Targeting the tumor-draining area : local immunotherapy and its effect on the systemic T cell response

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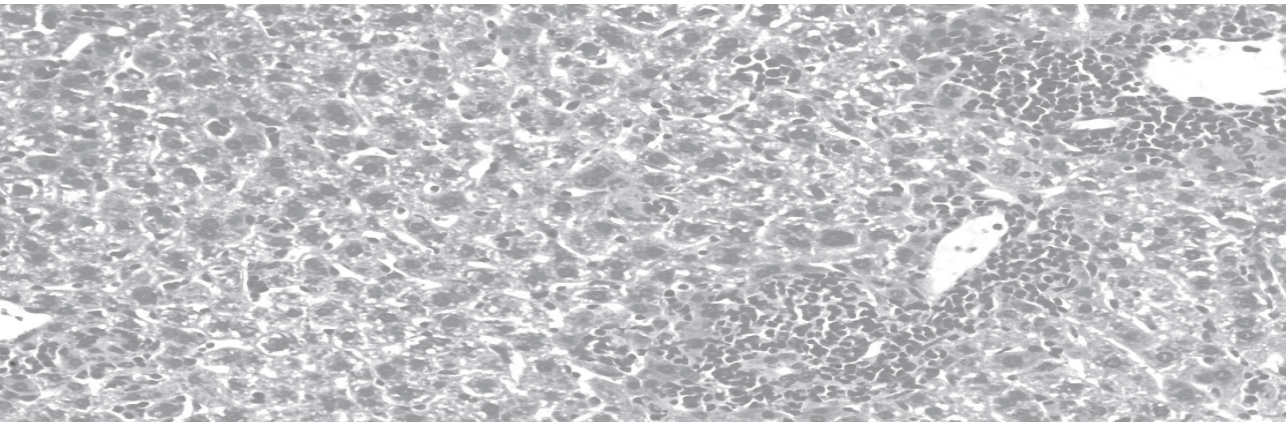
General introduction



General Introduction

Our immune system has evolved to protect us from disease and death caused by pathogens including bacteria and viruses. It comprises two separate but interacting compartments, the innate and the adaptive immune system. The innate immune response constitutes the first line of defense against pathogens that have broken through physical barriers such as the skin and mucosal layers. Innate immune cells, including macrophages, dendritic cells (DCs), as well as neutrophils, basophils, eosinophils and granulocytes harbor receptors (e.g. Toll like receptors) that recognize specific conserved patterns on pathogens known as PAMPs (pathogen associated molecular patterns). After recognition of PAMPs, the innate immune cells become activated which leads to enhanced phagocytosis of the pathogens by these cells and production of substances that can destroy the pathogens. In addition, immune signaling agents, such as cytokines and chemokines are secreted that cause inflammation and attraction of other immune cells. NK cells are rapidly responding innate immune cells, which cause destruction of virus-infected cells and tumor-cells by secreting granzymes and perforin that induce apoptosis. NK cells are regulated by an array of activating and inhibiting receptors on their surface (1). Compared to adaptive immune cells (i.e. B and T cells), innate immune cells have a limited repertoire of recognition and do not posses the ability to generate memory against pathogens, an exclusive characteristic of the adaptive immune system.

The adaptive immune system consists of B cells and T cells. B cells are responsible for the humoral response of the adaptive immune repertoire. Upon encountering cognate antigen, they divide from low numbers of precursor cells into large numbers. Subsequently, B cells either mature into memory B cells or into plasma cells, which secrete antibodies that can neutralize pathogens or tag them for destruction by the innate immune cells. The cellular component of the adaptive response consists of T cells. T cells are divided in $CD8^+$ T cells, cytotoxic T cells, and $CD4^+$ T cells, T helper cells and γδ T cells. T cell precursors originate in the bone-marrow and develop in the thymus from immature to mature T cells by T cell receptor (TCR) rearrangement and selection for specificity. In the thymus many epitopes (derived from self proteins) are presented by the individual's major histocompatibility (MHC) molecules, and T cells are selected based on affinity towards these MHC-presented epitopes. T cells with no affinity to the MHC/epitope complex die of neglect, because they are unable to recognize the basic structure of the MHC in which peptides are presented (lack of positive selection). T cells carrying T cell receptors with strong affinity for self-epitopes are eliminated, because they risk causing auto-immunity (negative selection). T cells with low affinity for self MHC-presented epitopes are allowed to expand

(positive selection) (2). After these selection processes the T cells leave the thymus and circulate in the periphery, residing mostly in secondary lymphoid tissue such as lymph nodes and spleen. This is where the naïve T cells will first encounter high affinity foreign epitopes.

Effective priming of T cells is dependent on 1. High affinity TCR engagement with MHC molecules presenting the cognate foreign epitope, 2. Interaction with costimulatory molecules such as CD80, CD86, and various TNF-(receptor) family molecules like CD40, OX40-L, 41BB-L and CD70, present on the cell surface of antigen presenting cells (APCs) such as DCs. TCR signals and costimulatory signals operate together in the immunological synapse to provide long lasting stimulation 3. Cytokines secreted by the APCs, mainly IL-2, IL-12 and IFN-alpha. The development of naive T cells into effector cells with different functions and kinetics, is controlled by the presence and strength of all three types of signals (TCR-, Costimulation-, Cytokine-mediated).

T lymphocytes

T cells are divided roughly in $CD8^+$ T cells, which recognize epitopes presented in MHC Class I molecules, and $CD4^+$ T cells, which recognize epitopes presented in MHC Class II molecules. $CD4^+$ T cell differentiation displays great plasticity, incorporating many subsets, some of which are definite, others retain the capacity to switch from one subset to another, according to present knowledge. There are at least 5 different categories known: T helper 1(Th_1), T helper 2 (Th_2), T helper 17 (Th_{17}), Regulatory T cells (T_{reg}), and Follicular helper T cells (T_{fh}). The subset distinction is based on the cytokine expression profile: IFN-gamma for Th_1, IL-4 and IL-5 for Th_2 and IL-17 for Th_{17}, or the transcription factor responsible for subset differentiation, T-bet for Th_1, Gata-3 for Th_2, Ror-γt for Th_{17}, FoxP3 for T_{reg} and Bcl6 for T_{fh} cells (3, 4). The subsets have different roles in the immune system. Th_1 cells activate DCs via CD40-CD40L interaction, effectively licensing the DC to prime $CD8^+$ T cells into CTLs (5, 6) and provide help to $CD8^+$ T cells via cytokine production. T_{reg} cells inhibit T cell responses against self-antigens, thereby keeping auto-immunity at bay. Both Th_2 and T_{fh} cells are involved in the activation of B cells, and Th_2 cells also are important in regulating the innate immune response against parasites.

$CD8^+$ T cells appear to be less heterogeneous but are also found to secrete various cytokines and various stages of activation and development, from naïve to full effector CTLs and memory cells, are found (7). Several reports describe the existence of regulatory $CD8^+$ T cells, but further studies are required in order to elucidate their precise physiological role.

Antigen presentation/DC

Antigens are generally proteins and sometimes carbohydrates nucleic acids or lipopeptides and can be derived from self molecules, pathogens and/or other non-self materials. Antigen is taken up by professional antigen-presenting cells (APCs); B-cells, DCs and macrophages, and processed for presentation. Material that is taken up can be either processed in endosomal compartments and loaded directly onto MHC II molecules, or proteins can be further cleaved by the proteasome into fragments which are then transported into the ER by the TAP transporter, where they can be loaded onto the MHC I molecule. Furthermore, material taken up can be stored in compartments that facilitate antigen supply to MHC Class I for several days (8). Peptides presented in MHC I molecules are either derived from the biosynthetic pathway, or in the case of DCs also from exogenously ingested proteins, a process referred to as cross-presentation (9). Cross-presentation is important in the priming of tumor-specific $CD8^+$ T cells, by APCs that have taken up necrotic tumor cell material and present it in MHC I molecules, either in the tumor or the tumor-draining LN (10). Peptides presented in MHC II molecules, present mainly on APCs and B cells, are generally derived from antigen that has been taken up by the APCs and becomes processed via the socalled endosomal route (11).

Lymph nodes

The lymph node (LN) plays a pivotal role in the immune system. Located at strategic places in the body, lymph nodes are the meeting point for different immune cells and create the architectural conditions for a productive primary or secondary immune response following first, respectively additional encounter with antigen. APCs arrive in the lymph node with the afferent lymph, while the majority of lymphocytes enter the LN from the bloodstream via high endothelial venules (12). Once in the T cell area or the B cell follicle DCs display, based on information received in the periphery, antigens in the context that instructs the lymphocytes to differentiate into adequate effector cells that are required for the situation at hand. Not only APCs appear in the LN through the afferent lymph, the interstitial fluid also drains to the LN into the subcapsular sinus. From here, the highly specialized conduit system distributes the fluid containing only molecules smaller then 70 kD to the B cell follicles and the T cell zone in the paracortex, where they can be taken up and presented by resident APCs (13, 14). An abundant influx of activated DCs, and danger signals, such as pro-inflammatory cytokines, heat shock proteins or uric acid can cause the lymph node to become reactive, swelling up to several times its original size, due to additional influx from both DCs via the afferent lymph and lymphocytes via the bloodstream, and reduced egress of lymphocytes from the LN (15-17). This

process accelerates the normal kinetics of APCs and lymphocyte interactions and provides a pro-inflammatory environment.

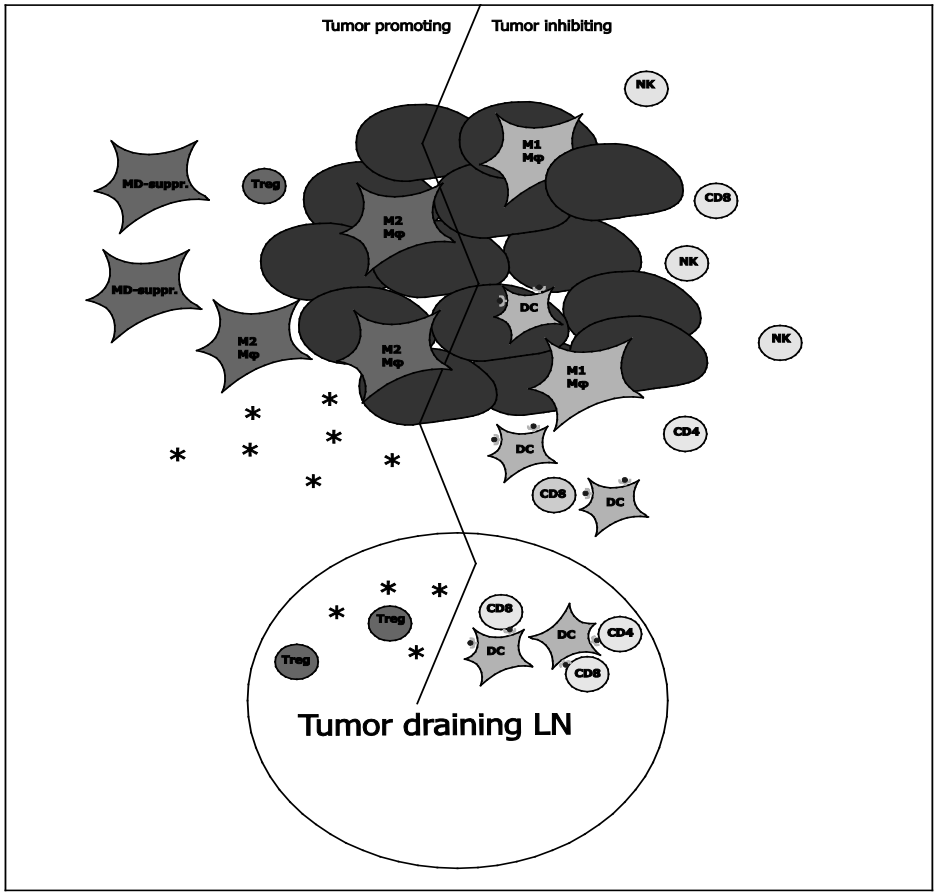


Figure 1: Schematic overview of immunological processes within the tumor microenvironment and tumor-draining LN. Within the tumor microenvironment and tumor-draining lymph nodes immune cells and processes are active, with opposing effects. Tumor promoting inflammation consists of cells like M2-macrophages (M2-Mφ), myeloid-derived suppressive cells (MD-suppr.) and T_{reg} cells and soluble factors like TGF-ß, IL-6 and VEGF. Tumor inhibiting immunity consists of cells like NK cells, M1-macrophages (M1- Mφ), $CD8^+$ T cells, $CD4^+$ T cells, and dendritic cells (DC).

Cancer immune surveillance

In 1891 the concept of cancer immune surveillance was first postulated, by William Coley, who described the ability of the immune system to recognize and possibly kill tumor cells (18). This notion was generally overlooked until Burnet revived it in the 1960's (19). He elaborated on the idea that the immune system is keeping emerging tumor cells in check, and is capable of preventing malignant cells from growing out. Not until decades later data from experimental animal models and descriptive studies in patients were published that started to

elucidate the molecular and cellular basis behind this theory, as reviewed by Swann and Smyth (20). In recent years significant numbers of T cells specific to tumor associated antigens have been identified in cancer patients, and several studies have correlated pro-inflammatory immune infiltration with improved prognosis (21, 22). The concept of immune surveillance was extended by Dunn et al. who described the phenomenon called immuno-editing; immunological pressure put upon malignant cells by lymphocytes ultimately causing the development of tumor cells capable of evading the immune system (23, 24). This evasion can be shaped by several different mechanisms, including MHC Class I downregulation, antigenic drift, secretion of immune suppressive agents, such as TGF-beta, IDO or IL-10, and attraction and expansion of regulatory T cells. Many immunotherapy strategies have been studied to intervene with these processes, with varying success rates.

Tumor immunotherapy

Modulating the immune system in order to clear tumors and metastases has been the goal of extensive studies in the past few decades. Strategies are generally based on either enhancing the tumor specific T cell repertoire, or blocking tumor induced immune suppression. Cancer immunotherapies generally employ one of four different methods; direct targeting of tumor-associated antigens by monoclonal antibodies, vaccination to drive effector T cell responses to tumor-associated antigens, adoptive transfer of tumor-specific T or NK cells or immune-modulating monoclonal antibodies.

Vaccination has long been used to strengthen the immune response against pathogens, and is now being used to boost anti-tumor T cell or B cell responses in various different forms. Among the most promising for tumor immune therapy are vaccinations with synthetic long peptides, and DCs loaded with tumor antigen (25-32). Each approach is aimed at expanding the tumor specific T cell repertoire and redirecting existing tumor specific T cells into pro-inflammatory effector cells.

Adoptive transfer strategies have employed tumor specific T cells present in tumor tissue or peripheral blood by expanding them in vitro and infuse them back into the patients. This process is laborious and costly; however, some promising results have been obtained, proving the potential of tumor-specific T-cells (33-36). New techniques, designed to increase the number of T cells recognizing tumor antigens by introducing T cell receptors through gene transfer are hopeful, and are currently being investigated for their potential to be used in the clinic (37, 38).

Monoclonal antibodies are now established as targeted therapies for several diseases including malignancies. Some antibodies target specifically tumor

cells, such as Herceptin and Rituximab, and tag thereby the tumor cells for destruction, leading to enhanced antigen presentation, which indirectly also enhances the anti-tumor immune response (39-41). Additionally, a plethora of immune-modulating antibodies is available, which can all be used to stimulate the anti-tumor immune response. Certain antibodies activate the $CD8^+$ T cell, such as 4-1BB agonists, and others activate DCs presenting tumor antigen, such as CD40 agonists, or block inhibitory signals for T cells, like PD-1 and CTLA-4 blocking antibodies (40-46). The use of monoclonal antibodies is expected to grow in the next few decades, with more molecules being investigated for their role in therapeutic settings.

Outline of this thesis

This dissertation deals with the role of local immune stimulation in the lymph node and tumor microenvironment and its effect on systemic $CD8^+$ T cell responses, in particular the anti-tumor $CD8^+$ T cell responses.

In **chapter 2** the use of a slow-release system is described to deliver the immune-activating agonistic CD40 antibody to the tumor-draining area, and the advantages of this method over systemic administration of the antibody. The local, slow-release administration was very effective in activating a systemic anti-tumor effector $CD8^+$ T cell response, to such an extent that a tenfold lower dose of antibody could be used without loss of efficacy. Adverse side-effects, analyzed by organ histology and liver enzymes in the blood, were much lower upon local anti-CD40 antibody delivery compared to systemic administration. The local delivery of anti-CD40 antibody resulted in a systemic anti-tumor $CD8^+$ T cell response, capable of clearing distant tumors expressing identical tumor antigens.

Chapter 3 shows that slow-release local administration of CTLA-4 blocking antibody can also activate a tumor-specific $CD8^+$ T cell response and cause tumor regression, while lowering systemic adverse side-effect as compared to systemic administration. CTLA-4 blocking antibody is being widely used in clinical trials, and its use has been complicated by induction of auto-immune disease. Here we show that using a local low dose injection of CTLA-4 blocking antibody in a slow-release formulation is equally effective in activating a tumor-specific $CD8^+$ T cell response, capable of eradicating tumor cells as systemic high dose treatment.

The influence of local lymph node activation on systemic T cell responses is further analyzed in **chapter 4**. $CD8^+$ T cell priming generally occurs in a locally inflamed lymph node, called a reactive LN, due to the presence of pathogens. The role of the inflammatory milieu on the priming and fate of $CD8^+$ T cells was studied by separating the TCR-MHC interaction from the inflammatory cues, by priming briefly in vitro followed by transfer to mice with or without a CpG-induced

reactive lymph node. The primary $CD8^+$ T cell response was not influenced by the presence of a reactive lymph node, however, after a boost vaccination in the memory phase, $CD8^+$ T cells primed in the presence of a reactive LN displayed a strong quantitative advantage over control $CD8^+$ T cells. The reactive LN, which remained swollen with enhanced cellularity for a pronounced period of time, was envisaged to act as a shelter for CD8+ T cells while undergoing contraction after the primary response.

In **chapter 5**, the advantages and disadvantages of the use of dextran-based microparticles as slow-release system for the delivery of immune-activating antibodies such as agonistic CD40 in the tumor-draining area are described. Dextran-based microparticles can be tailored to release antibodies in desired pharmacokinetics, leading to an even further decrease of adverse side-effects, as compared to previously described Montanide-ISA 51. However, dextran-based particles were unexpectedly found to have a stimulating effect on tumor-outgrowth. This effect coincided with the appearance of large, ulcerated swellings at the site of injection.

In **chapter 6**, the issues presented in this thesis are discussed. The knowledge gained in the work shown here, compared with and strengthened by related published work, is used to state the opinion that targeting the tumor-draining lymph node and/or tumor microenvironment for immune-activating therapy against tumors must be seriously considered.

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