Chapter 1

General introduction

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GENERAL INTRODUCTION

The immune system consists of distinct components that work in a highly cooperative manner to combat pathogenic infections and eradicate malignant cells from the body. The skin and mucosal tissues provide a physical barrier against pathogens; however when a pathogen is able to penetrate this first line of defence, the immune system becomes activated. The innate immune response, which includes macrophages and granulocytes such as neutrophils, is rapidly activated upon pathogen encounter due to the binding of pathogen-associated molecular patterns (PAMPs) to pathogen recognition receptors (PRR). The adaptive immune response, which consist of T lymphocytes as the cellular component and B lymphocytes as the recognition part of the humoral component, progresses after a few days of infection and acts in a highly specific manner due to the presence of antigen-specific receptors on the cell surface of T and B cells. Furthermore, B and T lymphocytes can differentiate into long-lived memory cells that become rapidly activated upon re-infection with an identical invading pathogen, thereby providing long-term protection. Dendritic cells (DCs) bridge the innate with the adaptive immune response as these cells recognize invading pathogens and subsequently activate the adaptive immune system (1).

1. DENDRITIC CELLS

Immature DCs are located at strategic places in close proximity with the external environment, e.g. the skin, and scavenge the body for invasion by foreign material. DCs contain intra- and extracellular PRRs, such as the toll like receptors (TLRs) that bind to specific PAMPs (2). Upon encountering a pathogen, DCs receive maturation stimuli that lead to processing and presentation of pathogen-derived peptides in distinct Major Histocompatibility Complex (MHC) molecules. Peptides derived from endogenously synthesized proteins, including viral proteins are presented in MHC class I molecules that can be recognized by the T cell receptor (TCR) of $CD8^+$ T cells (cytotoxic T cells). Exogenous material can either be presented in MHC class II molecules that are then recognized by $CD4^+$ T cells (helper T cells) or in MHC class I molecules. The latter is a process called cross-presentation (3). Furthermore, mature DCs express costimulatory ligands, produce pro-inflammatory cytokines and migrate to lymph nodes to activate distinct T cells. The finding that many viruses have evolved numerous immune evasion mechanisms that interfere with antigen presentation and DC maturation to promote their persistence, underscores the importance of DCs in eliciting immune responses (4).

2. T CELLS

T cell activation

T cell activation occurs when a naive T cell bearing a unique TCR encounters a DC presenting cognate antigen in the context of MHC molecules. Besides this first signal, complementary signals are required to augment TCR triggering and to promote T cell expansion, survival and effector functions. These signals can be provided by costimulatory receptor/ligand interactions (signal 2) and by cytokines including the type I interferons (IFNs) and interleukin-12 (IL-12) (signal 3) (5,6). Costimulatory molecules are divided in members of the Ig superfamily that comprise the principal costimulatory receptor CD28, which binds to B7.1 (CD80) and B7.2 (CD86), or members of the tumor necrosis factor (TNF)/TNF receptor superfamily

that include CD27/CD70, OX40/OX40L and 4-1BB/4-1BBL (7). CD27 and CD28 are constitutively expressed on naive T cells, whereas OX40 and 4-1BB are upregulated upon TCR triggering. Costimulatory ligands are upregulated upon DC maturation and thereby provide a stimulatory signal to T cells under inflammatory conditions. In **chapter 2**, a detailed description of costimulatory receptor/ligand interactions and their specific role in viral infections is provided. The impact of type I IFNs and IL-12 on T cell responses is unique in different viral settings as e.g. in lymphocytic choriomeningitis virus (LCMV) infection, type I IFNs are required for expansion and differentiation of LCMV-specific $CD8^+$ T cells, whereas in *Listeria monocytogenes* infection, IL-12 signals are critical (8-11). However, redundant roles for IL-12 and type I IFNs have been described as both signals trigger a common gene regulation program (12).

A T cell response can be distinguished in three distinct stages: 1) the expansion phase, 2) the contraction phase and 3) the memory phase. Upon T cell activation, clonal expansion occurs (13), and T cells migrate to the site of infection. Effector $CD8^+$ T cells can produce multiple cytokines, including interferon-γ (IFN-γ), TNF and interleukin-2 (IL-2). The cytotoxic effects of $CD8^+$ T cells to eradicate infected or malignant target cells are mediated via perforins and granzymes or by Fas ligand-mediated interactions. Based on the cell surface expression of CD127 and Killer cell-lectin-like receptor G1 (KLRG1), effector T cells can be subdivided in short lived effector cells (SLECs, $CD127^-/KLRG1^+$) and memory precursor effector cells (MPECs, $CD127^+/KLRG1^-$). Although these markers provide a correlation with either terminally differentiated or long-lived memory cells, forced expression does not dictate the fate of a T cell in either direction (14).

$CD4^+$ T cells can differentiate in diverse subsets upon activation, including T helper 1 (T_h1), T_h2, regulatory T cells (T_{reg}), T_h17 and T follicular helper (T_{FH}) cells. These T cell subsets have distinct cytokine profiles and facilitate unique immune responses (15). T_h1 cells provide help to $CD8^+$ T cells by licensing DCs via CD40/CD40L interactions, leading to upregulation of costimulatory ligands and cytokine production that subsequently facilitate $CD8^+$ T cell responses. In certain virus infections, $CD4^+$ T cell help is dispensable for the development of primary $CD8^+$ T cell responses, however, for memory $CD8^+$ T cell development the help of $CD4^+$ T cells is usually critical (16). Besides the helper function of $CD4^+$ T cells, IFN-γ derived from $CD4^+$ T cells can kill infected target cells as well (17). Furthermore, T_{FH} cells provide help to B cells and promote isotype switching of antibodies (18), whereas T_{reg} cells dampen the immune response.

IL-2 signals have been implicated to influence T cell responses and promote T cell expansion. IL-2 is produced by T cells upon antigenic stimulation and under certain inflammatory conditions DCs can produce IL-2 as well (19). IL-2 production by $CD4^+$ T cells is crucial for $CD4^+$ T cell differentiation and T_{reg} homeostasis critically depends on this cytokine. Moreover, IL-2 signals have been implicated to influence all aspects of a $CD8^+$ T cell response. In order to fully differentiate into memory T cells, $CD8^+$ T cells need to receive IL-2 signals during the primary phase (20). Furthermore, IL-2 signals are especially critical for secondary expansion of memory T cells (21), and this IL-2 has to be produced in an autocrine manner (22).

Memory T cells

Upon pathogen eradication the T cell response wanes in order to avoid immune mediated pathology. One mechanism of terminating a T cell response is by elimination of the antigen presenting DCs. Furthermore, co-inhibitory receptors on T cells including CTLA-4 that binds similar as CD28 (albeit with a higher affinity) to B7.1 and B7.2, and PD-1 that binds to PD-L1 and PD-L2, provide negative signals. T_{regs} can suppress the

immune response by expressing CTLA-4, but also by competing for IL-2 and secreting inhibitory cytokines such as IL-10 (23). The majority of the T cells will die by apoptosis in the contraction phase, but a small fraction of memory T cells subsists for a long period. Due to the higher frequency of antigen-specific memory T cells and the ability to rapidly expand and degranulate after re-exposure to cognate antigen, memory cells provide superior protection compared to naive T cells.

Memory T cells are able to produce multiple cytokines, and these polyfunctional T cells are associated with correlates of protection during infections (24-27). Furthermore, memory $CD8^+$ T cells can be divided into central memory T cells ($CD62L^+$, $CD127^+$, $CD27^+$, $CD28^+$, $KLRG1^-$, IL-2^{high}) that are mainly located within the lymphoid organs and effector memory T cells ($CD62L^-$, $CD127^-$, $CD27^{low}$, $CD28^{low}$, $KLRG1^+$, IL-2^{int}) that are localized in lymphoid organs but also in tissues. Central memory T cells have a superior expansion potential compared to effector memory T cells, but the tissue distribution of effector memory T cells might be more beneficial in certain infections. Furthermore, a memory T cell subset was recently characterized that is locally restricted within tissues such as the skin, and is not in contact with the circulation (28). These so-called tissue resident memory T cells express CD103 (α-chain of the integrin $\alpha_E\beta_7$) and provide rapid local protection upon encountering a similar pathogen (29).

The different subsets of T cells indicate the plasticity of the immune system. Vaccines should aim to elicit the best response for a particular situation; therefore it is crucial to understand which signals are involved in directing T cell responses, and lead to the heterogeneity of the effector and memory T cell subsets implicated in the different stages of an immune response.

3. B CELLS

B lymphocytes express B cell receptors (BCR) on their cell surface that recognize conformational epitopes of pathogens. Upon activation due to antigen binding to the BCR, B cells differentiate into plasma cells that secrete soluble forms of the BCRs, also known as antibodies. Antibodies consist of two heavy and two light chains, which both have constant and variable regions. Antibodies can neutralize pathogens by interfering with the capacity to infect cells. Furthermore, antibodies can coat certain infected cells and mark them for lysis by other cells of the immune system or by complement factors, a process termed antibody dependent cytotoxicity.

The first antibodies that are produced by B cells have an IgM isotype, but after obtaining help signals from $CD4^+$ T cells in the germinal centres, isotype switching occurs, and subsequently IgG, IgA and IgE antibodies can be produced. Class switching does not affect antibody affinity as only the constant region of the heavy chain is altered. High affinity antibodies are rather generated by random mutations in the variable regions of antibodies, a process termed somatic hyper mutation. Antibody isotypes contain multiple antigenic binding sites, therefore the overall strength of the antibody response binding to multivalent antigen is stronger and is indicated by the avidity. B cells expressing BCRs with a higher affinity and avidity, outcompete B cells expressing low affinity BCRs for survival signals in the germinal centre, subsequently leading to clonal selection of high affinity memory B cell clones.

4. VACCINATION

The antigen specificity and memory potential of the adaptive immune response is exploited in vaccination settings where attenuated pathogens, pathogen-specific proteins or peptides are used to establish protective immunity by inducing memory T cells, protective antibodies, or a combination of both. Vaccination is considered as one of the greatest achievements in human health management as it has eradicated devastating pathogens such as smallpox, and has decreased the morbidity due to infection with many pathogens by the administration of prophylactic vaccines early in life (30). However, vaccines against numerous (chronic) pathogens and cancer are not available. As T cell responses are critical for eradication in many of these situations, it is highly beneficial to elicit adequate T cell responses when designing novel vaccines. For instance, elimination of tumor cells is mostly dependent on T cell responses. Therapeutic vaccination has been used in tumor settings by inducing or boosting endogenous tumor-specific T cells, and these tumor-specific T cell responses correlate with protection (31). Requirements for vaccine-induced T cells include sufficient clonal expansion, polyfunctionality and the establishment of protective memory pools. Depending on the context, a combination of both T and B cells might be needed to induce protective immunity. Co-incident with the development of many synthetic vaccines, it is important to have a better understanding of factors that influence the adaptive immune response to these treatments and mimic the signals that occur during a natural infection. All of this information can be combined for the rational design of vaccines.

5. CYTOMEGALOVIRUS

Cytomegalovirus (CMV), a member of the β-herpes family, is omnipresent in the world's population (32). CMV establishes a lifelong state of latency and is never completely eradicated from the body. Healthy individuals however, do not suffer from CMV associated disease, but a CMV infection can have severe consequences in immune compromised individuals (e.g. transplant patients on immune suppressive drugs, HIV infected individuals). Furthermore, women who obtain a primary CMV infection during pregnancy are able to transmit a congenital CMV infection to the fetus which can lead to severe neurological disorders (33). Despite many attempts, no fully protective CMV vaccine exists to date (34). Therefore the development of a CMV vaccine is a top priority of national health organisations. As CMV replication is species restricted, mouse CMV (MCMV) has been used in experimental laboratory settings to study the mechanism of CMV infection in its natural host. This pathogen shares many properties with human CMV (HCMV), showing a similar course of infection as HCMV, is likewise shed via the saliva of infected subjects and features similar pathways of evasion of immune responses (35).

Upon CMV infection, a highly characteristic T cell population develops, that does not follow the normal pattern of T cell expansion and contraction (36,37), but gradually increases in time. These so-called inflationary T cells exhibit an effector memory phenotype and remain functional throughout life. Due to these characteristics, inflationary T cells might be a promising T cell subset to exploit in vaccination settings. The mechanisms and benefits of inflationary T cells are described in **chapter 3.**

7. OUTLINE OF THIS THESIS

The diverse repertoire of costimulatory receptors and specific ligands, suggests that each costimulatory receptor/ligand interaction has a unique function, nonetheless all interactions impact T cell expansion. It is unclear which signals are essential in a given situation and whether costimulatory molecules have unique or overlapping functions. Besides costimulatory signals, also IL-2 signals and type I IFNs promote T cell expansion. If these signals work in a cooperative manner is unknown. More understanding of the combined impact of signals that promote T cell expansion and differentiation could be valuable for improving current vaccination settings and immunotherapies.

In this thesis we assess how costimulatory signals, IL-2 and type I IFNs impact virus-specific immunity. We first evaluate the role of different costimulatory receptor/ligand pairs in acute and chronic virus infections (**chapter 2**). Subsequently, we focus on factors that impact CMV-specific immunity. In **chapter 3**, we discuss the benefits and implications of CMV-specific inflationary T cells. Next, we evaluate the importance of CD27/CD70 interactions for controlling CMV infection, and show that targeting CD70 might be a putative immune evasion mechanism exploited by MCMV (**chapter 4**). Furthermore, we demonstrate that the viral inoculum dose impacts memory T cell inflation as well (**chapter 5**). In the following chapter, we study factors that influence the humoral response towards CMV and show that specifically the viral dose impacts antibody levels, and repetitive antigenic stimulation influences antibody avidity **(chapter 6)**.

In **chapter 7** we examine the impact of IL-2 in driving virus-specific $CD8^+$ T cell expansion and we reveal that the relative amount of IL-2 predicts the secondary memory expansion potential of the entire $CD8^+$ T cell population. In **chapter 8**, we demonstrate that specific costimulatory signals mediated via B7.1/2 and CD70 are required for inducing IL-2 production in virus-specific $CD8^+$ T cells. Furthermore, in **chapter 9** we show that costimulatory molecules can operate in a highly redundant manner to initiate virus-specific T cell responses, which is dictated by pathogen-specific cues. Finally in **chapter 10**, a general discussion of these studies is provided.

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