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Time course of histomorphological changes in adipose tissue upon acute lipoatrophy

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Abstract *Background and aims:* Crown-like structures (CLS) are characteristic histopathology features of inflamed adipose tissues in obese mice and humans. In previous work, we suggested that these cells derived from macrophages primarily involved in the reabsorption of dead adipocytes. Here, we used a well-characterized transgenic mouse model in which the death of adipocytes in adult mice is inducible and highly synchronized. In this "FAT ATTAC" model, apoptosis is induced through forced dimerization of a caspase-8 fusion protein.
Methods and results: 0, 0.5, 1, 2, 3 and 10 days post induction of adipocyte cell death, we analyzed mesenteric and epididymal adipose depots by histology, immunohistochemistry and electron microscopy. Upon induction of caspase-8 dimerization, numerous adipocytes lost immunoreactivity for perilipin, a marker for live adipocytes. In the same areas, we found adipocytes with hypertrophic mitochondria and signs of organelle degeneration. Neutrophils and lymphocytes were the main inflammatory cells present in the tissue, and the macrophages were predominantly Mac-2 negative. Over the course of ablation, Mac-2 positive macrophages substituted for Mac-2 negative macrophages, followed by CLS formation. All perilipin negative, dead adipocytes were surrounded by CLS structures. The time course of histopathology was similar in both fat pads studied, but occurred at earlier stages and was more gradual in mesenteric fat.
Conclusion: Our data demonstrate that CLS formation results as a direct consequence of adipocyte death, and that infiltrating macrophages actively uptake remnant lipids of dead adipocytes. Upon induction of adipocyte apoptosis, inflammatory cells infiltrate adipose tissue initially consisting of neutrophils followed by macrophages that are involved in CLS formation.

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Introduction

The chronic subclinical inflammation of adipose tissue in obese mice and humans has been widely described in the literature [1,2]. Macrophages have been identified as a strong mediator of adipose tissue inflammation and dysfunction. Macrophage infiltration into adipose tissue is of clinical importance due to the tight correlation with the onset of insulin resistance [3–7], a major component of the metabolic syndrome [8,9]. While the presence of macrophages may dictate the tissue inflammatory environment subsequent to their infiltration, the underlying causes for the inflammatory phenotype of dysfunctional adipose tissue are not known. We hypothesized that inflammation is a direct consequence of adipocyte death [10]. Visceral adipose tissue is more inflamed during weight gain, suggesting that a higher rate of cell death occurs in the visceral pads compared to subcutaneous depot cells, possibly due to the fact that they are more prone to death [11]. Visceral fat accumulation is therefore more tightly linked to the metabolic syndrome [11–13]. Here we present new experimental data that strongly supports the hypothesis that macrophage infiltration is a primary and direct consequence of adipocyte death.

FAT ATTAC mice are transgenic mice that undergo adipocyte specific apoptosis after dimerizer administration that forces dimerization and activation of caspase-8 [14,15]. This tightly controlled, induced adipocyte apoptosis allows for high temporal resolution of the tissue events resulting from adipocyte death. We compared the morphology, immunohistochemistry and electron microscopy (EM) of two visceral fat depots upon dimerization and collected tissues at 0, 12, 24, 48, 72 h and 10 days after dimerizer to resolve the initiation of immune cell infiltration and CLS formation. We demonstrate that CLS formation occurs specifically at sites of dead adipocytes whose remnants need to be resorbed, a process that occurs primarily by activated macrophages.

Methods

Animals

The FAT ATTAC mice [15](“Fat Apotosis Through Targeted Activation of Caspase-8”) are a transgenic mouse model of inducible fat loss generated through the transgenic expression of a myristoylated caspase 8-FKBP fusion protein in adipocytes. Transgene expression is under the control of the aP2 promoter and is in this particular case expressed only in mature adipocytes [15]. The FKBP moiety was developed by Clackson and colleagues [16] that takes advantage of a mutated version of an FKBP domain that selectively binds a chemical dimerizer (AP20187, an FK506 analog) that force-dimerizes two mutant FKBP domains (but does not bind endogenous FKBP). In the FAT ATTAC mice, treatment of adipocytes with AP20187 (Ariad Pharmaceuticals), force-dimerizes a membrane-associated caspase-8 that activates downstream signaling cascades resulting in selective apoptosis of adipocytes. This is a very specific way to induce apoptosis, since this is the only pathway known to be triggered by caspase-8 [15].

Six- to 8-wk old male FAT ATTAC mice ($N = 11$) in the genetic background of FVB mice and wild type ($N = 8$) were used. Mice were provided ad libitum access to standard chow diet and water and maintained on a 12 h light/dark cycle. AP21087 was delivered on 4 consecutive days at 0.2 μg/g body weight. Mice were sacrificed at the respective time points (7am or 7pm) after 0 (basal), 12, 24, 48, 72 h and 10 days after dimerizer by exsanguination under isofluorane anesthesia. Cervical dislocation was used to verify death before tissues were collected.

Serum adiponectin analysis

Reduced circulating adiponectin was used to verify induction of adipocyte apoptosis. Approximately 10 μL of blood was collected from the tail vein of mice at 2 and 8 days post-dimerization, the plasma was reduced, separated on a Criterion TGX gel (BioRad, Hercules, CA), and immunoblotted with rabbit anti-serum against adiponectin as previously described [17].

Light microscopy

Mesenteric and epididymal fat depots were dissected and fixed by immersion in 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4 overnight at 4 °C, then dehydrated, cleared and paraffin-embedded. Three different biopsies of each depot were analyzed. For each sample, 3 μm-thick serial sections were obtained: the first was stained by hematoxylin & eosin to assess morphology, the others were processed for immunohistochemistry. Tissue sections were observed with a Nikon Eclipse E800 light microscope using a ×20 objective at ×200 final magnification, and digital images were captured with a Nikon DXM 1200 camera.

Immunohistochemistry

For immunohistochemistry 3-μm dewaxed serial sections were incubated with anti-Mac-2/galectin-3 (Cedarlane Laboratories, Canada) and anti-perilipin (kindly provided by Andy Greenberg, Tufts University, Boston, USA) primary antibodies according to the ABC method [18]. We used 3% hydrogen peroxide to inactivate endogenous peroxidase followed by normal goat or horse serum to reduce non-specific staining. Consecutive serial sections were incubated overnight (4 °C) with anti-Mac-2/galectin-3 (1:1000) and anti-perilipin (1:50) primary antibodies. Biotinylated HRP-conjugated secondary antibodies were goat anti-rabbit IgG (perilipin) and horse anti-mouse IgG (Mac-2/galectin-3; Vector Laboratories; Burlingame, CA, USA). Histochemical reactions were performed using Vector’s Vectastain ABC Kit and Sigma Fast 3,3′-diaminobenzidine as substrate (Sigma, St Louis, MO, USA). Sections were counterstained with hematoxylin.

Morphometry

1) CLS density was quantified by counting the total number of CLS in each section compared with the total number of adipocytes and was expressed as CLS number/10,000 adipocytes.