI = body mass index.

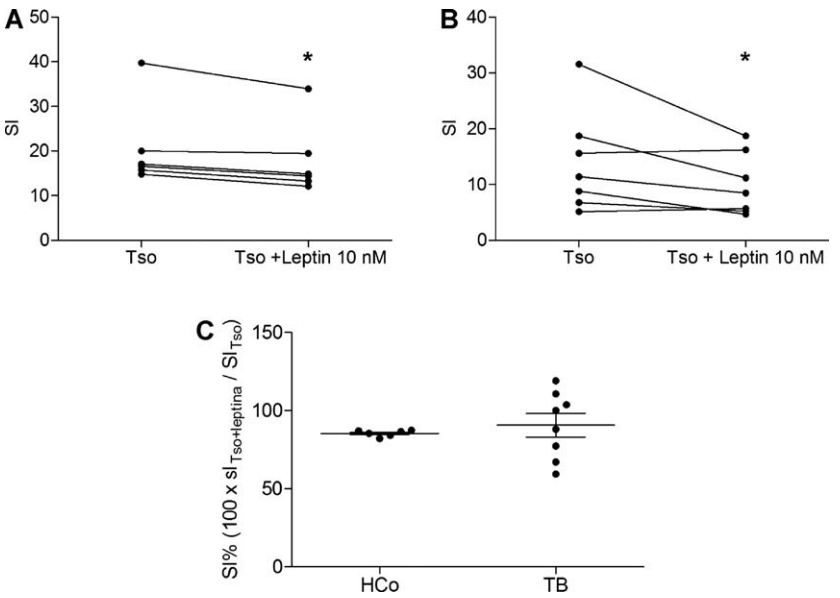

Figure 1 Stimulation index obtained when PBMC from **A)** healthy controls and **B)** TB patients were stimulated with Tso plus leptin 10 nM vs. Tso. PBMC co-cultured with leptin and Tso showed a statistically significantly lower SI than cultures treated with Tso alone, irrespective of the study groups ($P < 0.05$). **C)** Further comparison on the leptin-induced relative diminution of proliferation between controls and TB (% SI = 100 x $SI_{Tso\ +leptin}$ /SI_{Tso} considering SI_{Tso} as 100% stimulation), revealed no differences (mean ± SEM). SI = stimulation index; Tso = antigen obtained by the sonication of heat-killed H37Rv *Mycobacterium tuberculosis*; TB = tuberculosis; PBMC = peripheral blood mononuclear cells; SEM = standard error of mean.

Table 2 Levels of IFN-γ (pg/ml) in culture supernatants from PBMC challenged with Tso and different leptin doses*

Treatments	Healthy controls ($n = 7$) median (25–75 percentiles)	TB ($n = 8$) median (25–75 percentiles)	*P*
18-h cultures			
Basal	128.9 (46.82–146.0)	104.1 (97.92–114.8)	NS
Tso	259.9 (140.8–372.8)	110.9 (101.6–123.3)	NS
Leptin 0.01 nM	207.3 (83.47–309.1)	107.2 (89.30–112.2)	NS
Leptin 0.05 nM	224.9 (87.76–343.4)	104.1 (81.59–114.1)	NS
Leptin 0.1 nM	198.9 (83.56–312.0)	104.4 (95.41–123.9)	NS
Leptin 0.5 nM	313.2 (128.0–481.3)	110.1 (100.7–233.2)	<0.05
Leptin 1 nM	163.7 (107.4–400.2)	105.1 (92.64–132.1)	NS
Leptin 2 nM	161.8 (106.8–394.7)	113.6 (85.55–125.3)	NS
Leptin 5 nM	163.7 (111.7–395.3)	107.5 (88.77–123.9)	NS
Leptin 10 nM	194.0 (117.8–399.3)	105.8 (87.5–109.2)	NS
Leptin 15 nM	173.3 (128.0–253.3)	111.6 (87.04–118.5)	<0.05
4-day cultures			
Basal	4.7 (4.7–377.7)	4.7 (4.7–8.44)	NS
Tso	1016 (785.7–1181)	87.12 (59.27–490)	<0.02
Leptin 0.01 nM	875.9 (367.3–1172)	203.7 (57.42–494.3)	NS
Leptin 0.05 nM	814.6 (462.3–972.5)	199.6 (26.95–472)	<0.04
Leptin 0.1 nM	741.4 (416.5–1187)	299.7 (118.5–547.7)	NS
Leptin 0.5 nM	1005 (623–1180)	191.9 (32.61–448.1)	<0.01
Leptin 1 nM	890.5 (416.2–1109)	183.6 (52.67–494.1)	NS
Leptin 2 nM	1061 (377.2–1165)	128.7 (33.47–355.8)	<0.04
Leptin 5 nM	896.2 (357.5–1150)	38.39 (38.04–489.2)	<0.04
Leptin 10 nM	1013 (389.1–1133)	306.3 (55.96–483.5)	NS
Leptin 15 nM	1071 (718.2–1217)	413.7 (339.1–488.4)	<0.05

* TB patients showed lower IFN-γ production than controls, a difference that was statistically significant in some cases. Leptin did not exert a synergistic effect with Tso compared to cytokine secretion, at the doses assayed. Intragroup comparisons of IFN-γ production between Tso stimulated cultures and those exposed to Tso + different leptin doses revealed no significant differences (Friedman test).
IFN-γ = interferon-gamma; PBMC = peripheral blood mononuclear cells; Tso = antigen obtained by the sonication of heat-killed H37Rv *Mycobacterium tuberculosis*; TB = tuberculosis; NS = not significant.

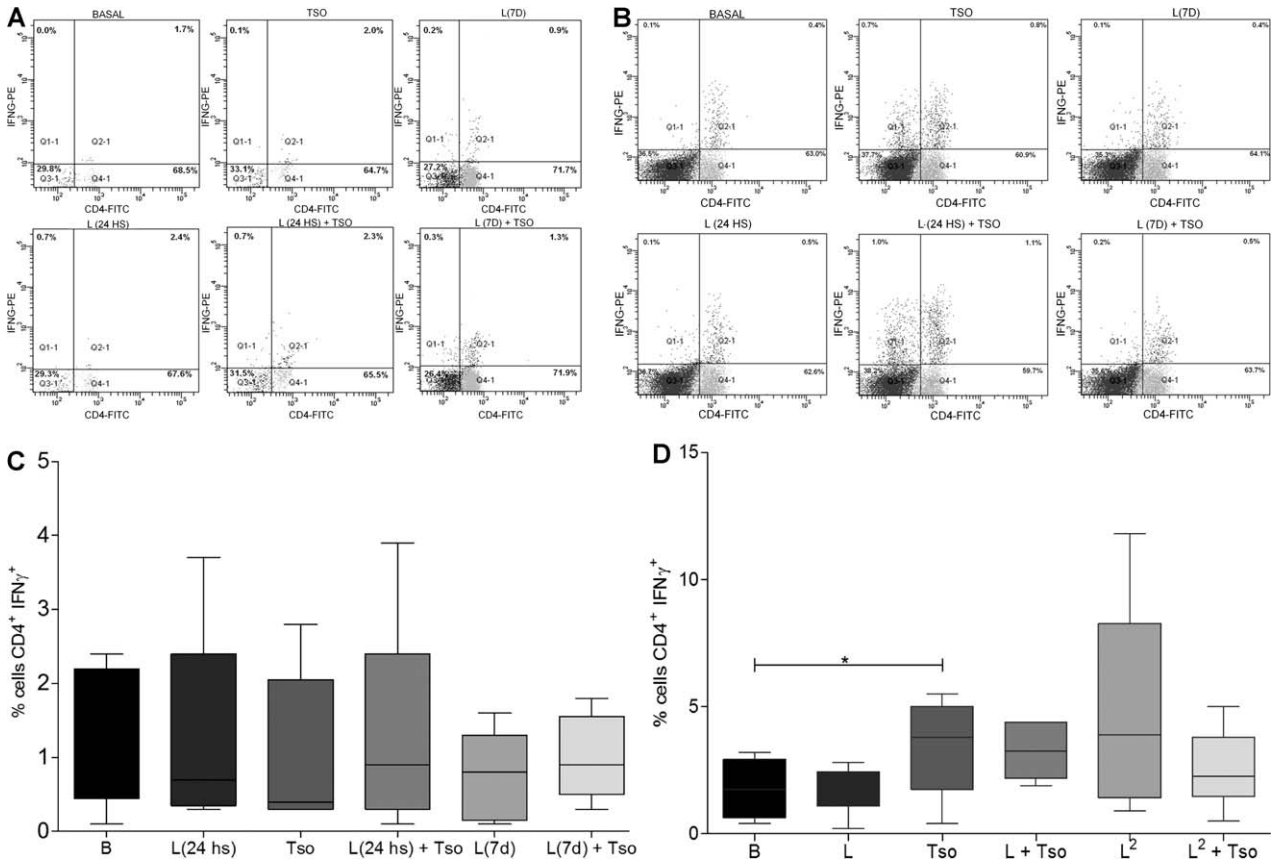

Figure 2 Intracytoplasmic IFN-γ production assessed by flow cytometry. Data obtained in **A)** TB patients and **B)** healthy controls. Box and whisker plots of the percentage of $CD4^+$ cells producing IFN-γ in **C)** TB patients ($n = 8$) and **D)** controls ($n = 7$). Box plots show 25–75 percentiles of data in each group with maximum and minimum values. Briefly, PBMC from TB patients and healthy controls were cultured over 6 days in the presence or absence of leptin 10 nM. The cells were then transferred to 24-well plates and stimulated with Tso for 24 h (Tso), leptin 10 nM (alone L[24h] or in combination L[24h] + Tso), and for 7 days (L[7d]; L[7d] + Tso) or no stimulation (**B**). In TB patients, there were no statistical differences between treatments. PBMC from controls showed significant IFN-γ production following stimulation with Tso (*$P < 0.05$), but leptin did not exert a synergistic effect with the antigen on this cytokine synthesis. IFN-γ = interferon-gamma; Tso = antigen obtained by the sonication of heat-killed H37Rv *Mycobacterium tuberculosis*; TB = tuberculosis.

plasma levels.[5,16,17] The increased risk of infection, together with a diminution in the $CD4^+$ T-cell population during hypoleptinaemia states, indicate an effect of leptin on immune function.[18] Ex vivo studies show that leptin activates the phagocytic activity in macrophages,[19] promoting pro-inflammatory cytokine production.[20] Leptin also facilitates the differentiation of immune cells towards a Th1 profile.[20] However, some controversy exists, as no effects were seen in human neutrophils stimulated with leptin at physiological doses.[21]

In the light of this, we performed several ex vivo and in vitro assays to study the role of leptin in the response of PBMC stimulated with mycobacterial antigens. We also analysed the effects of leptin on a monocytic cell-line THP-1 to approximate the effects of leptin on monocytes/macrophages. Decreased proliferation of PBMC from TB patients and controls was seen in Tso-stimulated cells exposed to leptin. Lord et al. showed that leptin inhibited the proliferation of PBMC activated with human anti-CD3 in a dose-dependent manner. In their work, leptin increased the proliferation of T-lymphocyte naïve cells while diminishing memory T-lymphocyte proliferation, suggesting that the net effect depends on the difference between naïve and memory T-lymphocytes.[14] These studies, together with the fact that the individuals in the present study were BCG-vaccinated, suggest that the Tso-induced rise in blastogenesis is mostly due to memory cells, with leptin co-stimulation resulting in a partly inhibited proliferation. As reported earlier, Tso-driven lymphoproliferation in TB patients was lower than in controls,[22] which is partly explained by the environment from which these cells are isolated, affecting proliferative capacity. Beyond the above, leptin exerted a similar inhibitory effect in both groups.

Given the protective role of IFN-γ in TB, we also studied the effects of leptin on its production. PBMC stimulated with anti-CD3 and depleted of LT naïve cells ($CD45RA^+$) showed increased IFN-γ production after 10 nM leptin treatment.[14] We then evaluated

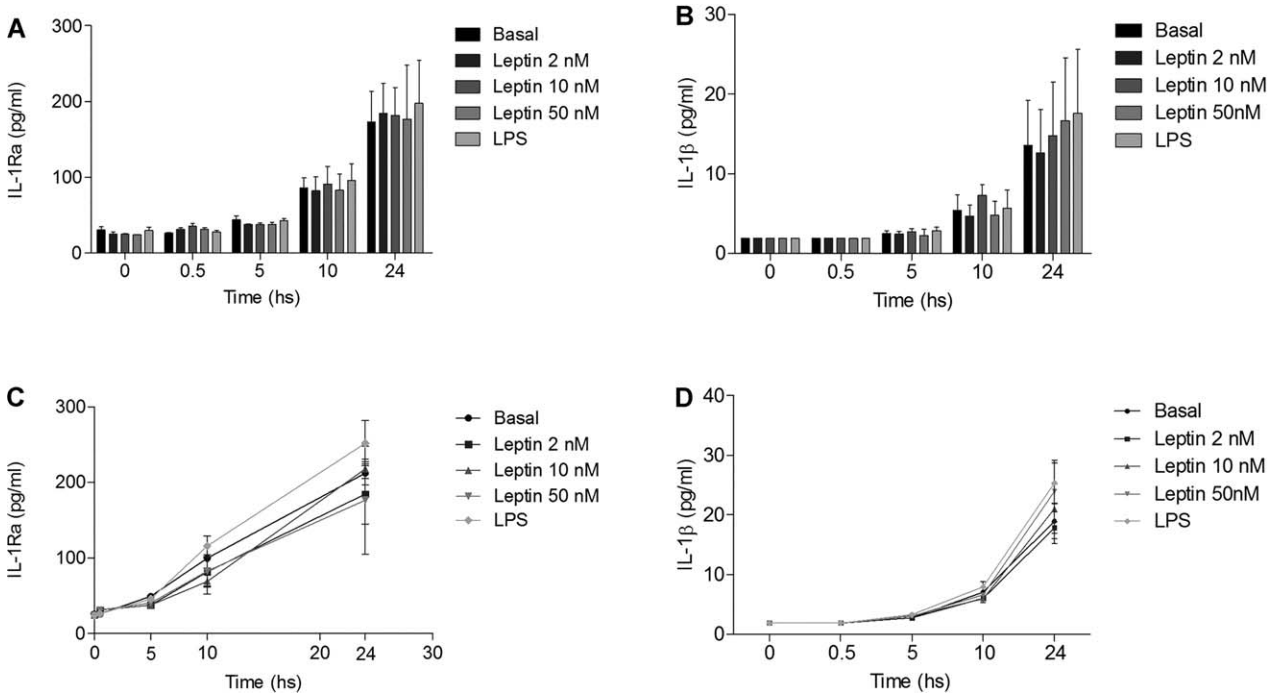

Figure 3 IL-1Ra and IL-1β secretion in culture supernatants of THP-1 cells treated with different doses of leptin. **A)** and **C)**: IL-1Ra. **B)** and **D)** IL-1β. Data are expressed as mean ± SD. Values at 24 h were significantly different from those at the remaining time-points (IL-1Ra, $P < 0.001$; IL-1β, $P < 0.001$). LPS = lipopolysaccharide; SD = standard deviation.

IFN-γ production in the culture supernatant of PBMC from TB patients and controls treated with Tso and several leptin doses after 18 h and 4 days of culture. No effect of leptin on IFN-γ production was seen, irrespective of the study groups. We evaluated intracytoplasmic IFN-γ production using flow cytometry to identify the proportion of IFN-γ-producing cells. Despite the increased IFN-γ production in PBMC from controls treated with Tso, leptin did not increase the number of IFN-γ producing cells, regardless of the presence or absence of mycobacterial antigens. In PBMC from TB patients, the effect of Tso stimulation on IFN-γ production was almost entirely absent. It is likely that leptin could not revert because cells were exhausted or were in an anergic state as a result of infection. A non-mutually exclusive possibility may be that Tso contains some mycobacterial factor blocking leptin effects on IFN production. No modifications in the percentage of $CD45RO^+$ cells were seen (data not shown). Furthermore, we analysed the effect of the hormone on PBMC cultured over 7 days or 24 h with 10 nM leptin. No differences in $CD4^+$ IFN-γ^+ cells were seen at either time-point evaluation. As regards the leptin receptor in PBMC, Sanchez-Margalet et al. demonstrated that ObRb expression is induced by leptins and HIV infection.[23] Transcript quantification of ObRb using RT-qPCR in PBMC from TB patients and controls revealed no between-group differences ($P = 0.71$, data not shown).

Leptin activates and induces the proliferation of monocytes and macrophages, and stimulates IL-6 and tumour necrosis factor-alpha synthesis.[24,25] Studies of THP-1 cells and PBMC from normal subjects demonstrated that leptin induces IL-1Ra production.[10] Our analysis of the expression and secretion of IL-1β and IL-1Ra in THP-1 cells revealed no modification by leptin treatment at nearly physiological concentrations. Our results are at variance with other studies on the effects of leptin on PBMC and THP-1 cells.[10] In most of these studies, cells were stimulated with PHA and/or leptin at supra-physiological doses. We employed a slightly supra-physiological concentration and Tso stimulation instead of PHA for a closer approximation to what happens in TB, which may account for such dissimilarities. Moreover, there seems to be no additive effect on immune and endocrine functions when leptin concentrations over the threshold level are employed. Leptin plasma levels <1 ng/ml coexist with changes in gonadal, hypophyseal and thyroid function, along with changes in peripheral lymphocyte distribution.[26,27] Such changes are totally or partly abolished when plasma leptin levels increase up to 2–3 ng/ml on leptin administration.[26,28] A study in normal individuals with decreased circulating leptin levels due to 72-h food deprivation revealed a modest reduction of $CD4^+CD45RA^+$ cells without compromising their proliferative and secretory capacity.[26] Leptin administration resulted in no major changes other than those induced by food deprivation. The short-term diminution of circulating leptin thus had little effect

on immune function. Furthermore, increased cytokine production was not observed in lean individuals with physiological or slightly supra-physiological systemic leptin levels.[26]

Our studies among TB patients suggest that their diminished leptin plasma levels may be considered a state of hypoleptinaemia,[5] for which leptin replacement treatment might be justified, as was recently proposed.[28] Nevertheless, circulating leptin in our patients did not fall as low as seen in severe states of hypoleptinaemia, where it was more likely to modify the immune function.[25] Medical decisions are based on an understanding of available evidence, in which supporting data are necessary to obtain a realistic view of the problem. Our results deal with an important topic worth considering when balancing the pros and cons of any intervention.

Acknowledgements

This work was supported by grants from the Fondo para la Investigación Científica y Tecnológica (PID 160 PAE 37245), the Ministry of Health, Secretariat for Science, Technology and Innovation of Santa Fe Province and the Florencio Fiorini Foundation.

Conflict of interest: none declared.

References

1 World Health Organization. Global tuberculosis report 2012. WHO/HTM/TB/2012.6. Geneva, Switzerland: WHO, 2012.
2 Ferraz J C, Melo F B S, Albuquerque M F P M, Montenegro S M L. Abath F G C. Immune factors and immunoregulation in tuberculosis. Braz J Med Biol Res 2006; 39: 1387–1397.
3 Toossi Z. Virological and immunological impact of tuberculosis on human immunodeficiency virus type 1 disease. J Inf Dis 2003; 188: 1146–1155.
4 Dietrich J, Doherty T M. Interaction of *Mycobaterium tuberculosis* with the host: consequences for vaccine development. APMIS 2009; 117: 440–457.
5 Santucci N, D'Attilio L, Kovalevski L, et al. A multifaceted analysis of immune-endocrine-metabolic alterations in patients with pulmonary tuberculosis. PLOS ONE 2011; 6: e26363.
6 Scarlett J, Jobst E, Enriori P, et al. Regulation of central melanocortin signaling by enterleukin1-β. Endocrinol 2007; 148: 4217–4225.
7 Martín-Romero C, Santos-Alvarez J, Goberna R, Sánchez-Margalet, V. Human leptin enhances activation and proliferation of human circulating T lymphocytes. Cell Immunol 2000; 199:15–24.
8 Goldberg A C, Eliaschewitz F G, Montor W R, et al. Exogenous leptin restores in vitro T cell proliferation and cytokine synthesis in patients with common variable immunodeficiency syndrome. Clin Immunol 2005; 114: 147–153.
9 Matarese G, Procaccini C, De Rosa V. The intricate interface between immune and metabolic regulation: a role for leptin in the pathogenesis of multiple sclerosis? J Leukoc Biol 2008; 84: 893–899.
10 Gabay C, Dreyer M, Pellegrinelli N, Chicheportich R, Meier C. Leptin directly induces the secretion of interleukin 1 receptor antagonist in human monocytes. Clin Endocrinol Metabol 2001; 86: 783–91.
11 Ozata M, Ozdemir C, Licinio J. Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. J Clin Endocrinol Metab 1999; 84: 3686–3695.
12 Hernández Pando R, Chacón Salinas R, Serafín López J, Estrada I. Immunology, pathogenesis, virulence. In: Palomino J C, Leão S C, Ritacco V, eds. Tuberculosis 2007: from basic science to patient care. 2007. Online publication. http://www.haikouvti.com/wxxz/tuberculosis2007.pdf Accessed May 2014.
13 Iikuni N, Lam Q L, Lu L, Matarese G, La Cava A. Leptin and inflammation. Curr Immunol Rev 2008; 4: 70–79.
14 Lord G, Matarese G, Howard J, Bloom S, Lechler R. Leptin inhibits the anti-CD3-driven proliferation of peripheral blood T cells but enhances the production of proinflammatory cytokines. J Leukoc Biol 2002; 72: 330–338.
15 Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int J Cancer 1980; 26: 171–176.
16 Keicho N, Matsushita I, Tanaka T, et al. Circulating levels of adiponectin, leptin, fetuin-A and retinol-binding protein in patients with tuberculosis: markers of metabolism and inflammation. PLOS ONE 2012; 7: e38703.
17 Yurt S, Erman H, Korkmaz G G, et al. The role of feed regulating peptides on weight loss in patients with pulmonary tuberculosis. Clin Biochem 2013; 46: 40–44.
18 Dardeno T A, Chou S H, Moon H S, Chamberland J P, Fiorenza C G, Mantzoros C S. Leptin in human physiology and therapeutics. Front Neuroendocrinol 2010; 31: 377–393.
19 Mancuso P, Gottschalk A, Phare S M, Peters-Golden M, Lukacs N W, Huffnagle G B. Leptin-deficient mice exhibit impaired host defense in gram-negative pneumonia J Immunol 2002; 168: 4018–4024.
20 Fernández-Riejos P, Najib S, Santos-Alvarez J, et al. Role of leptin in the activation of immune cells. Mediators Inflam 2010; 2010: 568343. Epub 2010 Mar 23.
21 Kamp V M, Langereis J D, van Aalst C W, van der Linden J A, Ulfman L H, Koenderman L. Physiological concentrations of leptin do not affect human neutrophils. PLOS ONE 2013; 8: e73170.
22 Mahuad C, Bay M L, Farroni M A, et al. Cortisol and dehydroepiandrosterone affect the response of peripheral blood mononuclear cells to mycobacterial antigens during tuberculosis. Scand J Immunol 2004; 60: 639–646.
23 Sánchez-Margalet V, Martín-Romero C, González-Yanes C, Goberna R, Rodríguez Baño J, Muniain M A. Leptin receptor (Ob-R) expression is induced in peripheral blood mononuclear cells by in vitro activation and in vivo in HIV-infected patients. Clin Exp Immunol 2002; 129: 119–124.
24 Loffreda S, Yang S Q, Lin H Z, et al. Leptin regulates proinflammatory immune responses. FASEB J 1998; 12: 57–65.
25 Sánchez-Margalet V, Martín-Romero C, Santos-Alvarez J, Goberna R, Najib S, González-Yanes C. Role of leptin as an immunomodulator of blood mononuclear cells: mechanisms of action. Clin Exp Immunol 2003; 133: 11–19.
26 Chan J, Matarese G, Shetty G, et al. Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans. Proc Natl Acad Sci USA 2006; 103: 8481–8486.
27 Khan S M, Hamnvik O P, Brinkoetter M, Mantzoros C S. Leptin as a modulator of neuroendocrine function in humans. Yonsei Med J 2012; 53: 671–679.
28 Matarese G, La Rocca C, Moon H S, et al. Selective capacity of metreleptin administration to reconstitute CD4+ T-cell number in females with acquired hypoleptinemia. Proc Natl Acad Sci USA 2013; 110: E818–E827.

RESUME

CADRE : La tuberculose (TB) est une maladie infectieuse avec hypercatabolisme caractérisée par un profond déséquilibre immuno-endocrino-métabolique comportant notamment une diminution du niveau plasmatique de leptine. La leptine semble être le lien entre l'état nutritionnel et le développement d'une réponse immunitaire protectrice.

OBJECTIF : Examiner les effets de la leptine sur la prolifération et la production de interféron-gamma (IFN-γ) par les mononucléaires du sang périphérique (PBMC) de patients tuberculeux et de témoins en bonne santé stimulés par des antigènes mycobactériens et un traitement par leptine ou pas. Comme les macrophages sont des cellules clés pour le confinement des mycobactéries, l'effet de la leptine sur la production d'interleukine (IL)-1β et d'IL-1Ra par les cellules de la lignée monocytaire THP-1 a également été étudiée.

RÉSULTATS : La leptine a diminué la capacité proliférative des PBMC sur la stimulation mycobactérienne et n'a eu aucun effet sur la production d'IFN-γ en termes de mesures dans les surnageants de culture ou en analyse intra-cytoplasmique par cytométrie de flux. Les études par polymérase en chaine en temps réel sur les monocytes de patients tuberculeux ont montré que l'expression du récepteur de la leptine était préservée. Par ailleurs, la sécrétion d'IL-1β et d'IL-1Ra par les cellules THP-1 n'étaient pas modifiée par le traitement à la leptine.

CONCLUSION : Les résultats actuels ne sont pas en faveur du traitement par leptine afin de corriger le déséquilibre immunitaire pendant la TB.

RESUMEN

ANTECEDENTES: La tuberculosis (TB) es una enfermedad infecciosa consuntiva caracterizada por un desbalance inmuno-endócrino-metabólico profundo que incluye la disminución en los niveles plasmáticos de leptina. Leptina pareciera constituir el nexo entre el estado nutricional y el desarrollo de la respuesta inmune involucrada en la eliminación de patógenos.

OBJETIVO: Examinar los efectos de leptina en la linfoproliferación y producción de IFN-γ en células mononucleares de sangre periférica (PBMC) de pacientes tuberculosos y controles sanos estimulados con *Mycobacterium tuberculosis* irradiado y sonicado con o sin el agregado de leptina. Considerando que los macrófagos son células clave en la contención de la infección con *M. tuberculosis* también se estudiaron los efectos de la leptina en la producción de interleucina (IL)-1β e IL-1Ra en la línea celular monocítica THP-1.

RESULTADOS: Leptina disminuyó la capacidad proliferativa de las PBMC cuando se las estimuló con antígenos micobacterianos y no ejerció efecto en la producción de IFN-γ tanto en las mediciones de sobrenadantes de cultivo como en el análisis por citometría de flujo de la citocina intracitoplasmática. Estudios de RT-PCR en PBMC de pacientes TB revelaron una expresión conservada del receptor de leptina. Además la secreción de IL-1β e IL-1Ra por parte de las células THP-1 no se vio modificada por el tratamiento con leptina.

CONCLUSIONES: Los presentes resultados no avalan una terapia de reemplazo con leptina con objeto de corregir el desbalance inmunológico en TB.