Metabolism and lipid mediators as regulators of innate immune cell function: implications for inflammation and immune responses

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Appendix

English Summary
Nederlandse Samenvatting
Resumo em Português
Curriculum Vitae
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English Summary

Background

The immune system is the main line of defence against the millions of potential pathogens and other dangers, such as possible cancerous cells and allergens, humans are exposed to on a daily basis. This system is a complex and intricate network of many different molecules, cells, tissues, and organs, all working in tandem, in a tightly regulated manner, albeit with different functions and mechanisms of action. The immune system can be divided into two main parts – the innate immune system and the adaptive immune system. The innate immune system acts in a fast and broad manner, with general specificity, being activated by generically conserved molecular patterns, such as PAMPs (pathogen-associated molecular patterns), which are present in pathogens/microbes and DAMPs (damage-associated molecular patterns), which are released by our own cells where they are damaged and/or attacked. Conversely, the adaptive immune system, although slower to act, is highly precise and efficient, recognizing a specific antigen, thus being able to zone-in on an individual type of pathogen or molecule.

However, without the initial input of the innate immune system, the adaptive immune system cannot be activated. This makes the innate immune system a key player in the overall immune response. Therefore, studying the mechanisms of action which govern innate immune cell function poses as an imperative endeavour to understand how we could enhance the activity of our immune system for our advantage, such as by improving the immune response against infections, increasing vaccine efficacy or boosting the anti-tumoral response. Conversely, knowing how to introduce a brake on immune responses could be important as well, such as in certain hyperinflammatory (e.g. severe COVID-19), or autoimmune diseases (e.g. rheumatoid arthritis), characterized by chronic inflammatory responses against our own antigens (also called self-antigens), which happens because our own immune cells start attacking our own body, due to loss of immune tolerance – in simple terms, our immune cells start seeing our own body as a threat which needs to be eliminated.

Two key cell types of the innate immune system are macrophages and dendritic cells (DCs), two main types of myeloid cells (Fig. 1). Macrophages are tissue resident immune cells responsible for the maintenance of homeostasis of tissues. In response to inflammatory signals, they can adopt pro-inflammatory functions characterized by the synthesis of pro-inflammatory cytokines (e.g. TNF and IL-6). A few examples of classical inflammatory signals in macrophages are:

- Stimulation of toll-like receptors (TLRs) by PAMPs and DAMPs, which activate different inflammatory pathways, depending on the TLR being stimulated;
- Binding of the Fc portion of antibodies (commonly called the "tail" of the *Y-shaped* antibody (Fig. 1)) to Fc receptors (FcR) present on the macrophages;

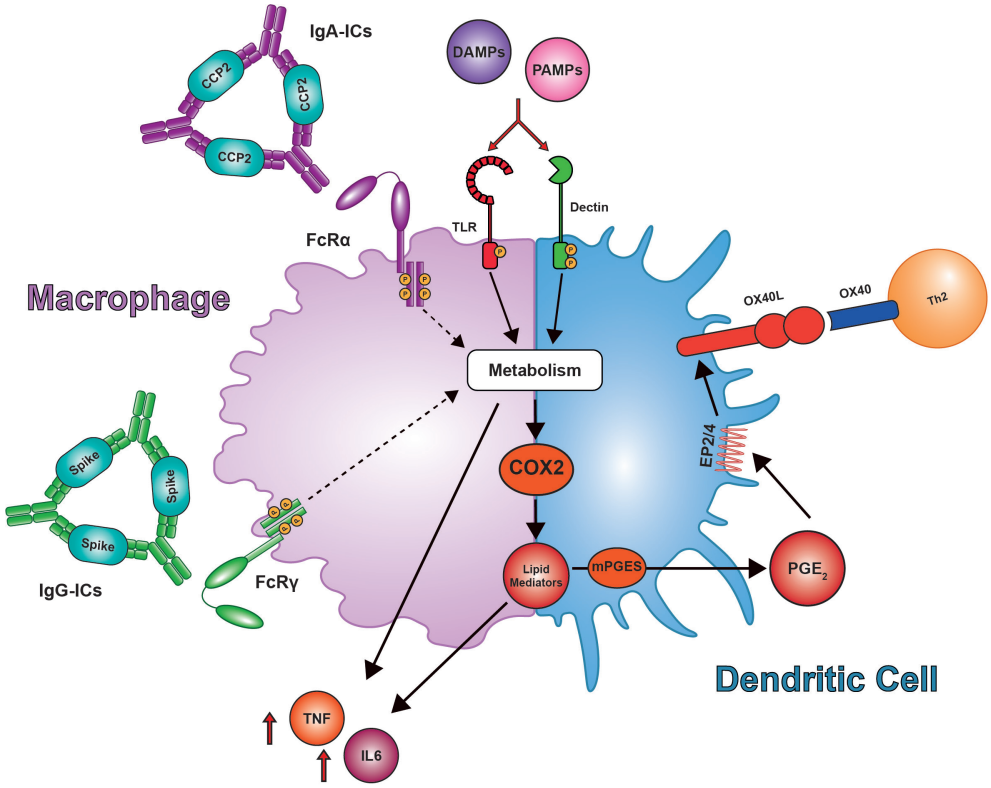

Figure 1: **Metabolism and lipid mediators play a central role in innate immune cell function.** Extrinsic stimuli induce metabolic changes in both macrophages and dendritic cells via PRRs (such as TLRs and Dectin), and/or via FcRs. These metabolic changes regulate the cellular immune response, via synthesis of COX2-dependent lipid mediators, and proinflammatory cytokines.

Predictably, these two stimuli can synergise and amplify the inflammatory phenotype of macrophages, thus mounting a stronger and more robust response against a pathogen, in cases where the Fc portion of the antibody engages an activating FcR.

While today it is known that macrophage differentiation and activation is a highly plastic process that exists on a spectrum, the two extremes of that spectrum are highly pro-inflammatory macrophages, commonly referred to as "M1-like", and anti-inflammatory macrophages/macrophages that promote the resolution of the inflammatory process (i.e. pro-resolving macrophages), commonly referred to as "M2-

like". In general, macrophages with the M1-like phenotype are tasked with patrolling and killing pathogens, through a process called phagocytosis and by the production of pro-inflammatory cytokines, while the M2-like promote wound healing, by producing anti-inflammatory cytokines, tissue regeneration and by engulfing dead cells, or cells undergoing cell death (i.e. apoptosis), through a process called efferocytosis.

On the other hand, the main function of DCs is to induce and direct the differentiation and activation of the adaptive immune system, especially through the presentation of antigens to T cells. Once DCs encounter an antigen, be it from an outside invader, such as a pathogen, or from an internal source (e.g. self-antigen or cancerous cell), they will undergo specific changes in activation, and either become immunogenic or tolerogenic. Immunogenic DCs will induce the differentiation and activation of cytotoxic CD8+ T cells, and/or CD4+ T helper cells, such as Th1, Th2 or Th17 cells, all with specific functions tailored to counter specific pathogens and immune challenges. Whereas tolerogenic DCs will instead induce the differentiation and activation of Treg cells, which work as built in immunologic brakes, that inhibit immune responses against a certain antigen, be it a self-antigen, to prevent autoimmune diseases, or against a foreign, but harmless, antigen such as those in foods.

In an impressive display of cellular coordination, the newly activated T cells, will migrate to the specific site where the presented antigen is located, and will oversee the immune response, by producing cytokines which will not only act on the innate immune system, such as the macrophages already present in the site, but also work as beacons to promote further migration of more innate immune cells to help fight against the specific threat, in the case of Th1, Th2 and Th17, or to instead stop the inflammatory immune response and promote tolerance, in the case of Tregs.

Additionally, DCs can also activate Tfh cells (T follicular helper cells), which are intimately involved in initiating and shaping responses by the *other branch* of the adaptive immune system – B cells. After this "activation trinity", between B cells, DCs, and Tfh, B cells will migrate to the specific site the antigen presented by the DCs originated from, where they will then produce and secrete antibodies. These antibodies, usually in the form of Immunoglobulin G (IgG) or Immunoglobulin A (IgA), are able to recognize the antigen and directly bind to it, forming immune complexes, where the *two little arms* of the Y-shaped antibody bind the antigen, while its "*tail*" i.e. its Fc part, sticks out (Fig. 1). These immune complexes are then able to be recognized by macrophages, via Fc receptors, which bind the Fc part, thus helping these macrophages undergo further pro-inflammatory activation. These mechanisms put DCs as the first target in a strategy of immune modulation, since they dictate the activation states of T cells and B cells, while it puts macrophages as the last target, since they are the final actors in this innate-adaptative-innate immunological chain.

One of the more recent fields of study in immunology which tries to understand how to modulate immune responses is immunometabolism. This area arose from the discovery that, depending on the function being performed, immune cells will undergo specific metabolic reprogramming, favouring certain metabolic pathways over others, not only for their energetic needs, but also for the synthesis of crucial metabolites to be used in their activation and differentiation stages. For instance, it was described that, *generally*, pro-inflammatory macrophages and immunogenic DCs rely on glycolysis and fatty acid synthesis, while pro-resolving macrophages and tolerogenic DCs instead rely on fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS). With this knowledge also came the hypothesis that, by modulating macrophage and DC metabolism, one could control their activation state. For example, by inhibiting metabolic pathways favoured by pro-inflammatory/immunogenic phenotypes, and promoting metabolic pathways favoured by pro-resolving/tolerogenic phenotypes, the cell would switch from a pro-inflammatory state to an anti-inflammatory state, and vice-versa.

One crucial pathway at the centre of immunometabolism is lipid metabolism. While lipids have long been seen as molecules for energy storage and building blocks for cell membranes, we now know that is not the case. Lipid metabolism plays a central role in the synthesis of metabolites used by immune cells for their respective functions. For example, fatty acid synthesis can lead to the formation of lipid droplets (small round pools of lipid molecules inside the cell, much like the oil droplets we see on the surface of soups and stews) in macrophages and DCs, which have been shown to play a role in immune functions, such as cytokine production, phagocytosis and antigen presentation. Alternatively, FAO was shown to be important as Acetyl-CoA from fatty acid oxidation can be used for histone acetylation, which is a way for the cell to control which genes are being expressed and/or repressed.

Additionally, lipid metabolism also includes the synthesis of lipid mediators. Lipid mediators are a group of molecules that play a highly important role in cell messaging. As the name suggests, these molecules originate from the oxidation of fatty acids, and act as chemical messengers. These mediators can act on the cell itself (autocrine signalling), on cells close by to the original cell (paracrine) or on cells far away from the cell of origin (endocrine signalling). These mediators are one of the main communicators between immune cells, being able to induce signalling pathways in macrophages and DCs that either promote the synthesis of proinflammatory cytokines and T cell priming, or instead dampen inflammation, by promoting tissue regeneration and immune tolerance. Thus, unravelling the mechanisms which lead immune cells to synthesize these lipid mediators and how these mediators act on the immune cells themselves is crucial to shed a light on possible therapeutic targets for intervention from an immunometabolism point of view.

Nevertheless, much still remains unknown. As mentioned above, *in general*, pro-inflammatory macrophages and immunogenic DCs tend to favour glycolysis and fatty acid synthesis, while anti-inflammatory macrophages and tolerogenic DCs tend to prefer oxidative phosphorylation and fatty acid oxidation. However, some studies have suggested that this immunometabolic dichotomy does not apply to all settings, showcasing that the metabolic reprogramming that macrophages and DCs undergo upon activation is dependent on a panoply of factors, such as cell differentiation state, tissue location, activation stimulus, and timing. Therefore, it is crucial to look at the cellular immunometabolic profile in specific contexts.

This context specificity also holds true for the role of lipid mediators. For example, while in some contexts one lipid mediator (e.g. Prostaglandin E_2) can induce a pro-inflammatory response in macrophages, or drive a Th1-priming response by DCs, in other contexts it can instead induce an anti-inflammatory response and/or drive a Th2 response.

Therefore, considering there is a crucial need to look into the metabolic requirements of macrophages and DCs in specific situations, this thesis aimed to map metabolic profiles and dependencies in different inflammatory and cellular contexts.

Outline of Thesis Chapters:

Chapter 2 constitutes a more in-depth theoretical introduction to this thesis. There you can find a background into the field of immunometabolism in the context of innate immunity, with specific focus on fatty acid metabolism in DCs and macrophages, and how both metabolic profile and immune function are intricately connected. We addressed the current evidence showcasing the importance of fatty acids (e.g. polyunsaturated fatty acids) and lipid mediators (e.g. prostaglandins) in affecting macrophage and DC function and metabolism. We also explored the multifaceted role of fatty acid metabolism in both promoting and inhibiting inflammation, having reached the conclusion that this showcases the need to study each specific context if we want to get a complete overview of the metabolic needs of immune cells and to find a way of efficiently modulating immune responses by targeting metabolism as a therapeutic approach.

In **chapter 3** we studied the role of SARS-CoV-2 anti-spike IgG (Fig. 1) in promoting an hyperinflammatory state in macrophages. IgG achieves this by inducing specific metabolic changes that prime these macrophages for excessive pro-inflammatory cytokine expression. By chemical inhibition of these metabolic pathways we could prevent IgG-induced hyperinflammation. On a similar note, in **chapter 4** we looked at

the role of IgA against CCP2 (citrullinated peptides, which are common self-antigens in the joints (Fig. 1)) in driving chronic inflammation in the context of rheumatoid arthritis. IgA achieves this by inducing a state of hyperinflammation in macrophages that is both dependent on certain metabolic changes and the synthesis of lipid mediators downstream of cyclooxygenase-2, thus identifying druggable targets with possible therapeutic applications in treating auto-antibody-driven chronic inflammatory diseases.

In **chapter 5** we describe how DCs recognize soluble egg antigens (SEA) from the parasitic helminth *Schistosoma mansoni* through Dectin-2 (a receptor on the cell surface of DCs), how they become licensed to induce the differentiation of Th2 cells, and how can DC lipid mediator synthesis be chemically targeted to mould their immune function within this context. It was previously shown that SEA signalled via Dectin-2 in DCs to induce the synthesis of PGE_2 (a type of lipid mediator called prostaglandin, downstream of an enzyme called cyclooxygenase-2). This PGE_2 acted autocrinally on the DCs and promoted the expression of OX40L, which is a molecule that licenses the DCs to prime a Th2 response.

We were able to build on that work and show that Dectin-2 directly binds to SEA in a manner that is dependent on the presence of high mannose glycans (molecules composed of several mannose sugar residues bound to each other). In the absence of these glycans, the binding of Dectin-2 was decreased, along with subsequent synthesis of PGE_2, the expression of OX40L and also the ability to prime Th2. Furthermore, we were also able to show that, when stimulating DCs with SEA, chemically inhibiting the synthesis of PGE_2 also decreased the expression of OX40L and their ability to prime Th2 cells. So, in short, this work provides new insights on how *Schistosoma mansoni* licences DCs to prime a Th2 response, and we were also able to identify possible molecular targets to control helminth-driven Th2 immune responses.

In **chapter 6** I provide an extensive discussion outlining the novel findings of this thesis and delve into future research suggestions to further decode the nuances of immunometabolism in the context of inflammatory responses, and the possible practical applications that this scientific information may have on society as a whole. I expose how lipid metabolism is intimately connected with macrophage and DC function, not only due to the metabolic reprogramming these cells undergo, but also via the synthesis of lipid mediators, which can act on the cells themselves (autocrine effect) and on their neighbours (paracrine effect). Specifically, I show how mediators downstream of cyclooxygenase-2 play a central role in driving both type 1 and type 2 immune responses, with DCs requiring PGE_2 to drive Th2 priming (type 2 immune response), but macrophages *seemingly* requiring mediators other than PGE_2 to acquire an *M1-like* phenotype (type 1 immune response). I also touch upon the

subject of how metabolic reprogramming is not a black and white picture and that we need to study metabolic requirements in specific contexts.

This was evidenced by that fact that the same stimulus (IgA) requires different metabolic pathways in different cells to induce inflammation (glycolysis in DCs vs mitochondrial metabolism in macrophages), or the fact that the same cell (macrophages) will use different metabolic pathways when stimulated with different antibodies to drive inflammation (mitochondrial metabolism for IgA vs glycolysis, pentose phosphate pathway and fatty acid synthesis for IgG). Finally, I also hypothesise about future applications for these discoveries, and how we could bridge these findings into clinical applications, potentially through the use of chemical inhibitors and/or activators to inhibit specific unwanted pathways and/or promote desired pathways to obtain a specific immune response – for example promote glycolysis and fatty acid synthesis in tandem with IgG-based therapies, or promote mitochondrial activity in tandem with IgA-based therapies to have a more robust immune response by macrophages, or inhibit PGE_2 in DCs, to downregulate unwanted type-2 immune responses and/or promote type-1 immune responses. However, it is important to always keep in mind that these ideas might not universally apply to all pathological contexts. Thus, after suggesting future perspectives on how to move forward, such as by incorporating spatial lipidomics with functional immune assays, and exploring what the results of this thesis might mean in the context of current urgent pathologies, such as cancer, obesity and age-associated neurological diseases, I conclude by reinforcing the message that to find efficient therapeutic targets we need to study this in each specific pathological context and have a cell-targeted approach.

Conclusion

In conclusion, this thesis contributes to the growing importance of lipid metabolism and Fc receptors in macrophage and DC biology, in the context of autoimmunity, chronic inflammation, cancer, obesity, and infection. Therefore, studying the effects of lipid mediators, FcR signalling, and the baseline immunometabolic phenotype of tissue-associated myeloid cells in the context of each disease, in tandem with lipidomics tools, could give us an insight into the mechanism underlying the cause of these pathologies, help predict disease development and progression, and potentially lead to new therapies to prevent, treat or cure these diseases.