Shaping innate immune responses: mechanisms that control type I interferon production

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General introduction

Type I interferons (IFN-I), which include multiple IFNα subtypes and IFNβ, constitute a family of powerful cytokines with diverse and versatile roles in (innate) immunity. IFN-I engage the ubiquitously expressed IFN-α/β receptor (IFNAR) to induce an anti-microbial transcriptional program marked by the expression of IFN-stimulated genes (ISGs) (1,2), suppress cell proliferation, and sensitize cells to apoptosis (3). IFN-I also act on various immune cells to help shape innate and adaptive immune responses (4,5). The multifaceted IFN-I-effector functions can protect against a wide variety of pathogens (6) as well as cancer (7). Hence, therapeutics, such as antiviral drugs, vaccine adjuvants, and anti-cancer drugs, are frequently aimed at inducing IFN-I expression.

There is, however, a dark side to IFN-I. Uncontrolled IFN-I production induces severe immunopathology, as seen in systemic lupus erythematosus (8) and genetic autoinflammatory disorders wherein IFN-I is constitutively produced, named interferonopathies (9). Persistent IFN-I production can also induce immunosuppression during chronic viral infection (10,11) as well as cancer (12-14). This highlights the importance of shaping IFN-I responses of appropriate quality (specificity, amplitude, and duration). To be able to predict, control, or tune IFN-I responses, a profound understanding of the mechanistic basis by which IFN-I are induced is required. It is the focus of the research described in this thesis to unravel molecular pathways that shape IFN-I responses.

Paramount to the induction of IFN-I are pattern recognition receptors (PRRs). PRRs detect conserved pathogen-associated molecular patterns (PAMPs) and host-derived danger-associated molecular patterns (DAMPs). Their activation initiates cell signal transduction cascades that culminate in the expression of cytokines, chemokines, and co-stimulatory molecules (15). Several PRRs can induce IFN-I expression, and the extent to which they do is highly dependent on how downstream signal transduction is relayed in cells. In recent years, it has become apparent that molecular mechanisms that control and limit the induction of IFN-I are tailored to the type of PRR activated as well as to specific cell types (16,17). By uncovering the delicate molecular machinery by which specific cells regulate IFN-I production, we increase our understanding of how to therapeutically intervene in cases where immune responses turn aberrant.

THESIS OUTLINE

In **chapters 2 to 4**, we focus on a family of membrane-bound PRRs, the Toll-like receptors (TLRs), which play a central role in innate immunity and several of which can induce IFN-I expression.

Chapter 2 reviews how TLRs and their associated molecular machinery relay signal transduction. We describe how synthetic TLR ligands and other chemical tools have aided the elucidation of TLR function. We discuss an emerging view

that signal transduction via specific TLRs is orchestrated in space and time within cells, which allows for tunable levels of IFN-I output. This concept is explained whilst focusing on the most extensively studied receptor TLR4, which - in macrophages - induces signals leading to NF-κB-dependent inflammatory cytokine production from the plasma membrane and signals leading to IFN-I secretion once internalized into endosomes. The concept of dynamic, localization-dependent tuning of signaling outcome is then extended to other TLRs, such as TLR2 and TLR9, for which the molecular insights are mostly derived from murine models. We end by pointing out the growing demand for novel reagents that provide the ability to conditionally control TLR activation for further elucidation of the complex spatiotemporal aspects of TLR signaling.

In **chapter 3**, we assess the feasibility of controlling TLR2 signaling through 'click-to-release' chemistry. We chemically modify a synthetic TLR2 ligand with a removable *trans*-cyclooctene (TCO) moiety and demonstrate in various cell models, both murine and human, that this renders the compound unable to trigger signaling. Conditional control of TLR2 activity is then demonstrated using a tetrazine, which liberates the TLR2 agonist and triggers near-instantaneous activation of TLR2 in cells pre-treated with the TCO-modified compound. Having illustrated the power of 'click-to-release' chemistry for precisely controlling TLR2 activation in a temporal manner, we speculate on how this chemistry may be further developed to impose spatial control, which will aid the elucidation of spatiotemporal patterns of TLR2 regulation.

In **chapter 4**, we scrutinize the molecular details of TLR2-induced IFN-I signaling in human myeloid cells, as it was unclear to what extent the localization-dependent signaling outcome found for murine TLR2 signaling is conserved across species. We demonstrate that human monocyte-like cells induce a modest level of IFNβ expression upon stimulation with diverse TLR2 agonists and unravel molecular machinery that drive this response. Strikingly, our results reveal that IFN-I signaling occurs from the cell surface (as does signaling towards NF-κB activation). This indicates that the outcome of TLR2 signaling is not as strictly split across separate cellular localizations in human monocytes as seen for TLR4 signaling in macrophages. Moreover, we identify that TLR2 responses differ between human monocyte- and macrophage-like cells: IFN-I production only occurs in the monocyte-like cells. From this, we draw the conclusion that the functional outcome of TLR signaling can differ not only depending on the cell type and TLR family member studied, but also depending on the differentiation state of a given cell.

In **chapter 5**, we explore mechanisms that tailor the production of IFN-I in tumor cells that are infected with oncolytic mammalian orthoreovirus (reovirus). Reovirus naturally infects tumor cells and is a promising therapeutic for oncolytic virotherapy, wherein tumor-targeted viruses are used to trigger cancer cell death.

Even though IFN-I counteracts viral infection and spread, oncolytic virotherapy may benefit from the production of some IFN-I due to their potent anti-cancer effector functions. We demonstrate that transformed human myeloid cells are permissive to reovirus infection and observe – analogous to our findings in chapter 4 – that their differentiation state dictates whether IFN-I are produced following infection. Albeit here, a reverse trend is observed: IFN-I expression is specifically induced by differentiated, macrophage-like cells. We then utilize the transformed macrophage-like cells to uncover factors that help drive IFN-I production in reovirus-infected cells. We discover that viral replication and IFNAR signaling both play a key role in augmenting reovirus-induced IFN-I production by enhancing the activation of IRF3, a transcription factor that drives IFN-I expression. We propose a positive feedback model to explain how the IFNAR, which commonly acts downstream of IFN-I secretion, can augment upstream signaling events that drive IFN-I expression. Tumor cells frequently perturb the IFNAR pathway for their own survival. We propose that such IFNAR-deficient tumors may need additional therapeutic intervention to further promote the secretion of IFN-I that follows reovirus infection.

In **chapter 6**, the main findings of this thesis are summarized and discussed in a broader perspective.

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