Function and homeostasis of murine splenic dendritic cell subsets

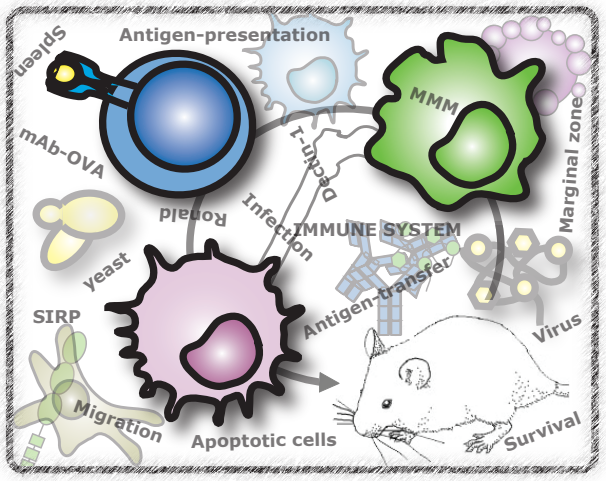
Backer, R.A.

2009

CHAPTER 6

GENERAL DISCUSSION

Summary and Perspectives



6.1 SUMMARY

The immune system protects us from harmful micro-organisms and tumor cells. In the immune system, dendritic cells (DCs) are specialized Ag-presenting cells that have a dominant role in the initiation of T cell responses. For this, DCs present intracellular Ags in MHC class I molecules to $CD8^+$ T cells or extracellular Ags in MHC class II to $CD4^+$ T cells, respectively. Next to these two classical Ag-presentation pathways, DCs are also able to cross-present extracellular Ags in the context of MHC class I to $CD8^+$ T cells. This cross-presentation pathway is important for the generation of primary $CD8^+$ T cell mediated responses against Ags that are not produced by the presenting DC itself, e.g. cell-specific viruses and tumor cells, which could otherwise not be presented to naive $CD8^+$ T cells.

DCs form a heterogeneous population in the mouse spleen. Splenic DCs can be subdivided into $CD8^+$ and $CD8^-$ DC subsets with different phenotype and localization. The specific localization of $CD8^+$ DCs in the white pulp and of $CD8^-$ DCs in the marginal zone (MZ) is regulated by chemokines, CCR7 and S1P[1-5]. In spleen, the $CD8^-$ DC subset specifically expresses SIRPα, which is a cell surface glycoprotein expressed by myeloid cells that was shown to be involved in migration of several cell types[6-8]. We showed that in the absence of functional SIRPα in SIRPα-Δ87 mutant mice the number of $CD8^-$ DCs in spleen is strongly reduced (chapter 2). Furthermore, SIRPα is involved $CD8^-$ DC migration by regulating integrin-mediated cell adhesion. $CD8^-$ DCs derived from SIRPα-Δ87 mutant mice express lower levels of MMP-9 and MMP-12 and are less mature in phenotype as compared to SIRPα-wt derived $CD8^-$ DCs. The study described in chapter 2 suggests that SIRPα plays an important role in the migration, the localisation and the homeostasis of $CD8^-$ DCs *in vivo*.

Next to differences in their homeostatic regulation splenic DC subsets also display different capacities to activate T cells. $CD8^+$ DCs are described as the cross-presenting DCs involved in $CD8^+$ T cell activation, while $CD8^-$ DCs are mainly involved in $CD4^+$ T cell activation. In chapter 3 of this thesis, we describe that yeast is efficiently cross-presented both *in vivo* and *in vitro*. Interestingly, $CD8^-$ DCs preferentially cross-presented the yeast-derived Ags thereby launching a $CD8^+$ T cell response, while both splenic DC subsets were able to present yeast-derived Ags in the context of MHC class II to $CD4^+$ T cells[9]. Since both DC subsets equally well phagocytosed yeast in a dectin-1 dependent manner, the observed differences in cross-presentation and cytokine production between the

subsets after stimulation with yeast could not be explained by differential uptake of yeast. These results strongly indicate that $CD8^+$ and $CD8^-$ DCs are specialized with respect to their differential capacities to activate naïve $CD4^+$ or $CD8^+$ T cells in response to stimulation with yeast.

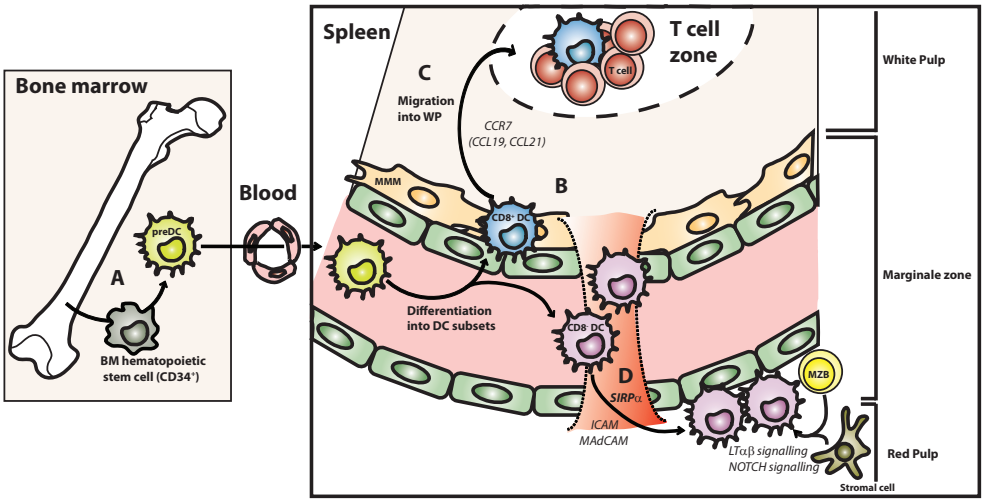
Efficient Ag-presentation is the main function of DCs in an organism. It has been shown that NADPH oxidase (NOX2) mediated ROS production in the phagosome is important for efficient cross-presentation, since ROS production controls the phagosomal pH and thereby the breakdown of Ags[10;11]. We therefore investigated in chapter 4 whether differences in ROS production between DC subsets might cause the differences in Ag-presentation capacities. $CD8^-$ DCs show higher mRNA expression of NOX2 subunits and are more efficient in total ROS production after PMA and yeast stimulation as compared to $CD8^+$ DCs. In addition, inhibition of ROS by the chemical compound apocynin reduced the *in vitro* capacity of splenic DCs to cross-present yeast-derived Ags. Taken together these data suggest that the differential Ag-presentation capacities of splenic $CD8^+$ and $CD8^-$ DCs could result from differentially regulated ROS production and its influence on Ag-presentation.

DCs phagocytose as well as endocytose Ags for efficient presentation to T cells. We observed in chapter 5 that $CD8^+$ DCs acquire Ags from $M\varphi$ for cross-presentation. Marginal metallophilic macrophages (MMMs) specifically transfer targeted Ags to $CD8^+$ but not to $CD8^-$ DCs. The transfer of Ags between MMMs and $CD8^+$ DCs can either result in T cell activation or T cell tolerance, depending on the use of an adjuvant. Since we could not observe migration of MMMs out of the MZ, we suggest that DCs migrate through the MZ and thereby acquire Ags form MMMs. This study is the first in assigning an important role to MMMs in the induction of primary $CD8^+$ T cell responses.

Based on the data presented in this thesis we conclude that, although $CD8^+$ and $CD8^-$ DCs are closely related in their origin, their homeostasis and Ag-presentation capacities are differentially regulated. Analysis of the T cell stimulation capacities of splenic DC subsets indicates functional specialization of $CD8^+$ and $CD8^-$ DCs in the elicitation of adaptive immune responses *in vivo*.

6.2 THE IMPORTANCE OF SPLENIC ARCHITECTURE FOR DC HOMEOSTASIS

$CD8^+$ DCs are mainly found in the T cell areas of the white pulp and



|Figure 1| DC migration and differentiation in the spleen.
(A) DCs originate from CD34$^+$ hematopoietic stem cells (HSC) in the bone marrow (BM). These HCS migrate via blood vessels to the spleen. **(B)** In the spleen, pre-DCs leave the blood in the marginal zone (MZ), which is surrounding the white pulp (WP), and undergo final maturation into CD8$^+$ and CD8$^-$ DCs. **(C)** CD8$^+$ DCs migration into the T cell zone within the white pulp is regulated by CCR7 and its ligands CCL19 and CCL21. **(D)** CD8$^-$ DCs migrate into the MZ and bridging channels, connecting the white pulp with the red pulp. This migration requires ICAM and MAdCAM mediated cell adhesion, which is regulated by the cell surface receptor SIRPα on CD8$^-$ DCs. Once located in the MZ, CD8$^-$ DCs are interacting with stromal cells and marginal zone B cells (MZB). These interactions include LTαβ- and NOTCH signaling, which are required for optimal CD8$^-$ DC survival and homeostasis in the MZ.

MZ, while CD8$^-$ DCs localize in the splenic MZ and bridging channels[12-15]. The data presented in chapter 2 show that defects in SIRPα signaling results in the absence of CD8$^-$ DCs in the spleen, whereas CD8$^+$ DCs were not affected. Since SIRPα is involved cell adhesion and migration, we hypothesize that SIRPα indirectly influences CD8$^-$ DC survival in the spleen (Figure 1). DCs are generated from hematopoietic stem cells in the bone marrow and arrive as pre-DCs in the spleen. Locally, these pre-DCs undergo final differentiation into CD8$^+$ and CD8$^-$ DCs[16;17]. ICAM- and MAdCAM-mediated interactions and transmigration through the endothelium are required for CD8$^-$ DCs to migrate into MZ[5]. Once arrived in the MZ, positioning of these CD8$^-$ DCs depends on S1P[5]. Subsequently, CD8$^-$ DCs receive survival signals including LTαβ and Notch signaling from stromal cells and MZ B cells[18]. In the absence of SIRPα, CD8$^-$ DCs show reduced ICAM- and MAdCAM-mediated adhesion, reduced migration through endothelium, and reduced expression of MMPs, suggesting that CD8$^-$ DCs in SIRPα mutant mice will not reach the MZ. Therefore, those CD8$^-$ DCs might not reach the required survival signals in the MZ, as indicated by the reduced RELb expression. Taken together these data

could explain the observed reduction in $CD8^-$ DC number in spleens of SIRPα mutant mice.

So far, we identified a role for SIRPα in the migration of $CD8^-$ DCs into the MZ. Once activated, $CD8^-$ DCs have to migrate further towards the white pulp in order to encounter Ag-specific T cells. Therefore, it would be of interest to determine the role of SIRPα in the migration of $CD8^-$ DCs from the MZ into the WP.

$CD8^-SIRP\alpha^+$ DCs can also be found in other (non-) lymphoid organs, such as skin, lymph nodes (LNs) and in the lamina propria of the intestinal tract. For Langerhans cells (LCs), one of the DC types in the skin, it has been described that CD47-SIRPα interaction is required for their migration to draining LNs. CD47-deficient LCs could not cross lymphoid endothelium and were therefore not able to enter the lymphatic system in order to reach the draining LNs[19;20]. As the function of SIRPα on DCs has been poorly investigated to date, it is unknown whether SIRPα only plays a role in $CD8^-$ DC migration into the spleen, or whether it is also plays an important role in DC migration in peripheral tissues, such as the intestine.

As SIRPα seems to be so important for $CD8^-$ DC migration and homeostasis an intriguing question is how $CD8^+$ DCs migration and homeostasis is regulated in the absence of SIRPα? The differential expression of SIRPα suggests that the specific localization of $CD8^+$ and $CD8^-$ DCs is important for splenic function, and that therefore specific migration and homeostasis of splenic DC subsets is regulated by different mechanisms. Further research on SIRPα is required to elucidate its role in DC migration and homeostasis in general.

6.3 $CD8^-$ DCS PREFERENTIALLY CROSS-PRESENT YEAST-DERIVED AGS

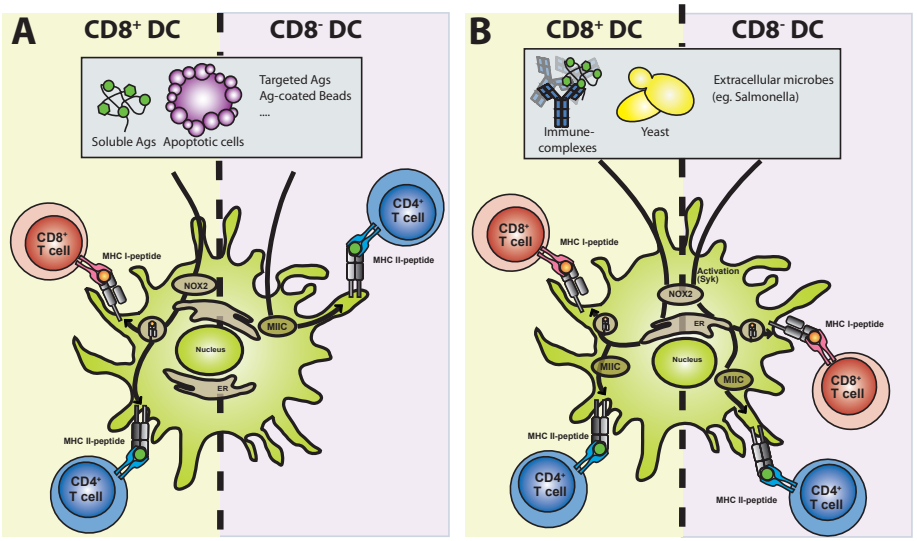
$CD8^+$ and $CD8^-$ DCs show different capacities to activate $CD8^+$ and $CD4^+$ T cells, respectively. $CD8^+$ DCs cross-present Ags, while $CD8^-$ DCs are specialized in Ag-presentation in the context of MHC class II molecules[21]. It has been well documented that $CD8^+$ DCs selectively cross-present cell-associated OVA, soluble OVA, and OVA-coated latex beads[22-24]. This, however, does not mean that $CD8^-$ DCs are not able to cross-present, in fact, in chapter 3 of this thesis we show that $CD8^-$ DCs are very well able to cross-present yeast-OVA. Interestingly, $CD8^-$ DCs, but not $CD8^+$ DCs were preferentially involved in the cross-presentation of these yeast-

derived Ags. In addition, only $CD8^-$ DCs produced cytokines in response to yeast. Thus $CD8^-$ DCs are the major cross-presenting DCs for fungal antigens. Also for bacterial Ags, such as *Salmonella* Ags, it has been shown that both $CD8^-$ DCs and $CD8^+$ DCs were able to cross-present[25]. Moreover, $CD8^-$ DCs are as efficient as $CD8^+$ DCs in cross-presenting OVA-immune complexes, as long as they are activated via $FC\gamma Rs$[26]. The differential capacity of $CD8^+$ and $CD8^-$ DC subsets to respond to certain Ags suggests that DC subsets represent specialized routes leading to the activation of adaptive immune responses *in vivo*, depending on the Ag encountered.

6.4 REGULATION OF AG-PRESENTATION BY DC SUBSETS

How DCs might differ in terms of T cell activation is not known. The differences between DC subsets might involve (A) Ag-uptake, (B) Ag-processing or (C) the Ag-presentation that subsequently lead to the differential T cell stimulation capacities of splenic DC subsets. $CD8^+$ and $CD8^-$ DCs differentially express Toll-like receptor subsets and other pattern recognition receptors, like DEC205, DCIR2 and Clec9a[21;27]. Since these receptors contribute to the uptake and further intracellular routing of Ags, their differential expression suggests that the observed differences between DC subsets can, at least partially, be explained by differential uptake of Ags.

Research by Dudziak *et al.* showed a difference between $CD8^+$ and $CD8^-$ DCs in their expression of genes involved in MHC class I versus class II Ag-presentation[21]. This study suggests that the differential Ag-presentation is due to DC intrinsic properties, more than to differences between the DC specific receptors. However, the differential expression pattern of MHC class I or class II components cannot totally explain all the subset differences. First of all, both DC subsets express high levels of MHC class I and II molecules on their cell surface, indicating that both $CD8^+$ and $CD8^-$ DCs are intrinsically able to generate class I and class II peptides. Also the role of DC subsets in priming only $CD4^+$ or $CD8^+$ T cells is not that selective. Next to their role in cross-presentation, $CD8^+$ DCs are also very important for MHC class II presentation of cellular Ag[28] and cross-presentation of immune-complexes and yeast occurred by both DC subsets[9;26]. Also for endogenous Ag, no differences between DC subsets in MHC class I presentation are observed, so it is not that the expression



|Figure 2| Differential antigen-presentation by DC subsets depends on the Ag source. **(A)** $CD8^+$ DCs and $CD8^-$ DCs differ in their capacity to activate $CD8^+$ and $CD4^+$ T cells. $CD8^+$ DCs are mainly involved in cross-presentation of soluble Ags, apoptotic cells and OVA-coated beads to $CD8^+$ T cells, while $CD8^-$ DCs present these Ags in the context of MHC class II to $CD4^+$ T cells. Ags that are taken up by DCs, end up in phagosomes. For cross-presentation it is important that Ags are prevented from total degradation by lysosomal proteases. For optimal protease activity, low pH in the phagosome is required. The NADPH oxidase NOX2 prevents phagosomal acidification by the production of reactive oxygen species (ROS), thereby controlling Ag-breakdown. In $CD8^+$ DCs, NOX2 is efficiently recruited to the phagosomes and Ags are rescued for transport into the cross-presentation pathway, resulting in MHC class I-peptide loading and $CD8^+$ T cell activation. On the other hand, it is suggested that NOX2 in $CD8^-$ DCs is not recruited to the phagosomes after uptake of the Ags. Therefore, Ags are not preserved, and only MHC class II restricted Ag-presentation is available since this pathway is less sensitive to Ag-degradation. **(B)** It is hypothesized that for cross-presentation, $CD8^-$ DCs have to be activated. Both $CD8^+$ and $CD8^-$ DCs are able to cross-present immune complexes (ICs), yeast and extracellular microbes like *Salmonella*. For ICs and yeast it is known that the uptake receptors, FcγR and dectin-1, respectively, are signaling via Syk, and the Syk signaling cascade is involved in ROS production. Therefore, after encounter of certain Ags, specific activation signals are inducing the recruitment of NOX2 to the phagosomes in both $CD8^+$ and $CD8^-$ DCs by, allowing subsequent cross-presentation.

of MHC class I processing pathways are insufficient, but rather that the ability to process certain Ags differs between DC subsets (Figure 2).

The differences in cross-presentation efficiencies can be explained by the differential regulation of ROS production by DC subsets. ROS, generated by the NADPH oxidase NOX2 complex, is involved in the regulation of protein degradation. ROS production prevents phagosomal acidification and thereby prolonging Ag-persistence[10]. After Ag-stimulation, $CD8^+$ DCs very efficiently localize their ROS production in their phagosomes thereby improving cross-presentation[11]. The phagosomal recruitment of NOX2

subunits in $CD8^+$ DCs is dependent on RAC2, in contrast to $CD8^-$ DCs, which recruit NOX2 to their cell membrane in a RAC1 dependent manner[11]. Thereby, $CD8^-$ DCs localize their ROS production not in phagosomes but at the cell surface for secretion (chapter 4). We hypothesized that after stimulation specific receptors, e.g. FcγRs and dectin-1, cross-presentation is favored in $CD8^-$ DCs by modulation and activation of cross-processing pathways and by directing ROS to phagosomes. This hypothesis is in line with the observation that the ITAM–based signaling pathway of FcγR utilizes Vav for the activation of the NOX2, thereby stimulating cross-presentation of particulate Ags[29].

The hypothesis underlying the studies described in this thesis is that, although there is an apparent division of DC labor, the function of DC subsets is rather flexible than static. In other words, the capacity to activate certain T cells is not so much depending on intrinsic capacities of DCs, but is depending on the type of Ag acquired and the subsequent activation of DCs. This hypothesis is supported by the observations made in chapter 3 on cross-presentation of yeast. As described, $CD8^-$ DCs are very well capable of cross-presenting immune complexes and yeast after uptake by Fc-receptors and dectin-1, respectively. Both receptors are able to signal via Syk[30;31], suggesting that activation of a Syk-Vav-RAC2-dependent pathway in $CD8^-$ DCs might be involved in cross-presentation of antigens via the activation of NOX2 in the phagosomes. Whereas the cross-presentation by $CD8^+$ DCs does not depend on the presence of the FcγR-chain, $CD8^-$ DCs lost their ability cross-present in the absence of FcγR-chain signaling. Therefore, $CD8^-$ DCs activation seems to be essential for their ability to cross-present, while $CD8^+$ DCs can cross-present independently of an additional activation signal.

6.5 COLLABORATION BETWEEN MMMS AND DCS IN T CELL ACTIVATION

As a lymphoid organ the spleen houses relatively large amounts of B cells, T cells, Mφ and DCs. All these cells are involved the induction and regulation of immunity against invading pathogens and tumor cells. Both Mφ and DCs efficiently take up extra-cellular Ags, but DCs are able to elicit primary immune responses whereas Mφ are not. Previous studies on the function of Mφ in the splenic MZ established the important role of MMMs in trapping and eliminating pathogens such as *Listeria monocytogenes* and *Lymphocytic choriomeningitis virus* (LCMV)[32;33]. In

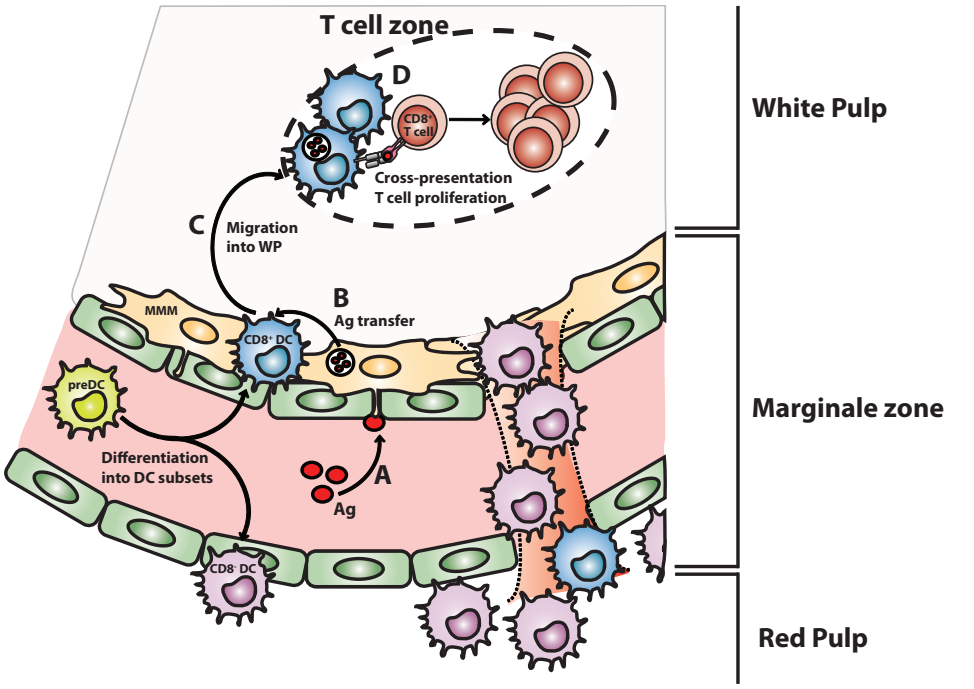
the absence of MMMs and marginal zone Mφ (MZMs), these pathogens cannot be controlled and dissemination to peripheral organs occurs early during infection. Siglec-1 on MMMs serves also as a phagocytotic receptor for sialyated pathogens such as *Neissera meningitidis* and *Porcine reproductive and respiratory syndrome virus*[34;35]. Another role of MMMs is in the uptake of apoptotic cells. Apoptotic cells up-regulate sialic acids[36-38], and are efficiently taken up via Siglec-1 and scavenger receptors on MMM[39-41]. Both the absence of type I scavenger receptors as well as loss of splenic Mφ caused loss of tolerance towards apoptotic cells resulting in the development of autoimmunity[40].

The $CD8^+$ DC subset is the main cross-presenting DC subset. Although recently $CD8^+$ DCs were also identified in the MZ[15], most $CD8^+$ DCs are located in the white pulp of the spleen. The white pulp is a secluded area and the permeability of its conduit system is very restricted by the size of the Ag[42]. Since the $CD8^+$ DCs that are located in the MZ are poor in Ag capture[15], large antigens may not reach $CD8^+$ DCs in the white pulp and therefore other cell types might be necessary to deliver Ag to these DCs. In chapter 5 we suggest a possible mechanism for $CD8^+$ DCs to acquire large Ags in the spleen. We found that MMMs in the MZ trap Ags and transfer these to cross-presenting $CD8^+$ DCs. We also show that Ag-transfer between MMMs and DCs is an additional mechanism to elicit $CD8^+$ T cell immunity and tolerance.

6.6 POSSIBLE MECHANISMS INVOLVED IN AG-TRANSFER BETWEEN APCS

Ags have been shown to be transferred between migrating and non-migrating DC subsets in skin[43;44], and the respiratory system[45]. In these studies DCs migrating from peripheral tissues were not involved in T cell activation themselves, but transferred Ag to lymph node resident $CD8^+$ DCs. In chapter 5 we demonstrate that a similar mechanism exists in the spleen and we are the first to show that Mφ are involved in Ag-transfer to $CD8^+$ DCs.

$CD8^-$ DCs are located in the MZ and are thus in close proximity of MMMs. Upon activation by TLR-ligands such as LPS, $CD8^-$ DCs migrate from the MZ to the T cell area within the white pulp[46-48]. Therefore, $CD8^-$ DCs could potentially be involved in the Ag-transfer process by bringing Ags from MMMs into the white pulp to $CD8^+$ DCs. However, our studies on sorted $CD8^-$ and $CD8^+$ DC subsets after MMMs targeting did not indicate



|Figure 3| Antigen-transfer between marginal metallophilic macrophages and CD8⁺ DCs: a model.

(A) Marginal metallophilic macrophages (MMMs) are strategically located at the inner rim of the marginal zone (MZ), surrounding the white pulp (WP) of the spleen. Most blood that enters the spleen is running through this MZ. MMMs sample the blood for blood-borne pathogens and other Ags, which are very efficiently taken up after recognition. **(B)** These Ags are transported from MMMs to newly incoming CD8⁺ DCs, which are on their way from the blood into the WP where they reside in the T cell rich areas. **(C)** Once arrived in a T cell zone, the acquired Ags are cross-presented by the CD8⁺ DCs, resulting in CD8⁺ T cell activation or CD8⁺ T cell tolerance. CD8⁻ DCs, which are under steady state located in the MZ, are thought not to be involved in the transfer of Ags from MMMs to CD8⁺ DCs.

uptake of Ag by CD8⁻ DCs. We therefore propose a scenario in which MMMs transfer Ags to newly incoming CD8⁺ DC precursors from the blood that are on their way to the T cell area (Figure 3). Where CD8⁺ DCs, or their precursors, exactly enter the spleen is currently unknown. Adoptively transferred DCs enter the spleen in the MZ[49], where also recirculating lymphocytes enter the spleen by leaving the blood[50;51]. This would implicate that DCs are passing MMMs on their way from the blood into the white pulp. Reis e Sousa *et al.* proposed that DCs receive their Ag in MZ bridging channels and subsequently migrate to the T cell area. DCs matured during this migration and induced T cell activation[48]. Therefore, it is tempting to speculate that CD8⁺ DCs acquire Ags from MMMs in a comparable way.

Also the question in which form the Ags (unprocessed Ags, peptides or MHC class I/peptide complexes) are acquired by $CD8^+$ DCs still needs to be answered. DCs are known to acquire Ag from live cells by a process known as 'nibbling' and possibly this is involved in the Ag-transfer between MMMs and $CD8^+$ DCs[52]. On the other hand, DCs can also sample Ags via gap junctions[53]. To answer these questions, further research is required.

6.7 FUTURE DIRECTIONS

In mice, DCs form a heterogeneous population of cells, which differ in phenotype, localization and function. Since the amount of human material to isolate DCs from is limited, most studies on human DCs are performed using blood-derived DCs. These DCs, however, are not complete representatives of all human DC subsets that could be isolated from fresh tissue. Currently, definitive markers for the discrimination of human DC subsets remain to be identified. DCs isolated from human tonsils and spleen show heterogeneity in the expression of their surface markers CD11c, CD4 and CD11b[54], suggesting that indeed several different DC subsets can be identified in man. Recently, a specific marker for the human equivalent of $CD8^+$ DC has been proposed[55]. However, expression of specific DC markers and cytokine production profiles of human DC subsets do not always resemble the murine system. Nevertheless, it is thought that DCs from mice and man have similar basic characteristics, therefore suggesting that data obtained by mouse studies can be translated, at least to some extent, to the human situation.

Since DCs are potent regulators of immune responses, they are considered prime targets for future therapeutic applications against cancer, infectious diseases and autoimmune diseases. To date, applications for DCs in vaccinations and immunotherapy are being developed. The outcome of these DC targeting strategies is dependent on the recognition, processing, and presentation of the targeted Ag. Therefore, it is of particular importance to investigate how the ability of $CD8^+$ and $CD8^-$ DCs to activate T cells is regulated. For the development of DC-based vaccines, autologous DCs can be generated *ex vivo* from bone marrow or blood precursors, loaded with antigenic peptides and subsequently re-injected into the patient. Anti-tumor immune responses can also be induced by *in vivo* targeting and activation of DCs specifically with mAbs or receptor-ligands coupled to tumor-Ags [56]. The different functions of murine DC subsets in the induction of T cell responses show that subset-

specific targeting is very important for the activation of desired T cell responses and should be taken into account in the development of new clinical applications.

While most methods of DC targeting are based on direct targeting of DCs, we show that indirect targeting of $CD8^+$ DCs via MMMs might be at least as efficient in the induction of $CD8^+$ T cell responses as direct DC targeting. Since these MMMs are very efficient in the uptake of various potentially hazardous particles and since MMMs are able to transfer these Ags to DCs, they could provide a very potent target for the development of new vaccines. Certain Ags can only be cross-presented by specific DC subsets. Targeting of these Ags to MMMs could circumvent this problem, thereby increasing the efficiency of a vaccine. Since also Siglec-1^+ Mφ, representing human equivalents of the mouse MMMs, have been detected near $DEC205^+$ DCs in human spleen[57], further studies on targeting MMMs would be of utmost interest for the development of optimal vaccination strategies.

6.8 CONCLUDING REMARKS

Ag-presentation by splenic DC subsets results in different T cell responses. In this thesis, we hypothesize that cross-presentation by DCs is tightly regulated. But why is such a regulation required? Our data indicate that $CD8^-$ DCs are involved in preferential cross-presentation of extracellular Ags, like *Salmonella*, yeast and immune complexes[9;25;26]. On the other hand, $CD8^+$ DCs are important for cross-presentation of cellular Ags. $CD8^+$ DCs are able to efficiently acquire these cellular Ags by specific receptors. For example, uptake of virus-infected or apoptotic cells is mediated by a recently-characterized C-type lectin Clec9a, specifically expressed on $CD8^+$ DCs[27]. $CD8^+$ DCs can also specifically acquire Ags from MMM. In contrast to $CD8^-$ DCS, it is important that cross-presentation by $CD8^+$ DCs is independent on the activation status of the cell. $CD8^+$ DCs are not only important during inflammation, but also under steady state conditions, without immune activation. Thereby $CD8^+$ DCs control the immune balance between activation and tolerance of $CD8^+$ T cells via cross-presentation of apoptotic cells[58-60].

By transferring Ag to $CD8^+$ DCs, Mφ and DCs combine their specific capacities during immune responses. Mφ themselves have poor T cell stimulatory capacities, but are efficient in uptake of many different Ags. This highly phagocytic capacity of Mφ, combined with the efficient T cell stimulatory capacity of DCs, greatly enhances the repertoire of Ags that

can be cross-presented. This mechanism enables the adaptive and innate immune system to rapidly interact with each other and thereby lead to an efficient and robust response to blood-borne Ags.

Understanding how differential antigen processing by these two DC subsets occurs, and how these DCs interact with other APCs could have important implications for understanding how T-cell responses are initiated *in vivo*, and perhaps for the design of new therapeutic vaccines.

REFERENCE LIST

1. Cyster JG. Chemokines and cell migration in secondary lymphoid organs. Science 1999;286:2098-2102.
2. Ohl L, Mohaupt M, Czeloth N et al. CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. Immunity. 2004;21:279-288.
3. Forster R, Schubel A, Breitfeld D et al. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 1999;99:23-33.
4. Martin-Fontecha A, Sebastiani S, Hopken UE et al. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. J.Exp.Med. 2003;198:615-621.
5. Czeloth N, Schippers A, Wagner N et al. Sphingosine-1 phosphate signaling regulates positioning of dendritic cells within the spleen. J.Immunol. 2007;179:5855-5863.
6. Zen K, Parkos CA. Leukocyte-epithelial interactions. Curr.Opin. Cell Biol. 2003;15:557-564.
7. de Vries HE, Hendriks JJ, Honing H et al. Signal-regulatory protein alpha-CD47 interactions are required for the transmigration of monocytes across cerebral endothelium. J.Immunol. 2002;168:5832-5839.
8. Fukunaga A, Nagai H, Noguchi T et al. Src homology 2 domain-containing protein tyrosine phosphatase substrate 1 regulates the migration of Langerhans cells from the epidermis to draining lymph nodes. J.Immunol. 2004;172:4091-4099.
9. Backer R, van LF, Kraal G, den Haan JM. CD8- dendritic cells preferentially cross-present Saccharomyces cerevisiae antigens. Eur.J.Immunol. 2008;38:370-380.
10. Savina A, Jancic C, Hugues S et al. NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. Cell 2006;126:205-218.
11. Savina A, Peres A, Cebrian I et al. The small GTPase Rac2 controls phagosomal alkalinization and antigen crosspresentation selectively in CD8(+) dendritic cells. Immunity. 2009;30:544-555.
12. Pulendran B, Lingappa J, Kennedy MK et al. Developmental pathways of dendritic cells in vivo: distinct function, phenotype, and localization of dendritic cell subsets in FLT3 ligand-treated mice. J.Immunol. 1997;159:2222-2231.
13. Steinman RM, Pack M, Inaba K. Dendritic cells in the T-cell areas of lymphoid organs. Immunol.Rev. 1997;156:25-37.
14. Leenen PJ, Radosevic K, Voerman JS et al. Heterogeneity of mouse spleen dendritic cells: in vivo phagocytic activity, expression of macrophage markers, and subpopulation turnover. J.Immunol. 1998;160:2166-2173.
15. Idoyaga J, Suda N, Suda K, Park CG, Steinman RM. Antibody to Langerin/CD207 localizes large numbers of CD8alpha+ dendritic cells to the marginal zone of mouse spleen. Proc.Natl.Acad.Sci.U.S.A 2009;106:1524-1529.
16. Naik SH. Demystifying the development of dendritic cell subtypes, a little. Immunol.Cell Biol. 2008;86:439-452.
17. Liu K, Victora GD, Schwickert TA et al. In vivo analysis of dendritic cell development and homeostasis.

Science 2009;324:392-397.

18. Ansel KM, Ngo VN, Hyman PL et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. Nature 2000;406:309-314.
19. Hagnerud S, Manna PP, Cella M et al. Deficit of CD47 results in a defect of marginal zone dendritic cells, blunted immune response to particulate antigen and impairment of skin dendritic cell migration. J.Immunol. 2006;176:5772-5778.
20. Van VQ, Lesage S, Bouguermouh S et al. Expression of the self-marker CD47 on dendritic cells governs their trafficking to secondary lymphoid organs. EMBO J. 2006;25:5560-5568.
21. Dudziak D, Kamphorst AO, Heidkamp GF et al. Differential antigen processing by dendritic cell subsets in vivo. Science 2007;315:107-111.
22. den Haan JM, Lehar SM, Bevan MJ. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. J.Exp.Med. 2000;192:1685-1696.
23. Pooley JL, Heath WR, Shortman K. Cutting edge: intravenous soluble antigen is presented to CD4 T cells by CD8- dendritic cells, but cross-presented to CD8 T cells by CD8+ dendritic cells. J.Immunol. 2001;166:5327-5330.
24. Schnorrer P, Behrens GM, Wilson NS et al. The dominant role of CD8+ dendritic cells in cross-presentation is not dictated by antigen capture. Proc.Natl.Acad. Sci.U.S.A 2006;103:10729-10734.
25. Yrlid U, Wick MJ. Antigen presentation capacity and cytokine production by murine splenic dendritic cell subsets upon Salmonella encounter. J.Immunol. 2002;169:108-116.
26. den Haan JM, Bevan MJ. Constitutive versus activation-dependent cross-presentation of immune complexes by CD8(+) and CD8(-) dendritic cells in vivo. J.Exp.Med. 2002;196:817-827.
27. Sancho D, Joffre OP, Keller AM et al. Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature 2009;458:899-903.
28. Valdez Y, Mah W, Winslow MM et al. Major histocompatibility complex class II presentation of cell-associated antigen is mediated by CD8alpha+ dendritic cells in vivo. J.Exp.Med. 2002;195:683-694.
29. Graham DB, Stephenson LM, Lam SK et al. An ITAM-signaling pathway controls cross-presentation of particulate but not soluble antigens in dendritic cells. J.Exp. Med. 2007;204:2889-2897.
30. Rogers NC, Slack EC, Edwards AD et al. Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. Immunity. 2005;22:507-517.
31. Sedlik C, Orbach D, Veron P et al. A critical role for Syk protein tyrosine kinase in Fc receptor-mediated antigen presentation and induction of dendritic cell maturation. J.Immunol.
32. Aichele P, Zinke J, Grode L et al. Macrophages of the splenic marginal zone are essential for trapping of blood-borne particulate antigen but dispensable for induction of specific T cell responses. J.Immunol. 2003;171:1148-1155.
33. Seiler P, Aichele P, Odermatt B et al. Crucial role of marginal zone macrophages and marginal zone metallophils in the clearance of lymphocytic choriomeningitis

virus infection. Eur.J.Immunol. 1997;27:2626-2633.

34. Vanderheijden N, Delputte PL, Favoreel HW et al. Involvement of sialoadhesin in entry of porcine reproductive and respiratory syndrome virus into porcine alveolar macrophages. J.Virol. 2003;77:8207-8215.
35. Jones C, Virji M, Crocker PR. Recognition of sialylated meningococcal lipopolysaccharide by siglecs expressed on myeloid cells leads to enhanced bacterial uptake. Mol.Microbiol. 2003;49:1213-1225.
36. Kim SM, Lee JS, Lee YH et al. Increased alpha2,3-sialylation and hyperglycosylation of N-glycans in embryonic rat cortical neurons during camptothecin-induced apoptosis. Mol.Cells 2007;24:416-423.
37. Morris RG, Hargreaves AD, Duvall E, Wyllie AH. Hormone-induced cell death. 2. Surface changes in thymocytes undergoing apoptosis. Am.J.Pathol. 1984;115:426-436.
38. Savill JS, Henson PM, Haslett C. Phagocytosis of aged human neutrophils by macrophages is mediated by a novel "charge-sensitive" recognition mechanism. J.Clin.Invest 1989;84:1518-1527.
39. Erwig LP, Henson PM. Clearance of apoptotic cells by phagocytes. Cell Death.Differ. 2008;15:243-250.
40. Wermeling F, Chen Y, Pikkarainen T et al. Class A scavenger receptors regulate tolerance against apoptotic cells, and autoantibodies against these receptors are predictive of systemic lupus. J.Exp. Med. 2007;204:2259-2265.
41. Miyake Y, Asano K, Kaise H et al. Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. J.Clin. Invest 2007;117:2268-2278.
42. Nolte MA, Belien JA, Schadee-Eestermans I et al. A conduit system distributes chemokines and small blood-borne molecules through the splenic white pulp. J.Exp.Med. 2003;198:505-512.
43. Allan RS, Smith CM, Belz GT et al. Epidermal viral immunity induced by CD8alpha+ dendritic cells but not by Langerhans cells. Science 2003;301:1925-1928.
44. Allan RS, Waithman J, Bedoui S et al. Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. Immunity. 2006;25:153-162.
45. Belz GT, Smith CM, Kleinert L et al. Distinct migrating and nonmigrating dendritic cell populations are involved in MHC class I-restricted antigen presentation after lung infection with virus. Proc.Natl.Acad. Sci.U.S.A 2004;101:8670-8675.
46. De ST, Pajak B, Muraille E et al. Regulation of dendritic cell numbers and maturation by lipopolysaccharide in vivo. J.Exp. Med. 1996;184:1413-1424.
47. Reis e Sousa, Hieny S, Scharton-Kersten T et al. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. J.Exp.Med. 1997;186:1819-1829.
48. Reis e Sousa, Germain RN. Analysis of adjuvant function by direct visualization of antigen presentation in vivo: endotoxin promotes accumulation of antigen-bearing dendritic cells in the T cell areas of lymphoid tissue. J.Immunol. 1999;162:6552-6561.
49. Austyn JM, Kupiec-Weglinski JW,

Hankins DF, Morris PJ. Migration patterns of dendritic cells in the mouse. Homing to T cell-dependent areas of spleen, and binding within marginal zone. J.Exp.Med. 1988;167:646-651.

50. Kraal G, Mebius R. New insights into the cell biology of the marginal zone of the spleen. Int.Rev.Cytol. 2006;250:175-215.
51. Lopes-Carvalho T, Foote J, Kearney JF. Marginal zone B cells in lymphocyte activation and regulation. Curr.Opin.Immunol. 2005;17:244-250.
52. Harshyne LA, Watkins SC, Gambotto A, Barratt-Boyes SM. Dendritic cells acquire antigens from live cells for cross-presentation to CTL. J.Immunol. 2001;166:3717-3723.
53. Neijssen J, Herberts C, Drijfhout JW et al. Cross-presentation by intercellular peptide transfer through gap junctions. Nature 2005;434:83-88.
54. McIlroy D, Autran B, Cheynier R et al. Infection frequency of dendritic cells and CD4+ T lymphocytes in spleens of human immunodeficiency virus-positive patients. J.Virol. 1995;69:4737-4745.
55. Sancho D, Mourao-Sa D, Joffre OP et al. Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. J.Clin. Invest 2008;118:2098-2110.
56. Tacken PJ, de V, I, Torensma R, Figdor CG. Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting. Nat. Rev.Immunol. 2007;7:790-802.
57. Pack M, Trumpfheller C, Thomas D et al. DEC-205/CD205+ dendritic cells are abundant in the white pulp of the human spleen, including the border region between the red and white pulp. Immunology 2008;123:438-446.
58. Melief CJ. Mini-review: Regulation of cytotoxic T lymphocyte responses by dendritic cells: peaceful coexistence of cross-priming and direct priming? Eur.J.Immunol. 2003;33:2645-2654.
59. Wilson NS, Villadangos JA. Regulation of antigen presentation and cross-presentation in the dendritic cell network: facts, hypothesis, and immunological implications. Adv.Immunol. 2005;86:241-305.
60. Rock KL, Shen L. Cross-presentation: underlying mechanisms and role in immune surveillance. Immunol. Rev. 2005;207:166-183.