Role of metabolic pathways and sensors in regulation of dendritic cell-driven T cell responses

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General introduction

Introduction

We are constantly exposed to microorganisms and parasites that can invade our body (i.e. infect) and have the capacity to cause disease (i.e. pathogenic). Yet we rarely become ill. This is because we have developed specialized molecules, cells and tissues that together are involved in our ability to resist infection (i.e. immunity) and collectively are known as the immune system. Immunology is the science that studies the immune system and Edward Jenner is generally recognized to have performed the first immunological experiment. He demonstrated in the late 18th century that inoculation with cowpox could confer protection against its more fatal cousin smallpox. As cowpox is caused by the vaccinia virus ('vacca' is Latin for cow), this procedure was called vaccination, a term now used for any form of immunization with a weakened microorganism or substitute thereof.

It is now well described that immune cells make drastic changes to their biology to transition between different life stages and efficiently deal with the threat of infection. These changes can include activation, growth, multiplication (i.e. proliferation), movement (i.e. migration), specialization (i.e. differentiation), gain of effector/regulatory functions and finally, the generation and maintenance of immunological memory. Over the past 10 years or so, immunologists have come to realize that these processes are underpinned by unique metabolic states, which ignited the field of immunometabolism.

Metabolism is the total sum of life sustaining chemical reactions in organisms and their cells. This can be broadly divided into on the one hand the breakdown of molecules to generate energy (i.e. catabolism) and on the other hand the synthesis of complex molecules from simple molecules (i.e. anabolism). The latter provides fundamental building blocks for the cell. Seminal work by Chawla and colleagues in 2006 [1] and Rathmell and colleagues in 2011 [2] showed that immune cells on opposite sides of the immunological spectrum – that is to say immune cells which are associated with the initiation and maintenance of inflammation (i.e. effector cells) versus immune cells which are associated with resolution or suppression of inflammation (i.e. regulatory cells) - can be distinguished based on their metabolic profiles. More importantly, they showed that gain of effector functions was dependent on breakdown of glucose into lactate when oxygen is abundant (i.e. aerobic glycolysis) followed by the synthesis of complex molecules, while the gain of regulatory functions was dependent on the breakdown of fatty acids to generate energy through oxidative phosphorylation (i.e. fatty acid oxidation or beta oxidation). This sparked the idea that immunity could be boosted by enhancing aerobic glycolysis in immune cells or diminishing their beta-oxidation, while immune

responses could be dampened by promoting beta-oxidation in immune cells or decreasing their aerobic glycolysis.

Dendritic cells (DCs) play a crucial role in the initiation and maintenance of effector T cell responses and regulatory T cell responses. They do so by providing antigen (signal 1), co-stimulation (signal 2) and cytokines (signal 3) to T cells. The strength of these combined signals determines whether the T cell becomes activated or unresponsive (i.e. anergic) and in the case of CD4+ T cells, the nature of these combined signals also determines whether the T cells becomes anti-bacterial/viral IFN producing T helper 1 (Th1) cells, anti-parasitic IL-4-producing T helper 2 (Th2) cells, anti-fungal/yeast IL-17 producing T helper 17 (Th17) cells or immune-suppressive regulatory T cells (Tregs). Effector CD8+ T cells are also known as cytotoxic T lymphocytes (CTLs) because they are specialized in secreting molecules that are toxic to cells (cyto = prefix meaning cell) which have become infected or cancerous.

Pioneering work by Pearce and colleagues in the early 2010s [3, 4] showed that the activation of inflammatory DCs is dependent on aerobic glycolysis. However, little was known about the metabolic properties of different DC subsets. Another unanswered question was if DCs with different T cell polarizing properties - that is to say they preferentially skew T cells towards a specific specialization (e.g. Th1 over Th2) - rely on distinct metabolic characteristics for their T cell polarizing ability. Moreover, while there has been a long-standing interest in understanding how the mechanistic target of rapamycin (mTOR) - a master regulator of anabolic metabolism [5] - controls DC biology and function, its role in shaping DC-mediated T cell responses is far from unambiguous [6]. In addition, there have been very few studies interrogating the role of AMP-activated protein kinase (AMPK) - a master regulator of catabolic metabolism and a negative controller of mTOR [7] - or its upstream activator liver kinase B1 (LKB1) in DC biology [8]. This is in part because no pharmacological inhibitors of AMPK and LKB1 were available.

Although it has been six years since the first major review on DC metabolism was published [9], its conclusion is as relevant as ever: “It will be important to more fully characterize how metabolism controls the immune priming function of DCs and whether metabolic manipulation of DCs can be used to alter their immune polarizing properties."

Thesis outline

This thesis aims to characterize the metabolic programs that DCs rely on for activation and polarization of different T cell responses, as well as elucidate the roles of mTOR and AMPK therein. As DCs play a central role in the establishment of protective immune response to infection and after vaccination and are critical regulators of tolerance to host self-antigens, manipulation of DC function through manipulation of DC metabolism may be an attractive therapeutic strategy.

Chapters 2 and 3 serve as extended introduction to this thesis, as they lay the theoretical and practical groundwork for the subsequent chapters. In **Chapter 2**, an updated overview is provided on publications that investigate the link between DC metabolism and their T cell-polarizing capacities. In **Chapter 3**, the workings of extracellular flux (XF) analysis, the method that revolutionized the field of immunometabolism a decade ago, are explained, and recommendations for XF analysis of DCs are given.

Chapters 4 to 7 explore and compare the metabolic profiles of *in vitro*-cultured DCs that preferentially prime either Th1 cells, Th2 cells, Th17 cells or Tregs after stimulation with known antigens or immunomodulatory compounds. In **Chapter 4**, the contribution of intracellular glycogen stores to early metabolic reprogramming of activated murine DCs is evaluated. In **Chapter 5**, metabolism is both the beginning and the end, as metabolic and other changes in human DCs are assessed after stimulation with tolerogenic/regulatory short-chain fatty acids. **Chapter 6**, summarizes and discusses the studies that have interrogated the role of cellular metabolism in controlling the function of various type 2 immune cells, including that of Th2-priming DCs. In **Chapter 7**, transcriptomic and metabolomic data are integrated to provide new insights in the poorly understood metabolic reprogramming of human DCs for Th2 priming. Together, these chapters paint a picture of how DCs with different T cell-polarizing properties can have unique metabolic profiles that might selectively be targeted to alter the nature of the ensuing T cell response.

In Chapters 8 and 9 the *in vivo* role of the LKB1/AMPK pathway and mTOR complex 1 (mTORC1) signalling in DC driven T cell responses is interrogated. Advantage was taken of murine models with DC-specific deletions of these master regulators of metabolism, which allowed the characterization of how DC metabolism shapes the adaptive immune response *in situ*. In **Chapter 8**, mice are studied with DCs that are deficient in LKB1 and are therefore expected to display defects in catabolic metabolism. In **Chapter 9**, mice are examined with DCs that are deficient in raptor, a component of mTORC1, and are

therefore expected to display defects in anabolic metabolism. Together these two chapters reveal that the general dogma of anabolic metabolism = pro-inflammatory versus catabolic metabolism = anti-inflammatory may not be generally applicable to DCs *in vivo*. Instead, the immunological outcome of metabolic editing of DCs seems to be greatly influenced by DC subset and tissue localization.

Finally, **Chapter 10** serves as a conclusion to this thesis, as it relates and integrates the work presented herein to studies on DC-driven T cell responses and other works on immunometabolism. It is moreover a representation of the topics the defendant wants to explore in the future and which he thinks may have potential to further mature the field of DC metabolism.

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