extinguishing metaflammation: mechanisms and therapeutic opportunities for immunological control of metabolic dysfunctions

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CHAPTER 1

General introduction

Obesity, type 2 diabetes and metaflammation

Chronic imbalance between energy intake and expenditure results in overweight and obesity. These conditions are defined by elevated body mass index (BMI; 25-29.9 kg/m^2 and >30 kg/m^2, respectively) as a gauge for adiposity and predispose for developing co-morbidities like type 2 diabetes, cardiovascular diseases, and some forms of cancer. Although preventable, obesity prevalence has reached epidemic proportions, afflicting over 650 million adults as of 2016 (1), and over 450 million suffered from type 2 diabetes in 2019 (2). Lifestyle interventions - encompassing either dietary, physical, behavioral, or a combination - have been proven efficacious in reducing obesity-associated risks and improving quality of life, yet post-intervention weight maintenance remains a considerable challenge (3). In fact, although mechanistically incompletely understood (4-6), formerly obese individuals are at risk for accelerated post-dieting weight regain (7). Although type 2 diabetes and other obesity-induced metabolic dysfunctions are drug-treatable, most entail symptomatic treatments, and new, innovative therapeutic strategies are still required to both provide alternatives to conventional medicine and act in concert with lifestyle interventions for alleviating disease.

Understanding the pathophysiology of obesity-induced metabolic dysfunctions may assist in developing such new therapeutic strategies. Chronic nutritional overload causes adipocyte hypertrophy and hyperplasia that eventually results in white adipose tissue (WAT) dysfunction, at least partly through hypoxia-induced adipocyte cell death (8, 9). This is believed to trigger inflammation, where recruited immune cells produce proinflammatory cytokines that generate a vicious circle exacerbating inflammation, as well as inhibit adipocyte canonical insulin signaling and promote lipolysis-derived fatty acid efflux. These events promote ectopic lipid deposition in skeletal muscle and the liver, contributing to development of non-alcoholic fatty liver disease (NAFLD). This disruption of skeletal muscle and liver homeostasis promotes local inflammation, hepatic gluconeogenesis and tissue-specific insulin resistance via proinflammatory cytokines and lipotoxicity, together contributing to whole-body insulin resistance and development of type 2 diabetes (10, 11) (**Figure 1**). Hence, type 2 diabetes is driven by so-called chronic, low-grade inflammation particularly in metabolic tissues, also coined metaflammation (12).

Conceivably, most research on metaflammation has focused on WAT, as this is considered the etiological origin of an inflammatory cascade that impairs whole-body insulin sensitivity. It is well-established that cells of both the innate and adaptive immune system accumulate in WAT during obesity and are either associated with or drive insulin resistance (13, 14). A key paradigm herein is the recruitment of monocytes through the monocyte chemoattractant protein 1 (MCP-1)-CCR2 axis and subsequent development into proinflammatory macrophages upon encountering the WAT inflammatory milieu (15-17). .

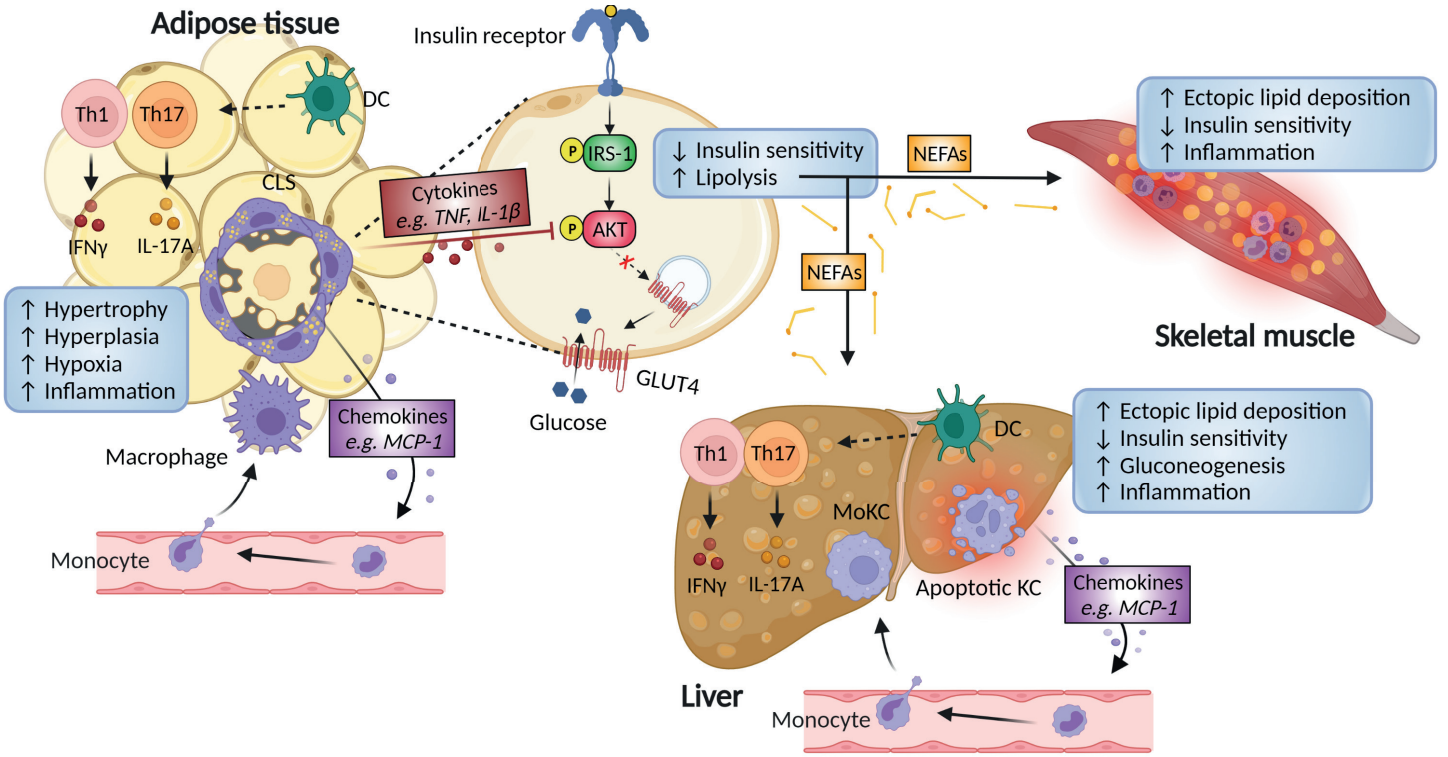

Figure 1. Pathophysiology of obesity-induced metabolic dysfunctions. See text for details. Th: T helper, DC: dendritic cell, IFNγ: interferon gamma, IL: interleukin, CLS: crown-like structure, TNF: tumor necrosis factor, MCP-1: monocyte chemoattractant protein 1, IRS-1: insulin receptor substrate 1, GLUT4: glucose transporter 4, NEFAs: non-esterified fatty acids, KC: Kupffer cell, MoKC: monocyte-derived KC. Created with BioRender.com.

Macrophage-derived proinflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin (IL)-1β, were shown to inhibit tissue-specific insulin signaling (18, 19) (**Figure 1**). Similarly, activation and impaired survival of Kupffer cells (KCs), the liver-resident macrophages, and recruitment of proinflammatory monocyte-derived KCs contribute to NAFLD pathogenesis and progression towards non-alcoholic steatohepatitis (NASH) (20-22) (**Figure 1**). Although well-established, the environmental and cellular changes dictating proinflammatory macrophage activation in the context of obesity are hitherto not fully understood, and identifying new mechanisms may provide novel therapeutic targets or strategies

In addition to macrophages, dendritic cells (DCs) were also shown to contribute to obesity-induced metabolic dysfunctions (23-25). DCs are specialized antigen presenting cells that bridge the innate and adaptive immune system by governing T cell responses dependent on the inflammatory and metabolic context, both aiding in defense against pathogens and maintenance of immune homeostasis. Obesity promotes DC accumulation in metabolic tissues, and mice lacking DCs or with impaired DC migration are protected against metaflammation and insulin resistance (23-25). Furthermore, both WAT and liver are populated by different T cell subsets. Here, interferon (IFN)-γ-producing $CD4^+$ T helper 1 (Th1) cells and IL-17A-producing Th17 cells increase during obesity, and are considered to contribute to insulin resistance (as reviewed in (26) and (27)). The T cell priming capacity of DCs is increasingly recognized to be driven by their cellular metabolic rewiring, facilitating co-stimulatory marker and cytokine expression necessary for skewing T cell differentiation (28). Accordingly, the metabolic microenvironment of DCs has considerable impact on its T cell priming functions (29). However, the mechanistic underpinnings of DC-mediated T cell priming in metabolic tissues in the context of obesity are yet incompletely understood.

Type 2 immunity, metabolic homeostasis and parasitic worms

During homeostasis, the maintenance of insulin sensitivity in metabolic tissues is under the control of the immune system. Specifically in lean WAT, Th2 cells and type 2 innate lymphoid cells (ILC2s) produce the canonical type 2 cytokines IL-4, IL-5 and IL-13, of which IL-5 maintains WAT eosinophil homing (30). These eosinophils are the principal producers of IL-4, and together with Th2 and ILC2-derived IL-4 and IL-13 promote alternative activation of macrophage through IL-4Rα and/or IL-13Rα1/2-mediated activation of the transcription factor STAT6 (30-33). Since tissue macrophages are well-established sentinels of homeostasis (34, 35) and this immunological circuit appears to culminate in alternative activation of macrophages, these cells are considered the effector cells that maintain tissue

insulin sensitivity (**Figure 2**). Although not as extensively studied, similar processes are believed to also contribute to maintenance of insulin sensitivity in the liver (36). One could imagine that restoring this impaired type 2 immunity environment in obese individuals may restore insulin sensitivity and mitigate obesity-induced metabolic dysfunctions.

Parasitic helminth worms are the strongest natural inducers of type 2 immunity (37). Indeed, cross-sectional studies have shown that individuals living in helminth-endemic areas are less likely to develop metabolic dysfunctions (38), and pharmacological elimination of helminths worsened metabolic parameters associated with insulin resistance (39, 40), indicative of an inverse correlation between helminth infection and metabolic dysfunctions. In line with this, experimental infection of obese mice with different helminth species (31, 41-43), as well as treatment with helminth-derived immunomodulatory molecules (41, 42, 44), induced type 2 immunity in metabolic tissues and improved whole-body insulin sensitivity. Together, this suggests that type 2 immunity, induced by helminths or their molecules, may hold promise in treatment of metabolic disorders, although causality and underlying mechanisms remain to be established.

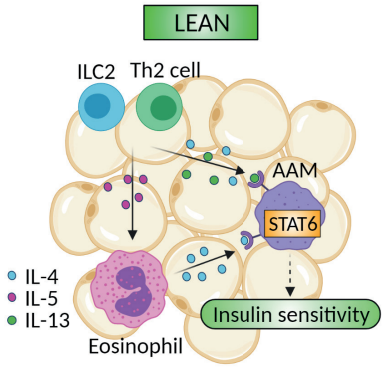

Figure 2. Type 2 immunity in lean WAT. See text for details. AAM: alternatively-activated macrophage. Created with BioRender.com

Thesis outline

Immunometabolism is a rapidly evolving new field at the intersection of immunology and metabolism that consists of two inter-related branches: [1] cellular immunometabolism, *i.e.* how intracellular metabolism dictates immune cell function; and [2] systemic immunometabolism, *i.e.* how immune cells control tissue-specific and whole-body metabolic homeostasis. The work presented in this thesis mainly focuses on how immune cells regulate tissue-specific and whole-body metabolic homeostasis in various experimental contexts. To

this end, we mainly used preclinical mouse models of obesity, insulin resistance and type 2 diabetes to dissect the underlying molecular mechanisms and explore various therapeutic strategies.

In part 1, we investigate the molecular mechanisms involved in the control of metabolic homeostasis by myeloid cells. **Chapter 2** is an example of cross-fertilization of the two arms of immunometabolism, investigating whether manipulation of macrophage metabolism affects immune cell function and, consequently, whole-body metabolism. Here, we investigate the effects of macrophage-specific deletion of ATP citrate lyase (Acly), a metabolic enzyme linking cellular metabolism to immune cell function, on inflammatory disorders such as obesity-induced type 2 diabetes. **Chapter 3** describes the discovery of a new role for the soluble form of the mannose receptor, a cell surface receptor involved in antigen binding and internalization, in the regulation of macrophage proinflammatory activation and metabolic homeostasis. Next, **chapter 4** summarizes and discusses the impact of the mannose receptor and other family members of the C-type lectins on regulating immune cell functions and their effects on metaflammation. Finally, in **chapter 5**, which constitutes another example of a study bridging the two arms of immunometabolism, we investigate how the nutrient sensor liver kinase B1 (LKB1) governs DC function in the context of obesity. Collectively, this section describes novel mechanisms that control metabolic homeostasis, providing potential new leads for therapeutic interventions.

In the second part of this dissertation, we investigate whether immunomodulatory (helminth) molecules improve metabolic dysfunctions of obese mice, and study the underlying mechanisms. **Chapter 6** first provides a literature overview on regulation of metabolic homeostasis by immune cells and the impact of helminths and their immunomodulatory molecules. Among these molecules are the immunomodulatory soluble egg antigens (SEA) of the helminth *Schistosoma mansoni*. In **chapter 7**, we next investigate how *S. mansoni* SEA and ω1, a type 2 immunity-inducing molecule present in *S. mansoni* SEA, may promote insulin sensitivity in a mouse model of diet-induced obesity. Finally, in **chapter 8** we explore the immunometabolic effects of a novel plant extract, named Totum-63 and developed for the treatment of pre-diabetes, on obese mice. Altogether, this part provides new insights into how immunomodulatory (helminth) molecules can regulate whole-body metabolic homeostasis.

To conclude, **chapter 9** highlights the main findings of this thesis and provides suggestions for future research investigating immunological control of obesity-induced metabolic dysfunctions.

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