Medicinal & Aromatic Plants
ISSN: 2167-0412

Torello et al., Med Aromat Plants (Los Angel) 2016, 5:6
DOI: 10.4172/2167-0412.1000275

Research Article Open Access

Extramedular Hematopoiesis in the Spleen of Obese Mice Modulation by the Alga *Chlorella*

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Abstract

In this study, Balb/C mice received standard or high-fat diet (HFD) and were treated with Chlorella for 5 days prior and 4 weeks after the onset of HFD. We demonstrate here, for the first time in the literature, that in HFD-induced obesity, the rapid decline in the number of granulocyte and macrophage progenitors (CFU-GM) in the bone marrow is associated with a continuous migration/increase of these cells into the spleen, a process characterized as extramedullary hematopoiesis (EMH). No changes in the size of the primitive (LSK), and reduction in the size of the granulocyte/macrophage (GMP) hematopoietic populations in the bone marrow were observed. We also found that increased expression of C-C chemokine receptor type 2 (CCR2) on GMP in the spleen might be a mechanism related to the migration of CFU-GM to this organ. Increased serum colony-stimulating activity (CSA) was also found in obese mice. IL-6 serum levels, measured at the end of the treatment (12 weeks), when impaired glucose tolerance was already established (22), was increased. Treatment with Chlorella restored to normal values the numbers of CFU-GM in the marrow and spleen, the percentage of GMP in the marrow, the expression of CCR2 on spleen GMP, the increased serum levels of IL-6, and further increased CSA compared to obese mice. These findings suggest the ability of Chlorella to modulate the shift in hematopoietic topographical hierarchy, probably due its anti-inflammatory properties.

Keywords: *Chlorella*; Obesity; Splenic hematopoiesis; CCR2 expression; IL-6 serum levels

Abbreviations: BM: Bone marrow; CFU-GM: Number of colony-forming units of granulocytes and macrophages; CSA: Colony-stimulating activity; LSK: Primitive hematopoietic cells ($Lin^-Sca\text{-}1^+c\text{-}Kit^+$); GLUT4: Glucose transporter type-4; GMP: Granulocyte and macrophage progenitor; CCR2: C-C chemokine receptor type 2; IL: Interleukin; FFA: Free fatty acid levels; LDL: Low density lipoproteins.

Introduction

Obesity is a worldwide epidemic that results in enormous costs to health-care systems [1,2]. Data from the World Health Organization (WHO) have shown that the incidence of obesity worldwide has doubled since the 1980s [3]. Obesity-associated inflammation is widely regarded as one of the major factors driving insulin resistance (IR) and the onset of type-2 diabetes (T2D). A hallmark of inflammation in obesity is the accumulation and expansion of visceral adipose tissue (VAT) macrophages with an inflammatory phenotype, which, along with the decrease in anti-inflammatory T-regulatory cells in the VAT, results in an imbalanced environment and is thought to drive IR and the progression to T2D in obese subjects [4]. In spite of the relevance of the effects of inflammatory states on the hematopoietic system, leading to cytokine dysregulation, disturbances in cell proliferation, self-renewal rates, metabolism and cell cycle, little is known regarding the changes in the hematopoietic system induced by the inflammatory state carried by obesity [5].

An important aspect observed during chronic inflammatory states is the appearance of extramedullary hematopoiesis (EMH), which consists in the ability of marrow cells to home, proliferate, and mature in extramedullary organs of adult animals. This involves pathophysiologic alterations in the stem cells and their microenvironment, enveloping extracellular matrix and stromal cells, in addition to local and systemic chemokine production [6]. Of importance here are our previous studies showing the relevance of the restoration of both the myelosuppression and the increased splenic EMH for recovering the homeostatic balance in the immunocompromised host, as observed during chronic inflammatory states such as infection and tumors [7-16].

The search for natural agents able to minimize the undesirable effects of the available pharmacological treatment for obesity [17,18] is receiving increasing attention [19,20]. In this context, *Chlorella*, a microscopic single-celled freshwater alga containing all the ingredients necessary to promote human health [21] has emerged as an alternative agent against obesity-related complications [22,23]. *Chlorella* is called an adaptogen, meaning it helps protect the body against various stresses, including physical and psychogenic [7,24-32].

Of relevance, the stimulation of the pool of hematopoietic stem cells and the activation of mature leukocytes consisted of important aspects of *Chlorella* effects on the hematopoietic system. Our previous studies demonstrate a significant recovery in the reduced number of myeloid progenitor cells (CFU-GM) in the bone marrow of immunosuppressed host, in consequence of biologically active cytokine release, which was observed in several experimental models of psychogenical and physical stress [7,11,26,28-30,32-36]. These results demonstrated that *Chlorella* up-regulates the production of colony-stimulating factors in the same manner as for CFU-GM, leading to appropriate production

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Received November 08, 2016; **Accepted** November 15, 2016; **Published** November 22, 2016

Citation: Torello CO, Paredes-Gamer EJ, Martins F, de Castro TCL, Saad JAM, et al. (2016) Extramedular Hematopoiesis in the Spleen of Obese Mice Modulation by the *Alga Chlorella*. Med Aromat Plants (Los Angel) 5: 275. doi: 10.4172/2167-0412.1000275

and efficient mobilization of early immune cells, which are crucial to the performance of the surveillance as well as the effector functions of the immune system. Importantly, the ability of the alga to reverse both the myelosuppression and the splenic EMH was related to its ability to recover the homeostasis [7,11,26,30,33].

In addition, clinical and experimental studies in the literature reported a series of biochemical and physiological effects of *Chlorella*, such as decreasing serum cholesterol fractions, triglycerides and glucose levels in addition to reducing body weight [37-41]. In a recent study [22], we demonstrated that prevention by the alga of high-fat diet (HFD)-induced insulin resistance in obese mice is due to improvement in insulin signaling pathway by increasing phosphorylation levels of IR, IRS-1 and Akt and reducing phosphorylation levels of IRS-1^{ser307}. We also found that *Chlorella* prevents HFD-induced dyslipidemia by reducing triglyceride, cholesterol and free fatty acid levels. Altogether our findings suggest that prevention by the alga of the deleterious effects induced by HFD is a good indicator for its use as a prophylactic-therapeutic agent against obesity-related complications.

In this context, the present study was designed to investigate the modulating therapeutic effects of *Chlorella* on the production of CFU-GM in the bone marrow of obese mice and their migration into the spleen (EMH). Colony-stimulating activity (CSA) and interleukin (IL)-6 levels in the serum were investigated. The numbers of primitive (LSK) and granulocyte/macrophage progenitor (GMP) hematopoietic populations in the bone marrow and the expression of C-C chemokine receptor type 2 (CCR2) on GMP cells in the spleen were also studied.

Materials and Methods

Mice

Six-week-old male Balb/C mice were maintained in a controlled environment (room temperature: 22 ± 3°C, humidity: 55 ± 5%), under specific pathogen-free conditions in a regimen of 12 h dark/light cycles. The animals were randomly divided into four groups (n=6 mice per group), as follows: standard rodent chow and vehicle (control-CT), standard rodent chow and *Chlorella* (CV), high-fat diet and vehicle (HFD) and high-fat diet+*Chlorella* (HFD+CV). The HFD consisted of 55% calories from fat, 29% from carbohydrate and 16% from protein, as described previously [22,42-44]. The animals received water and their respective diets ad libitum for the whole period. Body weight and fasting blood glucose were measured weekly [22]. CFU-GM, CSA assays, flow cytometric analysis of LSK, GMP and CCR2 were performed at 4 weeks of HFD intake. At the end of the experiment (12 weeks), IL-6 levels were measured. All animal studies were approved by the Animal Care and Use Committee at the State University of Campinas (process: 1987-1) and are in accordance with the guidelines for the Care and Use of Laboratory Animals.

Chlorella and treatment

The dried alga *Chlorella* (*Parachlorell beyerinckii* CK-5), previously identified as *Chlorella vulgaris* CK-5, a strain of unicellular green alga, was kindly provided by Research Laboratories, *Chlorella* Industry Co., Ltd. (Fukuoka, Japan). Previous study from our laboratory demonstrated the nutritional composition of the alga [22]. *Chlorella* was prepared in distilled water and doses of 50 mg/kg/day were given orally once daily by gavage of 0.2 ml volume/mouse for 5 days prior and 4 weeks after the onset of HFD. CT and HFD groups received vehicle (distilled water) only. In all groups, the experiments were performed during the morning, 24 h after the last administration of *Chlorella*. The selection of *Chlorella* dose was based on the preliminary dose-response studies performed in our laboratory [11,26]. Prophylactic-therapeutic administration was used in all our studies, since our aim is to investigate the modulating effects of *Chlorella* as a functionally whole food able to protect the host acting as a biological response modifier, what also justifies the use of the oral route for the administration of the alga.

Progenitor cell assays

CFU-GM assays were performed using bone marrow and spleen cells. The concentration of 1×10^5 cells/mL for bone marrow and 2×10^5 for spleen cells were cultivated in duplicate agar cultures in 35-mm Petri dishes. The medium used was Dulbecco's Modified Eagle's Medium (DMEM, Sigma Chemical Co. St. Louis, MO) containing 20% fetal bovine serum and 0.3% agar. Colony formation was stimulated by the addition of recombinant murine macrophage–granulocyte colony-stimulating factor (rmGM-CSF, Sigma) at a final concentration of 0.5 ng/mL. The cultures were incubated for seven days in a fully humidified atmosphere of 5% CO_2 in air and colony formation (clones >50 cells) was scored at 35X magnification using a dissection microscope [45].

Assay for serum colony-stimulating activity

The mice were bled from the heart under deep halothane anesthesia. Within each experimental group, the blood was pooled, left at 37°C for 30 min, and the clots were allowed to retract overnight at 4°C. Following centrifugation, the serum was removed and stored at - 20 °C. CSA was determined by measuring the ability of serum obtained from control and experimental groups to stimulate hematopoietic progenitor form CFU-GM (1×10^5 cells) from normal mice. The results were expressed as units of CSA/mL, where 1 unit/mL was defined as the lowest amount of CSA able to induce the formation of colonies [46].

Quantification of IL-6

Levels of IL-6 in the serum of obese mice were quantified by sandwich ELISA in microtiter plates (96-well flat-bottom maxisorp microplate-NUNC, Roskilde, DM) using the following Kit: anti-IL-6 (BD Biosciences, San Diego, CA, USA) Cytokine determinations were performed according to the ELISA protocol. Cytokine titers are expressed as pg/mL, calculated by reference to standard curves constructed with known amounts of recombinant cytokines.

Flow cytometric analysis

To determine hematopoietic cells populations, whole bone marrows and spleens were collected, cells were removed, fixed and labeled (1×10^6 cells). For the primitive population (LSK: $CD90^+Lin^-Sca\text{-}1^+c\text{-}Kit^+$), macrophage-granulocyte progenitor population (GMP: $Lin^-IL7R^-c\text{-}Kit^+Sca\text{-}1^-CD34^+CD16^{high}$) and CCR2 expression we used the following antibodies conjugated with different fluorocromes: CD90-FITC, Sca-1-Cy7-PE, c-Kit-APC, CD34-FITC, CD16-APC, IL-7R-PE, Lin (B220, CD3, TER119, GR1, CD11b)-PE, CCR2-FITC. The cells were collected using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) and data analyzes were performed using CellQuest software (BD Biosciences). The antibodies were purchased from BD Biosciences (San Diego, CA, USA).

Statistical analysis

Data were analyzed for statistically significant experimental differences using analysis of variance (ANOVA) followed by the Bonferroni test to compare data among all groups. Statistical significance was reached when $p<0.05$. In all cases, at least three independent experiments were conducted to warrant that the results were representative.

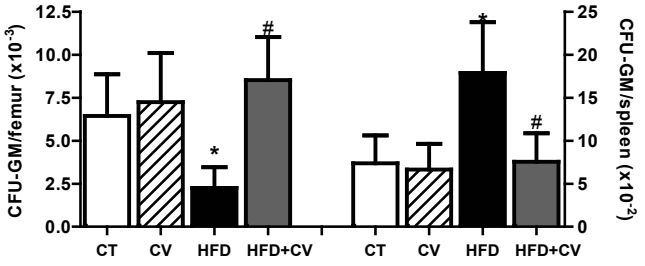

Figure 1: Number of bone marrow (left) and spleen (right) granulocyte-macrophage progenitors (CFU-GM) of mice fed on high-fat diet (HFD) and treated in a prophylactic–therapeutic manner with daily oral doses of 50 mg/kg Chlorella (CV). Treatment was given for 5 days prior and 4 weeks after the onset of HFD. Experiments were performed in the morning, 24 h after the last administration of CV. Controls (CT and HFD) received vehicle only. Results represent means ± SD of 6 mice per group. *p<0.05 vs. CT; #p<0.05 vs. HFD. ANOVA; Bonferroni Test. (CT- control; CV- Chlorella; HFD- high-fat diet; HFD+CV- high-fat diet+Chlorella).

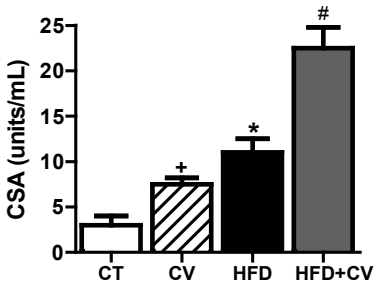

Figure 2: Serum colony-stimulating activity (CSA) in mice fed on high-fat diet (HFD) and treated in a prophylactic–therapeutic manner with daily oral doses of 50 mg/kg Chlorella (CV). Treatment was given for 5 days prior and 4 weeks after the onset of HFD. Experiments were performed in the morning, 24 h after the last administration of CV. Controls (CT and HFD) received vehicle only. Results represent means ± SD of 6 mice per group. +p<0.05 vs. CT; *p<0.05 vs. CT and CV groups; #p<0.05 vs. HFD. ANOVA; Bonferroni Test. (CT- control; CV- Chlorella; HFD- high-fat diet; HFD+CV- high-fat diet+Chlorella).

Results

Medullar and extramedullary hematopoiesis in obese mice

The growth and differentiation of bone marrow and spleen CFU-GM of HFD fed mice and treated with *Chlorella* are demonstrated in Figure 1. In HFD mice, bone marrow CFU-GM was significantly reduced (p<0.05), reaching levels approximately 2.8-fold lower than control values. Conversely, CFU-GM in the spleen was significantly increased, reaching levels 2.4-fold higher than controls. Treatment with *Chlorella* restored to control values the numbers of CFU-GM in the marrow and spleen. Importantly, no changes were produced by the alga in control animals.

Serum colony-stimulating activity (CSA) in obese mice

The presence of CSA in serum obtained from mice fed on HFD and treated with *Chlorella* is presented in Figure 2. CSA titers significantly increased (p<0.05) in HFD mice, reaching levels approximately 2.5-fold higher than control values. Treatment with the alga further increased (p<0.05) this serum activity, reaching values 2-fold higher than those observed in the HFD group and 7.5-fold higher than controls. Of importance, *Chlorella* also increased CSA in control group (p<0.05). This effect of the alga was consistent with our previous studies using different experimental models [11,26,30,32,33].

Primitive and granulocyte-macrophage progenitor hematopoietic populations

Most primitive hematopoietic population (LSK) and granulocyte-macrophage progenitor (GMP) hematopoietic populations in the bone marrow of HFD mice treated with *Chlorella* are presented in Figure 3. Although a tendency of reduced percentage of LSK cells was observed in mice receiving HFD, no significant differences were found in all groups studied. In relation to GMP population, significant reduction (p<0.05) in the percentage of this population was found in HFD mice, reaching values approximately 1.5-fold lower than controls. Treatment of HFD mice with the alga recovered the percentage of these cells to values similar to those of control group. No changes were produced by *Chlorella* treatment on both LSK and GMP populations of control animals.

Serum levels of IL-6

The effects of *Chlorella* on serum levels of IL-6 are presented in Figure 4. In HFD mice, a significant (p<0.05) increase (3-fold) was observed in the levels of IL-6 in the serum, compared to controls. Treatment with the alga restored IL-6 levels to values similar to those of control group. No changes in the levels of this cytokine were produced by the alga in control animals.

Expression of CCR2 on granulocyte-macrophage progenitor cells

The expression of CCR2 on granulocyte-macrophage progenitor

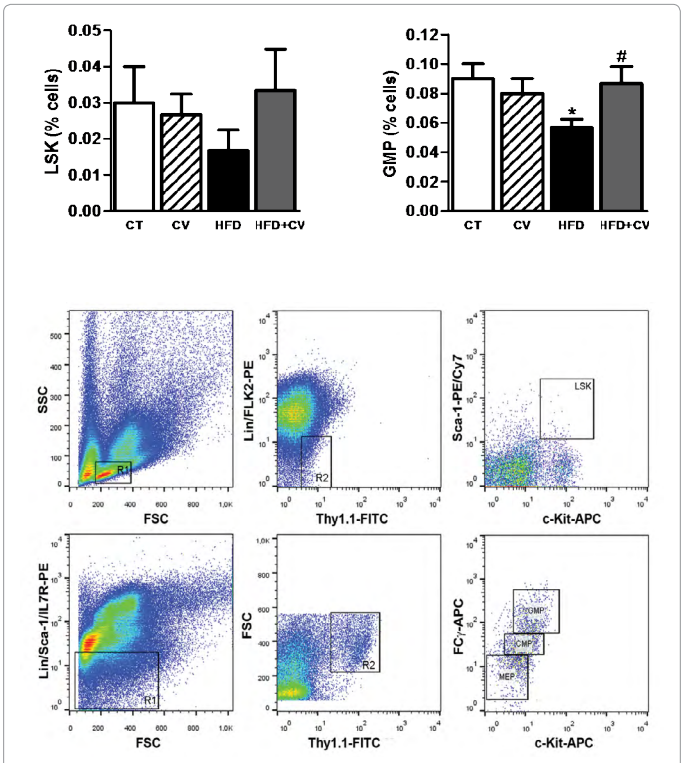

Figure 3: Quantification of **(A)** primitive LSK and **(B)** granulocyte-macrophage (GMP) populations in the bone marrow of mice fed on high-fat diet (HFD) and treated in a prophylactic–therapeutic manner with daily oral doses of 50 mg/kg Chlorella (CV). Treatment was given for 5 days prior and 4 weeks after the onset of HFD. Experiments were performed in the morning, 24 h after the last administration of CV. Controls (CT and HFD) received vehicle only. Results represent means ± SD of 6 mice per group. *p<0.05 vs. CT; #p<0.05 vs. HFD. ANOVA; Bonferroni Test. (CT- control; CV- Chlorella; HFD- high-fat diet; HFD+CV- high-fat diet + Chlorella). **(C)** Representative dot plots from flow cytometric analysis. Phenotypes: LSK: $CD90^+Lin^-Sca\text{-}1^+c\text{-}kit^+$; GMP: $Lin^-IL7R^-c\text{-}Kit^+Sca\text{-}1^-CD34^+CD16^{high}$.

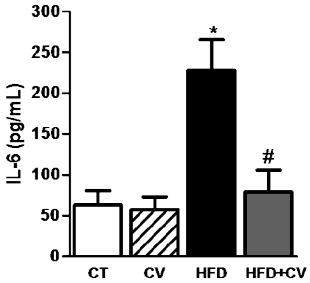

Figure 4: Levels of IL-6 in the serum of mice fed on high-fat diet (HFD) and treated in a prophylactic-therapeutic manner with daily oral doses of 50 mg/kg Chlorella (CV). Treatment was given for 5 days prior and 12 weeks after the onset of HFD. Experiments were performed in the morning, 24 h after the last administration of CV. Controls (CT and HFD) received vehicle only. Results represent means ± SD of 6 mice per group. *p<0.05 vs. CT; #p<0.05 vs. HFD. ANOVA; Bonferroni Test. (CT- control; CV- Chlorella; HFD- high-fat diet; HFD+CV- high-fat diet+Chlorella).

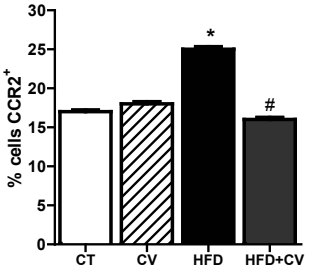

Figure 5: Quantification of granulocyte/macrophage progenitor (GMP) cells expressing CCR2 in the spleen of mice fed on high-fat diet (HFD) and treated in a prophylactic–therapeutic manner with daily oral doses of 50 mg/kg Chlorella (CV). Treatment was given for 5 days prior and 4 weeks after the onset of HFD. Experiments were performed in the morning, 24 h after the last administration of Chlorella. Controls (CT and HFD) received vehicle only. Results represent means ± SD of 6 mice per group. *p<0.05 vs. CT; #p<0.05 vs. HFD. ANOVA; Bonferroni Test. (CT- control; CV- Chlorella; HFD- high-fat diet; HFD+CV- high-fat diet+Chlorella). Phenotypes: GMP: $Lin^{-}IL7R^{-}c\text{-}Kit^{+}Sca\text{-}1^{-}CD34^{+}CD16^{high}$.

(GMP) cells in the spleen is presented on Figure 5. In HFD mice, the fraction of GMP cells expressing CCR2 increased (p<0.05), compared to controls. Treatment with *Chlorella* restored the percentage of these cells to values similar to those of control group.

Discussion

Obesity is characterized by a state of chronic inflammation and its effects on the hematopoietic system are poorly understood. In the present study, we demonstrate that in HFD-induced obesity, the rapid decline in the number of CFU-GM in the bone marrow is associated with a continuous migration of these cells into the spleen, which is characterized as extramedullary hematopoiesis (EMH). Treatment with *Chlorella*, in spite of not causing any effect on the number of CFU-GM in the bone marrow and spleen of normal mice, restored to control values both the reduced CFU-GM numbers in the bone marrow and the increased number of these progenitors in the spleen of obese mice. Corroborating these findings are our results with infected [7] and tumour-bearing mice [22]. In these two experimental models, the ability of the algae to produce cure (infection) or prolong survival (tumor) was related to the degree of reversion of both myelosuppression and the increased EMH, thus reinforcing the assumption that this modulating effect of the algae is relevant to its therapeutic activity.

Another important observation in this obesity model was the increased serum CSA, in spite of the reduced marrow CFU-GM numbers. These data corroborate our earlier reports in tumor-bearing mice [9,11,16,32,47], and this effect has been interpreted as a consequence of the activity of suppressor cells, particularly macrophages, in the spleen of these animals [48]. Some suppressor cells-derived colony-stimulating factors (CSFs) appear to induce at least two populations of immune suppressor cells. One population consists of mature macrophages, whose expansion becomes stimulated by the GM-CSF itself. A second population consists of less mature suppressor cells with a null phenotype, which appear in the bone marrow as a result of myelopoietic stimulation [49,50]. The connection between these findings becomes even more relevant considering the general association between obesity and several cancers, suggesting the presence of common underlying biological mechanisms. Different aspects of the pathophysiology of obesity, namely insulin resistance, adiposity, and low-grade chronic inflammation, may facilitate a cancer-promoting state [51]. These findings seem to indicate that the presence of EMH in obese mice might be an early indicator of the immunosuppressive environment fostered by immunosuppressive cells, being therefore a potential conduit by which components of obesity may increase risks for cancer. Importantly, treatment with *Chlorella* further increased CSA titers in obese mice, which was consistent with the restauration of normal production of CFU-GM in the bone marrow and prevention of splenic EMH. It is well known that the persistent elevation of CSF levels serves as a continuing stimulus that supports the survival, proliferation, differentiation, and end cell function of granulocytes and monocytes [52].

Robbins et al. [53] studied extramedullary hematopoiesis using a murine model of atherosclerosis, a chronic disease characterized by the accumulation of lipids and leukocytes in the arterial vessel wall [54-56], whose pathological mechanisms recapitulate many features of the inflammatory processes at work in obesity [4]. The authors demonstrated that hematopoietic progenitors progressively relocate from the bone marrow to the GM-CSF– and IL-3–rich splenic environment, where they clonally expand and differentiate into inflammatory monocytes and accumulate in lesions, giving rise to macrophages in the atheromata. Eventually, they ingest lipids and become foam cells, thus indicating that extramedullary sites supplement the hematopoietic function of the bone marrow by producing circulating inflammatory cells that infiltrate and generate lesions. In this context, our findings of a reduced percentage of GMP in the bone marrow, and increased expression of CCR2 on the surface of GMP cells in the spleen of obese mice corroborate these results. It is well known that monocytes, stem cell and progenitor cells are recruited to sites of inflammation via activation of this surface receptor by chemoattractant proteins [57,58]. Importantly, treatment with *Chlorella* restored GMP percentage in the marrow and CCR2 expression on GMP cells in the spleen to normal values.

With the purpose to demonstrate some additional mechanism by which the alga produces its modulating effects, we evaluated the production of serum IL-6, a multifaceted, pleiotropic cytokine that is a central player in the regulation of inflammation, hematopoiesis, immune responses, and host defense mechanisms [59]. In addition it is well known that increased systemic IL-6 expression is related with insulin resistance by impairing IRS1 phosphorylation [59-61]. Our results demonstrate that in obese mice serum IL-6 levels are significantly increased, thus corroborating previous findings in the literature [60]. Treatment of obese mice with *Chlorella* reduced to control values the increased levels of IL-6 in serum, which was consistent with our previously published findings showing the ability of the alga to reduce free fatty acid levels and prevent the development of insulin resistance in obese mice by increasing the phosphorylation levels of proteins such

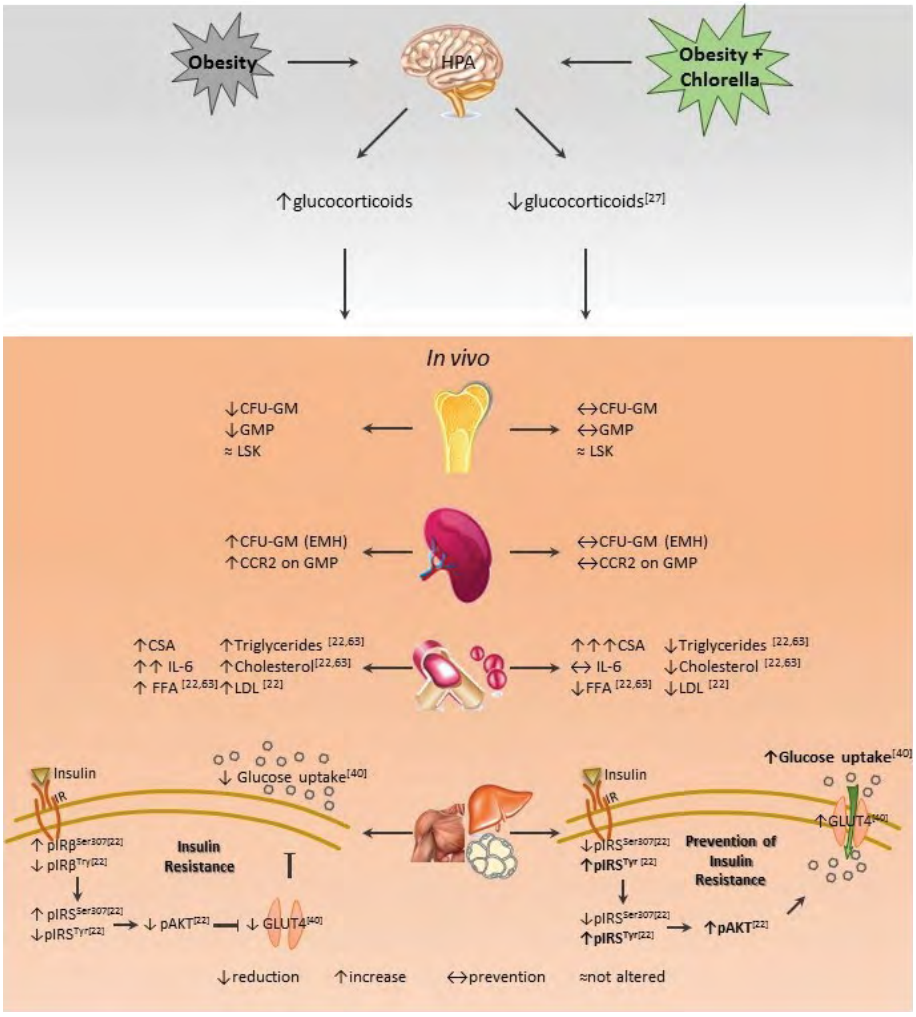

Figure 6: Review of our findings and those in the literature of the effects of Chlorella in obese mice. In the bone marrow, Chlorella restores to normal levels the production of CFU-GM and the number of GMP population. In the spleen, Chlorella prevents EMH and restores increased CCR2 expression on GMP cells. In the blood, Chlorella further increased CSA, reduced to normal levels IL-6 and restores to normal levels free fatty acid, triglyceride, cholesterol and LDL. In the liver, adipose tissue and muscle, Chlorella increased the phosphorylation in tyrosine levels of proteins such as IR, IRS-1, increasing the phosphorylation in Akt, translocation of GLUT-4 and increasing glucose uptake, thus contributing to prevention of insulin resistance. *Abbreviations*: BM: bone marrow; CFU-GM: number of colony-forming units of granulocytes and macrophages; CSA: colony-stimulating activity; LSK: primitive hematopoietic cells ($Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$); GLUT4: glucose transporter type-4; GMP: granulocyte and macrophage progenitor; CCR2: C-C chemokine receptor type 2; IL: interleukin; FFA: free fatty acid levels; LDL: low density lipoproteins.

as IR, IRS-1 and Akt [22]. Moreover, related to the fact that IL-6 has been implicated as a marker for visceral adiposity [62], recent studies [63] demonstrate the ability of *Chlorella* to modulate adipose tissue hypertrophy.

Altogether, our findings suggest that adjuvant colony-stimulating factors produced by *Chlorella* treatment, which act synergistically for highly enriched numbers of CFU-GM in combinations of modulatory cytokines, may inhibit suppressive effects of inflammatory processes on critical pools of hematopoietic progenitor cells leading to appropriate production and efficient mobilization of early immune cells, which are crucial to the performance of the surveillance as well as the effector functions in the organism.

Other findings in the literature support the ability of *Chlorella* to offset the major pathogenic mechanism underlying obesity. Hasegawa et al. [27] demonstrated the ability of the alga to inhibit the elevation of serum glucocorticoids induced by stress. It is well known that these hormones contribute to insulin resistance by counteracting insulin, promoting hyperglycemia-causing hepatic gluconeogenesis, inhibiting the peripheral utilization of glucose and impairing insulin-stimulated translocation of glucose transporter type-4 (GLUT-4) [64]. In this context, findings showing the ability of *Chlorella* to increase the expression of GLUT-4 are relevant. Moreover, the alga was demonstrate to attenuate oxidative stress by increasing antioxidant processes, thus suppressing inflammatory activation in peritoneal macrophages and liver of mice fed on an atherogenic diet, reducing DNA damage and lipid peroxidation in diabetic rats [65,66]. These findings support the ability of the alga to offset the increased oxidative stress observed during obesity, which is correlated to fat accumulation, representing a major pathogenic mechanism underlying the disease [67].

In conclusion, our pioneer findings showing the ability of the alga to modulate the shift in hematopoietic topographical hierarchy during inflammation are likely to have significant biological, diagnostic, and therapeutic implications in the treatment of insulin resistance. A summary of our findings and those in the literature mentioned here of the effects of *Chlorella* in obese mice is presented in Figure 6.

Acknowledgements

Fundação de Amparo à Pesquisa (FAPESP) (Proc.2014/10634-0, 2011/50903-1, 2010/50100-3), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Proc. 301006/2015-6) for financial support, and Juliana F. Vecina for technical contribution.

Conflict of Interest

The authors declare that they have no conflict of interest.

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