Initiation and regulation of specific immune responses by keratinocytes and dendritic cells. Role of cytokines and chemokines linking innate and specific immunity

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Summary

Protective immunity to pathogens depends on efficient immune responses adapted to the type of pathogen and the infected tissue, which is mediated by polarized T helper type 1 (Th1) or Th2 cells. The skin is constantly exposed to microbial pathogens and other stressful stimuli. Since the primary function of the skin is to provide an effective barrier against the outside world, it harbours a rapid and efficient host defense system exists that is triggered once the physical barrier has been broken. Keratinocytes are the major constituent of the skin and may act as natural immune cells capable of pathogen recognition, and they have an important role in initiating and perpetuating the activation of both innate and specific immune responses. Immature dendritic cells (DC) are strategically located in tissues that represent pathogen entry routes (the skin and mucosal surfaces), where they continuously monitor the environment. Upon contact with pathogens, or tissue-derived inflammatory factors induced by these pathogens, DC undergo a program of maturation that, amongst others, is associated with the acquisition of surface expression of T cell costimulatory molecules and migration through the lymph, toward secondary lymphoid organs. In this way, DC become the most potent antigen-presenting cells, the only ones capable of activating naive T lymphocytes and of initiating specific immune responses.

In this thesis, studies are described on the role of two distinct cell types, keratinocytes and DC, in the initiation and regulation of the class of the immune response.

Pathogens express pathogen-associated molecular patterns (PAMPs) that are recognized, amongst others, by Toll-like receptors (TLRs) present on innate immune cells. In Chapter 2 we examine the expression profile of TLRs by primary human keratinocytes and address the question whether these pathogen-recognition receptors play a role in cytokine and chemokine production by keratinocytes in response to different PAMPs. We demonstrate that human keratinocytes constitutively express mRNA for TLR1, 2, 3, 4, 5, 6, 9 and 10, but not for TLR7 or 8. Keratinocytes respond to TLR2, 3, 4, 5, 6 and 9 ligands, which indicates that they may play a critical role in alerting the immune system to the presence of pathogens and forming a link between innate and specific immunity.

DC play a key role in establishing the class of immune response against invading pathogens and they reside, in an immature state, in the epithelia in close contact with keratinocytes. Chapter 3 addresses whether and how soluble factors derived from pathogen-activated keratinocytes induce maturation and functional polarization of DC. Based on the
analysis of monocyte-derived DC, we found that double-stranded (ds)RNA-activated keratinocytes release TNF-α and IFN-α, that induce the maturation of DC. Moreover, the IFN-a and IL-18 released by these keratinocytes induce mature DC that strongly biased the development of Th1 cells from naive Th cells. These findings suggest that keratinocytes can contribute to the development of selective Th1/Th2 responses through the induction of maturation and functional polarization of DC. They also point to a novel role for keratinocytes as initiators and regulators of cutaneous T cell-mediated inflammation.

Upon activation with microbial compounds of cytokines, human monocyte-derived DC may mature into effector DC that promote the development of Th1- (DC1) or Th2- (DC2) *in vitro*. These functionally polarized DC populations, and nonpolarized DC0, may vary in their capability to attract other immune cells. Chapter 4 investigates the differential production of both inflammatory and homeostatic chemokines by these different DC populations. It is shown that DC0 and DC1, but not DC2, selectively express elevated levels of inflammatory chemokines. In addition, we show that the production of Th1-attracting chemokines is restricted to DC1. Since all inflammatory chemokines tested are expressed constitutively by mature DC, we propose a novel role for mature DC present in inflamed tissues in orchestrating the immune response by recruiting appropriate leukocyte populations to the site of pathogen entry.

Peripheral blood contains several DC subsets. The smallest population of DC is characterized by the expression of the blood DC antigen 3 (BDCA3) of which the function and ligand are still unknown. Chapter 5 describes the phenotypic and functional characteristics of BDCA3<sup>hi</sup> DC subset and compared them to BDCA1<sup>+</sup> myeloid and BDCA4<sup>+</sup> plasmacytoid DC. We show that, in addition to TLR1 and TLR3, BDCA3<sup>hi</sup> DC uniquely express TLR10 and upon activation with the TLR3 ligand dsRNA acquire the capacity to promote Th1 responses, albeit less potently compared to BDCA1<sup>+</sup> DC. Immunohistochemical analysis of human tissue revealed the presence of BDCA3<sup>+</sup> cells in both lymphoid and non-lymphoid tissues. This study suggests that BDCA3<sup>hi</sup> DC, due to their narrow TLR expression, have a limited capacity to recognize pathogens, but are as flexible in their capacity to bias the development of Th1/Th2 cells, as other DC subsets.

Atopic dermatitis (AD) is an eczematous skin disease associated with a generalized bias of Th2 cells. In Chapter 6 we questioned whether circulating DC in AD patients, compared to healthy controls, differ in their phenotype and function. We show that BDCA1<sup>+</sup> DC from AD patients have a selective and dramatic reduced capacity to produce IL-12p70, whereas
BDCA4+ DC show reduced IFN-α. Accordingly, even after maturation in the presence of an extremely potent Th1 stimulus (dsRNA+IFN-γ), BDCA1+ DC from AD patients induced considerably less IFN-γ-producing Th cells, compared to BDCA1+ DC from healthy controls. This study suggests that the defective IL-12 and IFN-α production by DC may contribute to the maintenance of the allergic state in AD patients, as well as their increased susceptibility to skin infections with pathogens that require protective IFN-α and Th1 cell responses.

In Chapter 7 we discuss how keratinocytes and immature DC, as innate immune cells, support specific immunity by the expression of soluble factors, with special emphasis on the role of chemokines herein. In addition, it is discussed how DC-derived chemokines amplify antigen-specific lymphocyte responses.