Case report

In vivo induction of the autophagic machinery in human bone marrow cells during Leishmania donovani complex infection

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1. Introduction

Autophagy is a self-catabolic pathway that preserves cellular homeostasis through lysosomal digestion of macromolecules and organelles. It promotes cell survival in periods of stress and starvation by providing nutrients and energy and by removing damaged or toxic proteins and organelles [1,2]. Even though it is thought to represent a survival process, autophagy induces a non-apoptotic cell death if not properly regulated [3]. A large spectrum of diseases has been associated with autophagy, rendering it intriguing to investigate its role in both physiological and ill-stated conditions [4].

Microtubule-associated protein 1 Light Chain 3 (MAP1-LC3) and Autophagy

Bone marrow macrophages

LC3B

Autophagy is a homeostatic process promoting cell survival in periods of stress. The induction of the autophagic machinery has also been implicated in both innate and adaptive immunity. Leishmania donovani, which is the causative pathogen of visceral leishmaniasis, is an intracellular parasite that invades and multiplies in bone marrow macrophages. We describe the induction of host cell autophagic machinery during acute natural bone marrow infection by L. donovani complex, detected by LC3B immunoblot. The successful treatment with liposomal amphotericin B resulted in the resolution of this phenomenon. Even though the role of autophagy in parasite biology has been previously studied, our findings show for the first time the in vivo host cell LC3B conversion as a marker of the induction of the autophagic machinery during infection with Leishmania parasite in real time conditions.

2. Brief report

A 63-year-old male patient, who was complaining for fever, weight loss and malaise for a period of ten months, referred to our Department for the evaluation of his pancytopenia.
Bone marrow examination revealed aplasia under the presence of abundant *Leishmania* parasites in both intracellular (inside macrophage vacuoles) and extracellular sites (Fig. 1A). The majority of bone marrow macrophages (nearly 95% of total macrophages) were infected. *L. donovani* existence was confirmed using PCR amplification method according to Disch J et al. [13]. Thus, the diagnosis of visceral leishmaniasis was set and liposomal amphotericin B was administered to the patient at a dose of 1 mg kg\(^{-1}\) day\(^{-1}\) for 21 days. At the end of the treatment course, bone marrow was free of parasites and its morphology was partially restored. In addition, bone marrow macrophages with multiple vacuole-like formations free of parasites were observed (Fig. 1B). The percentage of vacuolated macrophages ratio was about 80%. However, because of the persistence of low fever and IgM hypergammaglobulinemia, a second treatment course with liposomal amphotericin B at the same dose was decided. The third bone marrow examination, performed two months later, did not reveal any abnormal morphological characteristics in macrophages, while there were no symptoms or laboratory findings suggesting resistance of the disease.

Prompted by both recent publications which implicated the autophagic mechanism in the defense against intracellular pathogens and the presence of inclusions resembling autophagy-like vacuoles in bone marrow aspirate (Fig. 1B), we comparatively examined bone marrow specimens before and after the first treatment course as well as after remission, for the presence of increased autophagic flux. In order to detect the autophagic process, we estimated the LC3B-I to LC3B-II conversion by immunoblot. Bone marrow mononuclear cells were isolated with single layer centrifugation, as previously described [14]. Cell lysates were electrophoresed in 13% SDS-PAGE gel and transferred to PVDF membrane. LC3B-I conversion to LC3B-II was detected with a specific polyclonal Ab (LC3B Antibody #2775, Cell Signaling). Gel Pro Analyzer 3.1 was used for the densitometric analysis of the scanned immunoblots. The integrated optical density (IOD) of each blot lane was measured and the results were plotted.

A higher LC3B-II/LC3B-I ratio was observed in the sample collected before the initiation of the treatment with liposomal amphotericin B (Fig. 2, lane and bar II). In addition, the sample collected after the end of the first treatment course (Fig. 2, lane and bar III) showed slightly up-regulated autophagic process compared to control bone marrow specimens (Fig. 2, lane and bar I, lane and bar IV) and the sample collected after the administration of the second treatment course and the successful treatment (Fig. 2, lane and bar V).

### 3. Discussion

*L. donovani* is an obligate intracellular parasite that survives, differentiates and multiplies in the reticulo-endothelial system macrophages. Autophagy has been proven a central cell cycle mechanism of several parasites [15–17], playing a crucial role in the differentiation and virulence of the pathogens [17], while the induction of host cell autophagic machinery has been recently correlated with increased

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**Fig. 1.** May Gruvald–Giemsa staining of bone marrow macrophages. (A) Presence of intracellular (bold line) inside vacuoles (dash line) and extracellular leishmania (light line) parasites in the bone marrow before the initiation of the first treatment course. (B) Vacuolated bone marrow macrophages after the end of the first treatment course.

**Fig. 2.** Western blot analysis of LC3B-I to LC3B-II conversion in cell lysates. A. Western blot analysis data indicate an increased autophagic activity in bone marrow cells of the patient before the administration of the treatment [II]. After the end of the first treatment course the LC3B-II/LC3B-I ratio was remarkably decreased [III]. The LC3B-II/LC3B-I ratio of the sample collected after the administration of the second treatment course, which resulted in successful treatment, [IV] was comparable with bone marrow cell lysates from individuals negative for Leishmania infection; representative data [I, IV]. B: LC3B-II to I ratio as measured by densitometry. (I: control sample, LC3BII/I: 0.45, II: sample collected before the administration of treatment, LC3BII/I: 6.73, III: sample collected after the administration of the first treatment course, LC3BII/I: 0.65, IV: control sample, LC3BII/I: 0.22, V: sample collected after successful treatment, LC3BII/I: 0.16).
parasite load of *L. amazonensis* in macrophages [8]. However, to the best of our knowledge, the induction of host cell autophagy during Leishmania infection has not been reported, till now.

In this manuscript, we describe the induction of the autophagic machinery during natural bone marrow infection by *L. donovani*, by demonstrating the conversion of LC3B-I to LC3B-II during acute infection. Clinical improvement, regulation of the autophagic process and macrophage morphology exhibited parallel evolution during the course of the disease. After the treatment with liposomal amphotericin B and the elimination of parasites from the bone marrow, LC3B-II/LC3B-I ratio was still greater (1.44 fold) compared to control samples. At that point, the presence of macrophages with inclusions resembling autophagy-like vacuoles was the only abnormal morphological characteristic, which could indicate a possible activation of the autophagic process.

In conclusion, we report, for the first time, the induction of host cell autophagy during Leishmania natural infection. Considering that autophagy pathway has a dual role in both in host cell, promoting pathogen clearance or pathogen survival in host cells, LC3B immuno-blot or other assays for monitoring autophagy might provide useful information for the host cell-intracellular pathogen interaction during the course of a natural infection. The definition of this role would be the first step for the investigation of the impact that pharmacological interventions in the autophagic machinery could have in the defense against pathogens.

References