Summary for people outside the field

Identification of signaling pathways that mediate recognition of pathogens by the immune system

Introduction

Our immune system continuously protects our body from infection with pathogens such as bacteria, viruses and fungi. Responsible for this protection are white blood cells, which comprise a large variation of cells that all have their own specific function. An important white blood cell for initial recognition of pathogens is the dendritic cell. After recognition of the pathogen, the dendritic cell becomes activated and travels to the lymph node. In the lymph node, the activated dendritic cell stimulates resident T cells, which subsequently effectively combat the invading pathogen. Hence, dendritic cells play a crucial role in orchestrating immunity against pathogens.

However, efficacious immune responses against pathogens require different types of T helper cell responses. Thus far, at least four classes of T helper cell responses have been identified: T helper 1 for immunity against viruses and intracellular bacteria; T helper 2 for parasites; T helper 17 for fungi; and regulatory T cells for preventing excessive immune activation. After pathogen-recognition, the dendritic cell secretes specific proteins, so-called cytokines, which skews T helper cells to become T helper 1, 2, 17 or regulatory. Since skewing of T cell responses is crucial for eradication of pathogens, discrimination between different types of pathogens by dendritic cells is essential.

For discrimination between pathogens, dendritic cells are equipped with several proteins that are expressed both intra- and extracellularly, known as pattern recognition receptors. Different pathogens are recognized by a distinct set of receptors. Moreover, binding of pathogens to receptors results in the activation of specific signals that are “sent“ into the dendritic cell. Since every receptor induces its own specific signaling pathway, dendritic cells are able to discriminate between different pathogens and thereby mediate pathogen-specific T helper responses.

Aim of the study

In recent years, the signaling pathways for several receptors expressed by dendritic cells have been identified. However, for two important pathogen-receptors, DC-SIGN and dectin-1, the signaling pathways were still largely unknown. DC-SIGN recognizes a plethora of pathogens including viruses such as HIV-1 (AIDS) and MV (measles) and bacteria such as *Mycobacterium tuberculosis* (tuberculosis) and *Helicobacter pylori* (gastric ulcer). Dectin-1 recognizes several fungal pathogens including *Candida albicans* (candidiasis). Since knowledge about signal transduction pathways of these receptors will not only lead to a better understanding of the generation of immunity against pathogens, but could also lead to new insights for potential new therapies, the aim of this thesis was to identify the signaling pathways of DC-SIGN and dectin-1.

Results

In Chapters 2 to 4 the interaction of pathogens with DC-SIGN was studied. Previous studies have shown that mycobacteria such as *Mycobacterium tuberculosis* bear a mannose-cap (a sugar-structure at the outer surface of the bacterium), that plays an important role in the generation of an immune response against this pathogen by binding to DC-SIGN on DCs. In Chapter 2 we demonstrate that the mannose-cap, in

contrast to the previous findings, does not play a major role in the mycobacterium-host interaction.

In Chapter 3 we describe the identification of the DC-SIGN signaling pathway. We show that binding of mycobacteria, viruses and yeasts to DC-SIGN modulates the immune response against these pathogens by activation of signaling protein Raf-1, which results in enhanced production of specific cytokines.
Interestingly, the effect of pathogen-binding to DC-SIGN differs depending on the pathogen involved: mannose-bearing pathogens (viruses and mycobacteria) differently modulate the immune response compared to fucose-bearing pathogens. In Chapter 4 we show that this effect results from the activation of different signaling pathways. Mannose-bearing pathogens induce a signaling pathway mediated by a complex of proteins including LSP1, KSR1, CNK and Raf-1, while in contrast fucose-pathogens disengage this complex. This difference in signaling forms the basis for pathogen-specific T helper cell polarization by DC-SIGN.

Subsequently, we have studied the signaling pathways of dectin-1, a receptor that plays a crucial role in immunity against fungal infections. Previous studies have shown that dectin-1 induces a signaling cascade via the protein Syk, but it was not clear how this signaling pathway results in T helper 1 and 17 responses. In Chapter 5 we show that dectin-1 also induces a signaling pathway via Raf-1. The ultimate immune response is the result of an intricate interaction of these two pathways. Syk activates the proteins p65, c-Rel and RelB, while Raf-1 enhances the activity of p65 and attenuates RelB activity. Only the combination of both signaling pathways will result in T helper 1 and 17 activation that is required for an efficient anti-fungal immune response.

Besides activating T cells, dectin-1 also contributes to clearance of fungal pathogens. This process of uptake of particles is called phagocytosis. Binding of fungi to dectin-1 mediates phagocytosis of the pathogen, but the signaling pathway involved was still unclear. In Chapter 6 we show that protein LSP1 is essential for phagocytosis of fungi. LSP1 is associated with dectin-1 and is required for the uptake of both heat-killed and live fungal pathogens.

Conclusions
Recapitulating the results of this thesis, we have identified the signaling pathways of two important pathogen receptors, DC-SIGN and dectin-1. These findings offer new insights into the generation of pathogen-specific immune responses. Eventually, knowledge about these signaling pathways can lead to new therapeutic strategies against infections or for the generation of new vaccines.