Plasmacytoid Dendritic Cells Contribute to the Spatio-temporal Distribution of Megakaryocytes in the Bone Marrow

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München, November 22, 2024

Jutta Weitz

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List of Abbreviations

BDCA2 blood dendritic cell antigen 2

BMP4 bone morphogenic protein 4

BSA bovine serum albumin

BST2 bone marrow stromal antigen 2, also known as tetherin

c-mpl thrombopoietin receptor

CDC common dendritic cell progenitor

cDC common dendritic cell

CLP common lymphoid progenitor

CMP common myeloid progenitor

CXCR4 C-X-C chemokine receptor type 4

DMS demarcation membrane system

ECM extracellular matrix

EPO erythropoietin

FLT3L FMS-like tyrosine kinase 3 ligand

GM-CSF granulocyte-macrophage colony-stimulating factor

HSC hematopoietic stem cell

IFN Interferon

IL interleukin

IRF7 Interferon regulatory factor 7

M-CSF macrophage colony stimulating factor

MEP megakaryocytic-erythrocytic progenitor

MHC II major histocompatibility complex II

MK megakaryocyte

MKP megakaryocyte progenitor

MPP multipotent progenitor

PACAP Pituitary adenylate cyclase-activating polypeptide

PBS phosphate-buffered saline

pDC plasmacytoid dendritic cell

PECAM platelet endothelial cell adhesion molecule

PF4 platelet factor 4

PFA paraformaldehyde

SCF stem cell factor

SDF-1 stromal cell-derived factor 1

TLR toll like receptor

TPO thrombopoietin

VIP Vasoactive intestinal peptide

vWF von-Willebrand factor

1 Abstract

Megakaryopoiesis describes the maturation from haematopoietic stem cells (HSCs) to megakaryocytes (MKs). Megakaryocytes are large and scarce cells in the bone marrow which produce platelets. The production of platelets is achieved by fragmentation of the MK cytoplasm, which ultimately leads to MK apoptosis. Multiple studies have studied megakaryopoiesis, all trying to elucidate responsible factors for the differentiation and maturation of HSCs. This works aims to investigate the role of plasmacytoid dendritic cells (pDCs) in the regulation of megakaryopoiesis.

Cell quantification of pDCs, megakaryocytes and megakaryocyte progenitors (MKPs) in the bone marrow was performed by confocal microscopy and fluorescence-activated cell sorting (FACS). Confocal microscopy was also used to characterize the cell-to-cell interactions between pDCs and MKs or MKPs, as well as the spatial distribution of these cells in the bone marrow.

We observed a high number of pDC-MK interactions in the bone marrow of untreated mice. Next, pDCs were ablated in genetically modified mice, pDC-DTR, using the diphtheria toxin. Here, the number of MKs and MKPs significantly decreased after 3 days of pDC depletion. In addition, the recruitment of MKs and MKPs towards the vasculature – which is necessary for platelet production – was impaired and the remaining population of MKs showed an increased ploidy, suggesting that they were older than the MKs in steady state. Together, these results indicate that pDCs may influence the maturation and migration of MKs and MKPs towards the vascular niche.

We hypothesize that pDCs can sense the apoptotic MKs at the end of their platelet-producing-life and induce MKP proliferation in order to replace the dying MKs. Since pDCs are the major producer of interferon α (IFNα), treatment with IFNα was used to investigate its possible role in the signaling between pDCs and MKPs. In our experiment, this treatment showed no effect on the number and the distribution of MKs and MKPs in our experiment. However, due to the limitations os our setup, the role of IFNα in megakaryopoiesis remains to be clarified.

2 Introduction

This study investigated the interaction between plasmacytoid dendritic cells (pDCs) and megakaryocytes (MKs) in the murine bone marrow. pDCs constitue a special subset of dendritic cells, found in the peripheral tissue and in the bone marrow, where they originate. They are part of the innate, as well as the adaptive immune system. Their main function is the detection of viral or bacterial DNA or RNA and in consequence, the secretion of type I interferon α (IFNα) via the Myd88/IRLF pathway [2]. MKs main characteristics are their size and polyploidy, which allows them to generate approximately 3000 platelets each [3]. The maturation and differentiation of megakaryocyte progenitor cells (MKPs) into MKs is called megakaryopoiesis. This process is closely regulated in order to maintain platelet homeostasis. The main regulator of megakaryopoiesis is thrombopoietin (TPO), however, it is known that multiple other cytokines can also influence megakaryopoiesis [4, 5].
This study aims to understand the role of pDCs in megakaryopoiesis, more precisely on the spatial distribution of MKs and MKPs. Murine bone marrow stainings were used to observe and quantify the interactions between pDCs and MKs or MKPs. The whole mount bone marrow stainings allowed us to preserve the morphology of the bone marrow and therefore to observe the spatial distribution of the cells in relation to the vasculature. By using genetically modified mice, we were able to study the spatial distribution of MKs and MKPs under various conditions. Fluorescence-activated cell sorting (FACS) was used to confirm the manual cell countings after pDC depletion.
In chapter 3, the scientific background about pDCs and MKs will be described in more detail: their functions, their formation and the regulation of their maturation. In chapter 4, the used materials and the process of the whole mount bone marrow staining will be described, as well as the FACS analysis. The results of each experiment will be explained in chapter 5. Finally, the results will be interpreted and put into context in chapter 6. The dissertation will finish with an outlook about the topics which would be worth investigating in the future.

3 Scientific background

3.1 Megakaryocytes

When injury occurs, the bleeding will only stop when a clot is formed by platelet activation and coagulation. Platelets are small cytoplasmic fragments which circulate in the blood and carry different platelet granules with clotting mediators. They are one of the main actors in preventing bleeding by forming thrombi or clots (synonyms). But this is not their only role in mammals: they are also involved in the process of inflammation, in the innate immunity, neoangionesis and tumor metastasis [6, 7]. Understanding the regulation and the formation of platelets is an important step for the maintenance of human health. Platelets are produced by megakaryocytes. Megakaryocytes are very large (20-100 µm), polyploidic, and rare cells (ca. 0.01% of all nucleated bone marrow cells) found in the bone marrow. [8]. Megakaryocytes produce approximately 3000 platelets each by releasing 100-500 µm long cytoplasmic extensions, called proplatelets, into the bloodstream [3]. These proplatelets are then fragmented in small pieces called platelets [9]. There is evidence that the final platelets shedding occurs in part in the lung vasculature, where the turbulences at the bifurcations favor mechanical fragmentation of proplatelets [10]. In the bone marrow, billions of cells of different cell types form not only the anatomic structure, but also the ideal habitat secreting many different kinds of cytokines and chemokines that regulate quiescence, differentiation and proliferation of the hematopoietic stem cells (HSC). This area is located close to the endosteum and is therefore called the endosteal or osteoblastic niche [11].

3.1.1 Megakaryopoiesis

The traditional concept of megakaryopoiesis starts with a long-term HSC, which looses quiescence and becomes a short-term HSC. These HSC are self-renewal and pluripotent cells, which can develop into any type of blood cell. The short-term HSC differentiates

into a multipotent progenitor (MPP). Hematopoiesis then bifurcates into the myeloid and the lymphoid lineage: MPPs can develop either into a common myeloid progenitor (CMP) or a common lymphoid progenitor (CLP). As MKs derive from the myeloid lineage, the CMP then becomes a megakaryocytic-erythrocytic progenitor (MEP) which possesses both thrombopoietin receptor or myeloproliferative leukemia protein c-mpl and erythropoietin receptors. MEP differentiate into megakaryocyte progenitors (MKPs) which finally become mature MKs. The terminal differentiation into MKs include the synthesis of specific platelet proteins, such as lysosomes, dense and alpha granules, the demarcation membrane system; and endomitosis. Endomitosis, which is actually mitosis with failure of the late cytokinesis [12, 13], leads to polyploidy. As the cytoplasm volume increases proportionally to the ploidy, polyploidy is believed to be a way to increase platelet production and metabolic pathways. Although endomitosis can stop at any level, ploidy in MKs can reach up to 128N, meaning 128 complete chromosome sets [14, 15].

However, this is a very long process and does not provide an explanation for rapid MK maturation during inflammation or acute blood loss. Various studies have proposed different explanations for faster MK development. In 2013, Sanjuan-Pla *et al.* have described platelet-biased stem cells, which express von-Willebrand factor vWF and are at the apex of HSC hierarchy. These cells are able to produce significantly more platelets than vWF- stem cells [16]. In addition, Haas *et al.* described stem cell-like MKP. These cells are as quiescent as HSCs but can differentiate more quickly when activated during acute platelet demand [1]. Fig. 3.1 illustrates the traditional concept of megakaryopoesis, as well as the stem cell-like MKPs. Altogether, these studies give us a better insight in the process of megakaryopoiesis, while its details remain to be completely understood.

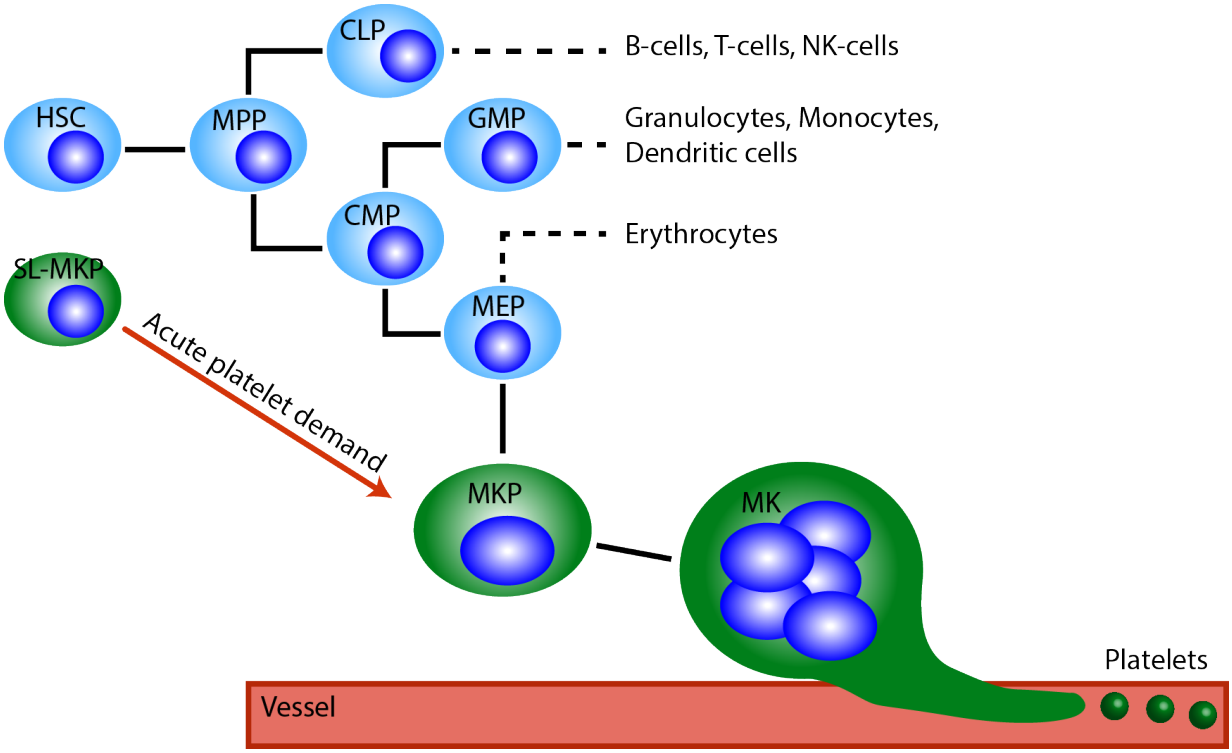

Figure 3.1: **Illustration of megakaryopoiesis**, taken from Haas *et al.* [1]. This scheme illustrates the different steps of megakaryopoiesis and the migration of the mature MK towards the vasculature for the release of platelets. MK = megakaryocyte; HSC = hematopoietic stem cell; SL-MKP = stem cell-like MKP; MPP = multipotent progenitor; CMP = common myeloid progenitor; CLP = common lymphoid progenitor; MEP = megakaryocytic-erythrocytic progenitor.

3.1.2 Spatio-temporal distribution of megakaryocytes and their progenitors

While HSC are located in the osteoblastic niche, close to the endosteum, mature MKs are located in the vascular niche, where the release of platelets into the vessels occurs. The proximity to the vasculature is also due to the need of oxygen, as a low-oxygen environment inhibits the maturation and formation of proplatelets [17]. MKs were thought to mature at the osteoblastic niche and then migrate towards the vasculature to form proplatelets [18]. Recently, another study could observe that only MKP migrate, whereas mature MK remain immobile close to the vessels. Fu and colleagues found out that there are non-motile and motile MKPs, which show a displacement towards the vessels. While non-motile MKPs grow constantly, motile MKPs are smaller and remain the same size. These data show that

the maturation from MKPs to MKs takes place locally in situ [19].

3.1.3 Regulation of the megakaryopoiesis

The regulation of megakaryopoiesis is complex, with many different factors that need to be considered. There are intra- and extracellular mechanisms involved. Thrombopoietin (TPO) is the key protein to regulate MK development [20, 21]. TPO is produced in the liver parenchymal cells and binds to c-mpl, which induces gene transcription enhancing MK proliferation and maturation. Geddis *et al.* observed in 2002 that after TPO treatment, the size, number and ploidy of MK was increased, in addition to elevated platelet levels [4]. TPO also binds to c-mpl on platelets and is degraded in the circulation. A platelet loss leads to less elimination and therefore increased levels of TPO and thus stimulates MKP proliferation and maturation [22]. TPO is also necessary for the survival of HSC, together with IL-3 and SCF [23]. However, TPO does not directly affects proplatelet formation by MKs, it mainly influences MKP proliferation [24, 25].

Besides TPO, there are also other cytokines that seem to influence megakaryopoiesis. For example, interleukines such as IL-6, IL-3, IL-11 and SCF also affect MKP differentiation *in vitro*. However, they seem to play a weak role *in vivo*. Erythropoietin (EPO) does also affect platelet formation, the specific role of EPO still remains unknown [14, 5]. Another player is stromal cell-derived factor 1 (SDF-1) and its receptor CXC chemokine receptor type 4 (CXCR4). SDF-1 is produced by stromal, endothelial and dendritic cells in the bone marrow, as well as by MKs themselves. This cytokine may induce endomitosis and proplatelet formation through Ras homolog family member A (RhoA) inactivation [26]. The RhoA GTPase is part of a signaling pathway which regulates actin polymerization and myosin activation. The migration of MKPs towards the vasculature during maturation plays another major role in this process. This seems to be linked to the chemoattraction of MKP and towards endothelial cells and the contact of both cells [27]. The platelet endothelial cell adhesion molecule (PECAM) is also indispensable for the MKP migration [28]. Despite their high CXCR4 expression, MKs react poorly to SDF-1 [29, 30]. After inflammation or an acute blood loss, the responsiveness of MK or MKP to SDF-1 can be regulated by cleavage fragments of the third complement component (C3a and des-ArgC3a) [31]. There is also evidence that bone morphogenic protein 4 (BMP4) and Interferonγ can promote MKP differentiation, whereas IFNα, Pituitary adenylate cyclase-activating

polypeptide (PACAP), vasoactive intestinal polypeptide (VIP) and platelet factor 4 (PF4) have been shown to inhibit it.
During the migration of MKP from the osteoblastic towards the vascular niche, they are influenced by the extracellular matrix (ECM) and the stroma surrounding them. For example, collagen type I, which is found in the osteoblastic niche, inhibits platelet formation. Other components of the ECM, which are found in the vascular niche, such as collagen type IV, fibronectin, laminin, fibrinogen and vWF are known to upregulate platelet production [32, 33, 34].

3.1.4 Thrombopoeisis

After completing the maturation process, MKs start to form long cytoplasmic extensions called proplatelets. These then disassemble into platelets which are packed with the granules and proteins from the former MK cytoplasm [35]. There are three principal molekular components involved in the formation of platelets. The demarcation membrane system (DMS) functions as a reservoir of membrane, β1-tubulin [36] as part of the cytoskeleton and Rho GTPases, which regulate platelet formation. The MKs remain with their multilobed nucleus until the entire cytoplasm has fragmented and become platelets. When only a rim of cytoplasm remains, MK become apoptotic and are phagocytosed by macrophages [37]. While it is commonly agreed that MKs possess an extrinsic and intrinsic apoptotic pathway and that their apoptosis follows the intrinsic pathway, recent studies have found out that apoptosis is dispensable for platelet formation [38, 39, 40] and that caspases are not necessary for the generation of platelets. The role of apoptosis in platelet shredding thus remains unclear.

3.1.5 Changes in megakaryopoiesis under platelet depletion

Another study done by Fu *et al.* looked at the changes in megakaryopoiesis under platelet depletion. As the number of MKs remains constant after platelet depletion, they were interested in the mechanism of urgent MK maturation. The acute platelet loss indeed led to an increased loss of cytoplasm in MKs. They could observe that the number of MKP increases after platelet depletion, as well as the fraction of MKPs close to the vasculature. Direct TPO treatment led to similar numbers of MKPs and even higher numbers of MKs. However, after MK depletion, the numbers of MKPs increases but without an increased

association with the vessels [19].

3.2 Plasmacytoid dendritic cells

Plasmacytoid dendritic cells (pDCs) are a special subset of dendritic cells and where first described in humans in 1950 [41] and identified in mice in 2001 [42]. Since then, they have become the subject of multiple studies examining their role in the immune responses against infection, inflammation and tumor progression. The development and the function of pDCs have aspects of both the adaptive and the innate immune system. The main role of pDCs is the secretion of IFNα and other cytokines in response to an acute viral infection [2]. But it has been shown that they also play a part in the pathogenesis of auto-immune diseases, cancer tolerance and the chronicity of the HIV infection [43].

3.2.1 The development of pDCs

Plasmacytoid dendritic cells develop in the bone marrow before they reach peripheral tissues, such as the thymus, through the blood stream. Traditionally, pDCs are believed to originate from the myeloid lineage, more precisely from a common DC progenitor (CDC), which also generates common dendritic cells (cDC). Onai *et al.* identified a clonogenic progenitor downstream of CDC [44]. This progenitor expresses high level of transcription factor E2-2, which is characteristic for the pDC lineage. After exposure to macrophage colony stimulating factor (M-CSF) and TPO, E2-2 is upregulated and leads to the differentiation of the progenitor cell to a pDC. [45]. However, pDCs can also derive from the lymphoid lineage. Even if lymphoid and myeloid pDCs may differ, both are able to activate lymphoid-specific genetic programs [46].
There are many factors involved in the regulation of pDC development. The most important factor is FMS-like tyrosine kinase 3 ligand (FLT3L), which is crucial for pDC generation [47]. Besides FLT3L, type I IFN and M-CSF also support pDC development [48, 49]. In contrast, granulocyte-macrophage colony-stimulating factor (GM-CSF) inhibits pDC development [50, 51].

3.2.2 The function of pDCs

Sensing of pathogens

The main role of pDCs is the recognition of viral deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) via their Toll-like receptors (TLR) 7 and 9, respectively. TLR7 senses RNA, whereas TLR9 senses viral and endogenous DNA. Their activation leads to the secretion of IFNα and other pro-inflammatory cytokines [2]. The uptake of the viral nucleic acids is a mechanism independent of a viral infection. Although the detailed mechanisms are yet to be completely understood, there is evidence that some viruses are bound by antibodies and taken up through fragment crystallizable region (Fc) receptors. There are inhibitory Fc receptors, activated during a memory response [52], and activating Fc receptors, which will induce IFNα production. These activating Fc receptors can bind endogenous DNA and thus probably have a part in autoimmune diseases, for example systemic lupus erythematosus (SLE) [53]. The production of IFNα or other pro-inflammatory cytokines is regulated by the compartment in which the nucleic acids are detected. For instance, viral DNA or RNA which is detected in endosomes will activate the myeloid differentiation primary response 88 (Myd88)/interferon regulatory factor 7 (IRF7) pathway, which leads to IFNα secretion. If it is detected in the endolysosomes, it will activate the NF-κB pathway and induce the production of pro-inflammatory cytokines [54, 55]. Besides TLR7 and TLR9, pDCs can also express TLR2 and TLR12 which can detect *Toxoplasma gondii* profilin [56] and bacterial polysaccharide A [57]. Although the TLRs seem to be the most important receptors in the anti-viral function of pDCs, other cytosolic nucleic acid receptors have just begun to be explored. For example, retinoic acid-inducible gene I (RIG-I) can detect the production of viral nucleic acid when the cell has been infected [58].

Source of IFNα

As described above, the main role of pDCs is the time-limited and amplitude-limited secretion of IFNα in response to a viral infection [59]. This IFNα secretion is very important at the beginning of the infection, especially when the virus reaches the circulation. During a local infection, pDCs only are involved if the local immune response does not resolve the virosis. An example is the Newcastle disease virus, which causes a pulmonary infection. In the lung, IFNα is mainly prodced by alveolar macrophages. pDCS will be only involved when the macrophages are eliminated [60]. However, the IFNα production of pDCs may not always be beneficial. Especially during chronic viral infections, such as an HIV-infection,

a dysregulated activity of pDCs could contribute to the chronicity of the infection [43]. A study from 2014 also found that excessive IFNα production, for example in response to the influenza virus, can lead to uncontrolled inflammation and apoptosis of bronchial epithelium [61].

pDCs as antigen presenting cells

Although pDCs are commonly known for their role in the innate anti-viral immunity, recent studies have discovered that pDCs also have antigen-presenting properties. The expression of major histocompatibility complex II (MHC II) molecules and the co-stimulatory receptors CD40, CD80 and CD86 give pDCs the ability to present antigens to $CD40^+$ cells, although not as effective as cDCs [62, 63]. Depending on the context, this presentation can either lead to an immune response or induce tolerance. When pDCs are activated via TLR, they will act as immunogenic. If they are unstimulated or activated through other receptors, they can promote tolerance, for example to tumor cells, alloantigens or harmless antigens. However, the role of pDCs in tolerance induction and its precise mechanisms are not yet completely understood. There have been some studies which explored the reaction of pDCs to activation through different receptors. On one hand, targeting antigens through blood dendritic cell antigen 2 (BDCA2) suppresses antigen-specific $CD4^+$ T-cell response upon 2^{nd} exposure and therefore promotes immunological tolerance [64]. On the other hand, targeting bone marrow stromal antigen 2 (BST2) with TLR agonists results in a protective immunity, mediated by T-cells [65]. The function of pDCs as a link between innate and adaptive immunity, contributing to induction and inhibition of an immune response, is very complex and has just begun to be explored.

Contribution of pDCs to different diseases

There is evidence that pDCs contribute to autoimmune diseases [66], such as systemic lupus erythematosus (SLE) [67], psoriasis [68, 69, 70] or type I diabetes [71] . In SLE, the antinuclear antibodies form immune complexes with endogenous DNA and can thus activate pDCs via TLR7 or TLR9. From this point of view, it is not surprising to find elevated IFNα levels in the blood of SLE patients. Depletion of pDCs blocks their abilities to produce IFN and could represent an option of treatment [72]. It would be crucial to know which other consequences the depletion of pDCs can promote. But not only SLE comes with an IFNα signature, it also seems to play a role in tuberculosis as the expression of the IFNα inducible transcripts correlates with the severity of the disease [73]. Last, but not

least, pDCs also contribute to the progression of some cancers. Indeed, a high number of pDCs is found in the microenvironment of some tumors. These pDCs seem to be tolerogenic rather than immunogenic, which probably leads to the poor prognosis associated with the pDC recruitment. In 2004, Treilleux *et al.* studied the possible contribution of pDCs to breast cancer [51] and a similar study was performed in 2011 on ovarian cancer [74]. Recently, pDCs have also gained importance in cancer immunotherapy with clinical trials on pDC-based cancer vaccines [75].

4 Materials and Methods

4.1 Mice

Wildtype mice (C57BL/6J) were pursued from The Jackson Laboratory. Genetically modified mice with the possibility to deplete pDCs (BDCA2-DTR, Strain 014176) were purchased from the Jackson Laboratory. Mice with green fluorescend protein (GFP) labelled pDCs (SiglecH-GFP) were bred at the Biomedical Center (BMC) and at the Walter-Brendel-Centre for experimental medicine, and kindly provided by Dr. Marco Colona and Dr. Susanne Stutte.

4.2 Reagents and Antibodies

Dipheria toxin was used to deplete pDCs in the pDC-DTR mice. 250ng/25g ml were injected i.p. over 3 consecutive days. For platelet depletion, we used 100 µl of anti-GPIb antibody (R300) per mouse and its negative control, non-immune rat antibodies (C301), also with a dose of 100 µl per mouse, both purchased from emfret analytics. After 12 hours of antibody injection, the mice were sacrificed. HOECHST, anti-GFP Antibody (A-11122), anti CD144 Antibody (16-1441-85) and the secondary antibodies goat anti-rat AF647 (A-21247), goat anti-rabbit AF488 (A-11034) and goat anti-rat AF594 (A-11007) were purchased from Invitrogen Life Technologies, BD Biosciences (Allschwil, Switzerland), or eBioscience (Vienna, Austria). CD41-FITC antibody (133904), anti-CD42d antibody (148502), and anti CD41 purified antibody (133901) were purchased from BioLegend (San Diego, US). The goat anti-armenian hamster AF647 secondary antibody (ab173004) was purchased from abcam.

4.3 Whole-mount immunofluorescence staining

First, sedation of the mice was performed using Isoflurane. The mice were then killed by cervical displacement. Sternum, humerus, femur and tibia were harvested and fixed with 4 % paraformaldehyde (PFA) at room temperature (RT) for 30 min. After this, the bones were incubated with 15 % sucrose for 2 h at 4°C, followed by 30 % sucrose overnight at 4°C. On the following day, the bones were ingrained in Tissue-Tek® O.C.T. Compound (Sakura) and frozen on dry ice. The samples were stored at -80°C. For staining, the samples were first cut on Histo Serve NX70 Cryostat (Epredia) until the myeloid tissue was fully revealed. The samples were blocked and permeabilized with 10 % Normal Goat Serum (Thermo Fisher Scientific) and 0.5 % Triton X-100 (Sigma-Alrich) for 45 min RT and then blocked with 10 % Normal Goat Serum overnight at 4°C. The first antibody was diluted 1:100 with phosphate-buffered saline (PBS); incubation was done overnight at 4°C. After incubation, the bones were washed 3x with PBS before beeing incubated with the secondary antibody (1:100 dilution) for 2 h at RT. If using a conjugated antibody, the bones were directly incubated with the conjugated antibody (1:100 dilution) overnight at 4°C. HOECHST was used to label the nuclei (1:1000 dilution). CD41-FITC or -PE and CD42d (hamster) combined with goat anti-hamster AF 647 were used to label MKs and MKPs. CD144 (rat) combined with goat anti-rat AF 594 was used to label the endothelial cells in the vessels. Anti-GFP (rabbit) was used to amplify the GFP signal from the bones of the SiglecH-GFP mice. For the imaging, LSM 880 confocal microscope with Airyscan module (Zeiss) was used with excitation wavelengths of 633 nm, 561 nm, 488 nm and 405 nm. The images were acquired with a 20x magnification objective and analyzed manually using the ZEN blue software. For each bone, the MKs and MKPs and either their distances to the vasculature or their interactions with pDCs were quantified on 10 images (Area = 455 nm x 455 nm; Depth = 15-20 µm, 1,5 µm step size). MKs and MKPs were differentiated by the staining, the size, and the shape of the cell and the number of nuclei. MKPs express higher CD41 levels, whereas mature MKs express lower CD41 and higher CD42 levels. MKPs are smaller than 20 µm, have only one nucleus and have less cytoplasm than MKs. These criteria were adopted in order to differentiate between MKs and MKPs, and to measure the distances from MKs and MKPs to the vasculature. Fig.4.1 shows the staining used to differentiate between MKs and MKPs. Fig.4.2 shows the adopted technique to measure the distances between MKs/MKPs and the vasculature.

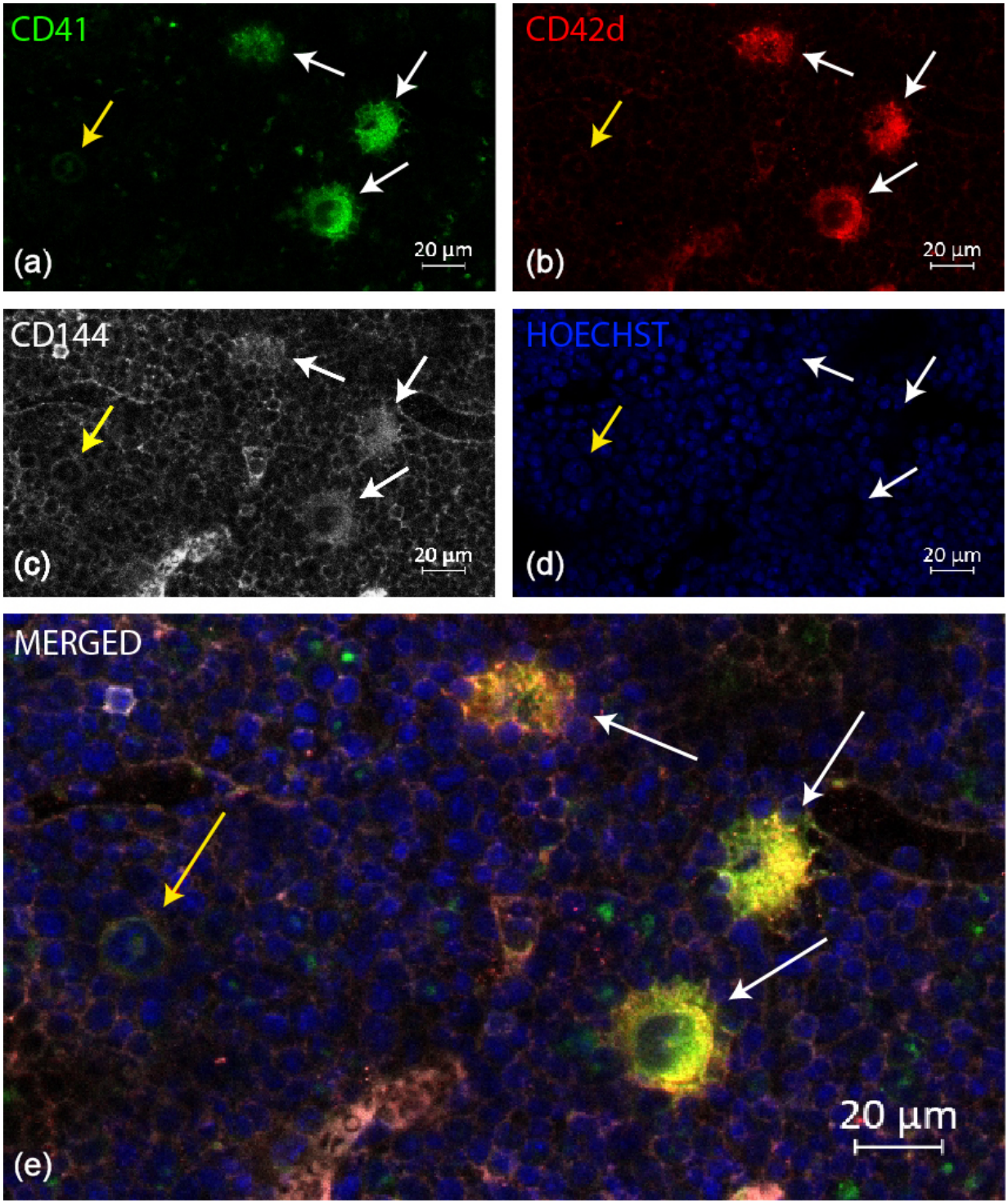

Figure 4.1: **Example of a whole mount bone marrow staining.** (a) shows the CD41 staining, which labels MKs and MKPs. (b) shows the CD42d staining, which is enriched on MKs. (c) shows the CD144 staining, which labels the endothelial cells of the vessels. (d) shows the HOECHST staining, which labels all nuclei. (e) shows all channels merged. The yellow arrow indicates MKPs, the white arrow indicates MKs. MKPs are smaller, rounder and have less cytoplasm than MKs. MKPs express less CD42d (CD42d-low) than MKs (CD42d-high). The scale bar is 20 µm.

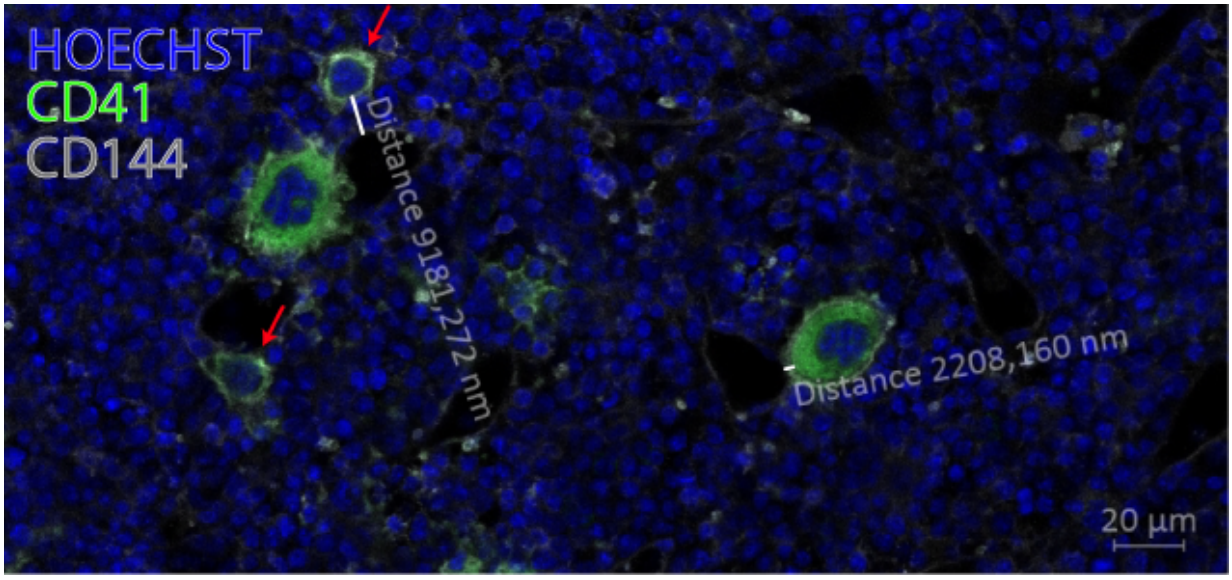

Figure 4.2: **Example of a whole mount bone marrow staining.** CD41 (green) labels MKs and MKPs, CD144 (grey) labels the vessels and HOECHST (blue) labels all nuclei. The red arrows point at the MKPs. The white bars show how the distances were measured for each cell. The scale bar is 20 µm.

4.4 Fluorescent Activated Cell Sorting

For the Fluorescent Activated Cell Sorting (FACS), C57BL/6J and pDC-DTR mice were used. First, after anesthetizing and sacrificing the mice, the femurs were harvested. They were then flushed with FACS Buffer (PBS + 1 % bovine serum albumin (BSA) using a 26G-needle. A 20G-needle was used to transform the flushed bone marrow into a single cell suspension. After centrifugation with 300 x g for 10 min at 4°C, the supernatant was carefully decanted thrown away. The pellet was then re-suspended in 100 µl PBS + 1 %BSA. Antibodies were added in a concentration of 1:100 and the cells were incubated for 1 h at 4°C. After incubation, 5 ml PBS were added to the suspension to wash it. The suspension was centrifuged again (300 x g, 10 min, 4°C) and after decanting the supernatant, the pellet was re-suspended in 200 ml PBS. For the MK and MKP the following antibodies were used: CD41-FITC, CD42d-APC and propidium iodide (PI) for cell viability. The cells were analyzed with a Gallios Flow Cytometer (Beckman Coulter). The gating strategy is shown in Fig.4.3. For the ploidy, the same antibodies were used but the cells were re-suspended in 0,5 ml PBS (instead of 0,2 ml). 1 % PFA was then added very slowly and the cells were incubated for 10 min at 4°C in the dark. After washing

with 5ml PBS and centrifuging, 500 µl DNA staining buffer (PBS + 2 mM $MgCl_2$ + 0.05% Saponin + 0.01 mg/ml Propidium iodide + 10 U/ml RNAse A) was added and the cells were incubated overnight at 4°C in the dark. The following day, the cells were directly analyzed by FACS.

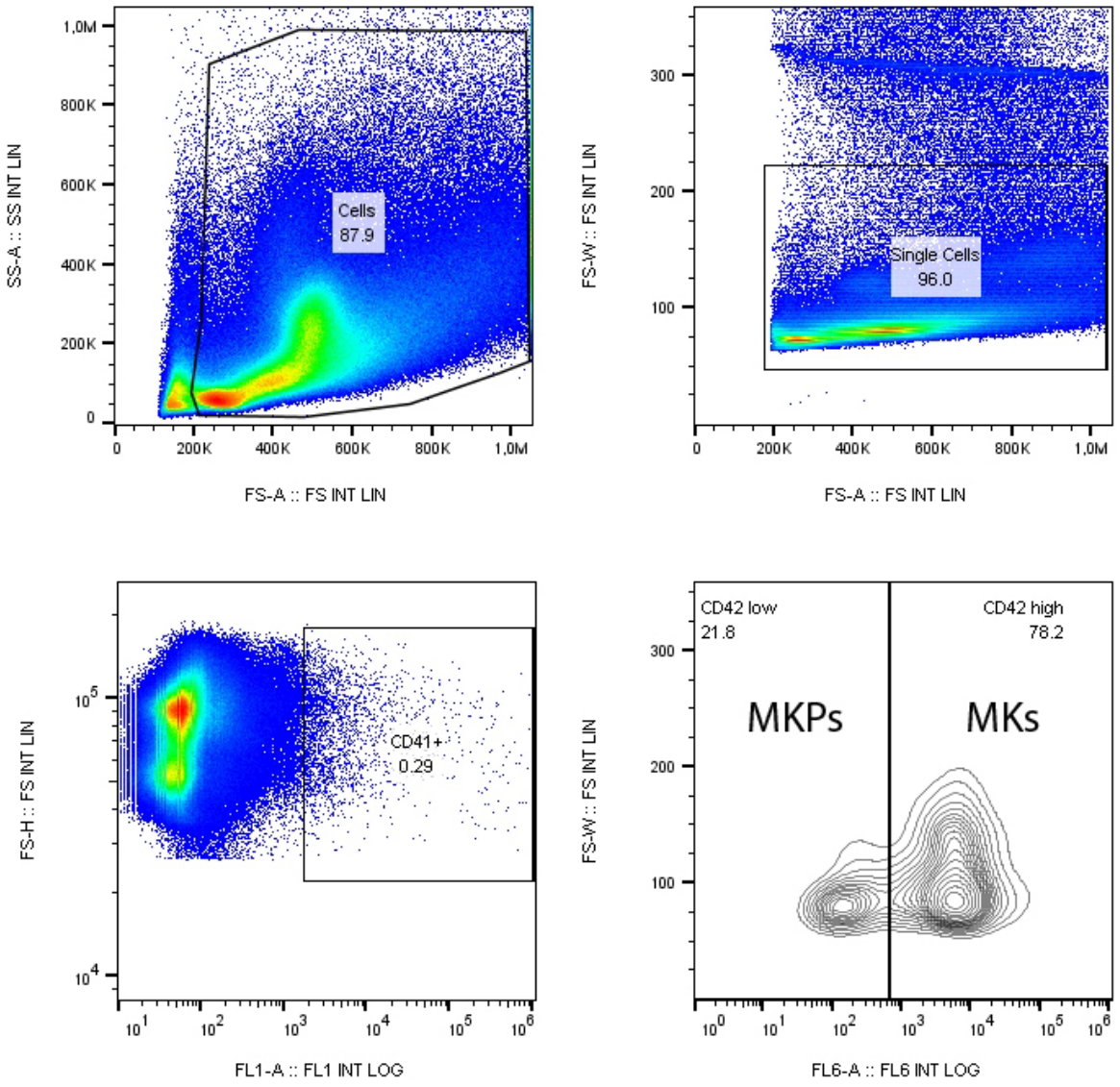

Figure 4.3: **Gating Strategy for identifying MKs and MKPs.** We used CD41-FITC (FL1) and CD42d-APC (FL6). MKs were defined as CD41+/CD42d high; MKPs were defined as CD41+/CD42d low.

5 Results

5.1 Characterization of MK/MKP numbers and pDC-MK/MKP interactions

To investigate and quantify the numbers of MKs and MKPs, the cell counting was done using sternum, tibia and femur bone marrow stainings. As shown in Fig.5.1, the numbers of MKs and MKPs in the sternum are comparable with the numbers of MKs and MKPs in long bones. Thus, numbers obtained by cell counting from long bones and sterna were pooled.

To have the intrinsic labelling for pDCs, a reporter mouse called SiglecH-GFP was used. SiglecH is a sialic acid–binding Ig-like lectin, that is expressed by pDCs and therefore, used as maker for these cells. The long bones and sternum were harvested and immunolabeled, showing the presence of interactions between pDCs and MKs, as shown in Fig.5.2. A significant number of MKs were in a very close spatial relationship with one or more pDCs. In some cases, the pDCs were located inside the MKs, as embedded in their cytoplasm.

In order to quantify these observations, MKs and MKPs were separated into four groups: MKs/MKPs with no pDC interactions, MKs/MKPs interacting with 1 pDC, MKs/MKPs interacting with 2 pDCs and MKs/MKPs interacting with 3 or more pDCs. The cells were counted manually and the results displayed in Fig.5.3. The results show that in average, 31% of the MKs and 15% of the MKPs interact with at least one pDC in steady state Fig.5.4.

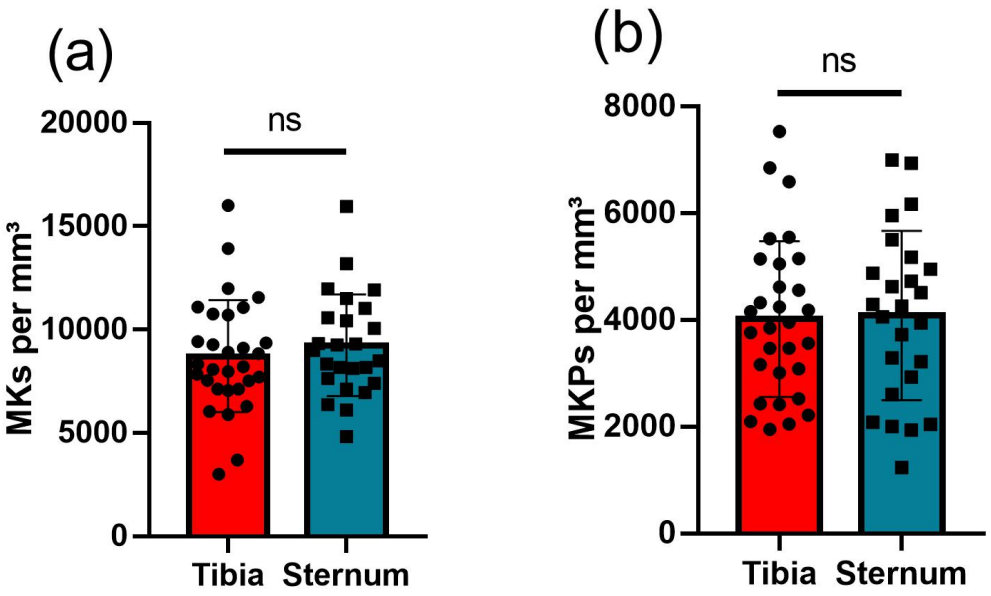

Figure 5.1: **Characterization of MK and MKP numbers in the bone marrow.** (a) MK numbers in the bone marrow. (b) MKP numbers in the bone marrow. The cells were counted in 3 tibias and 3 sterna of C57Bl/6J mice, with 10 images per bone. Statistics were done using the unpaired t-test. Mean + SD.

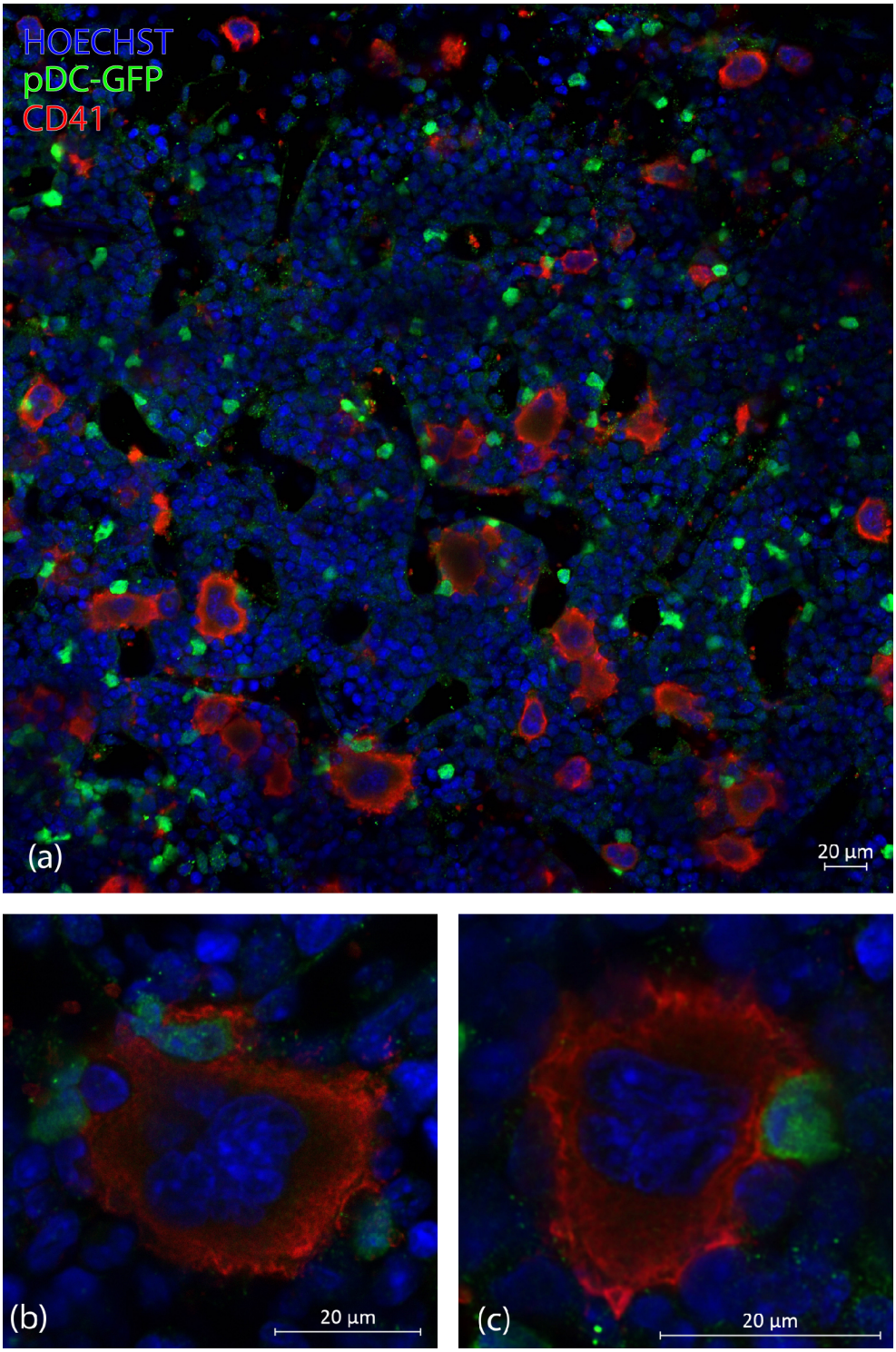

Figure 5.2: **pDCs interacting with MKs in the bone marrow**: representative whole mount bone marrow staining of a SiglecH-GFP tibia with 20x magnification. CD41 (red) labels MKs and MKPs, Siglec H-GFP (green) labels the pDCs and HOECHST (blue) labels all nuclei. (a) Overview with multiple MK/pDC interactions. (b) Close-up of one MK interacting with 3 pDCs. (c) Close-up of one MK interacting with 1 pDC. The scale bar is 20 µm.

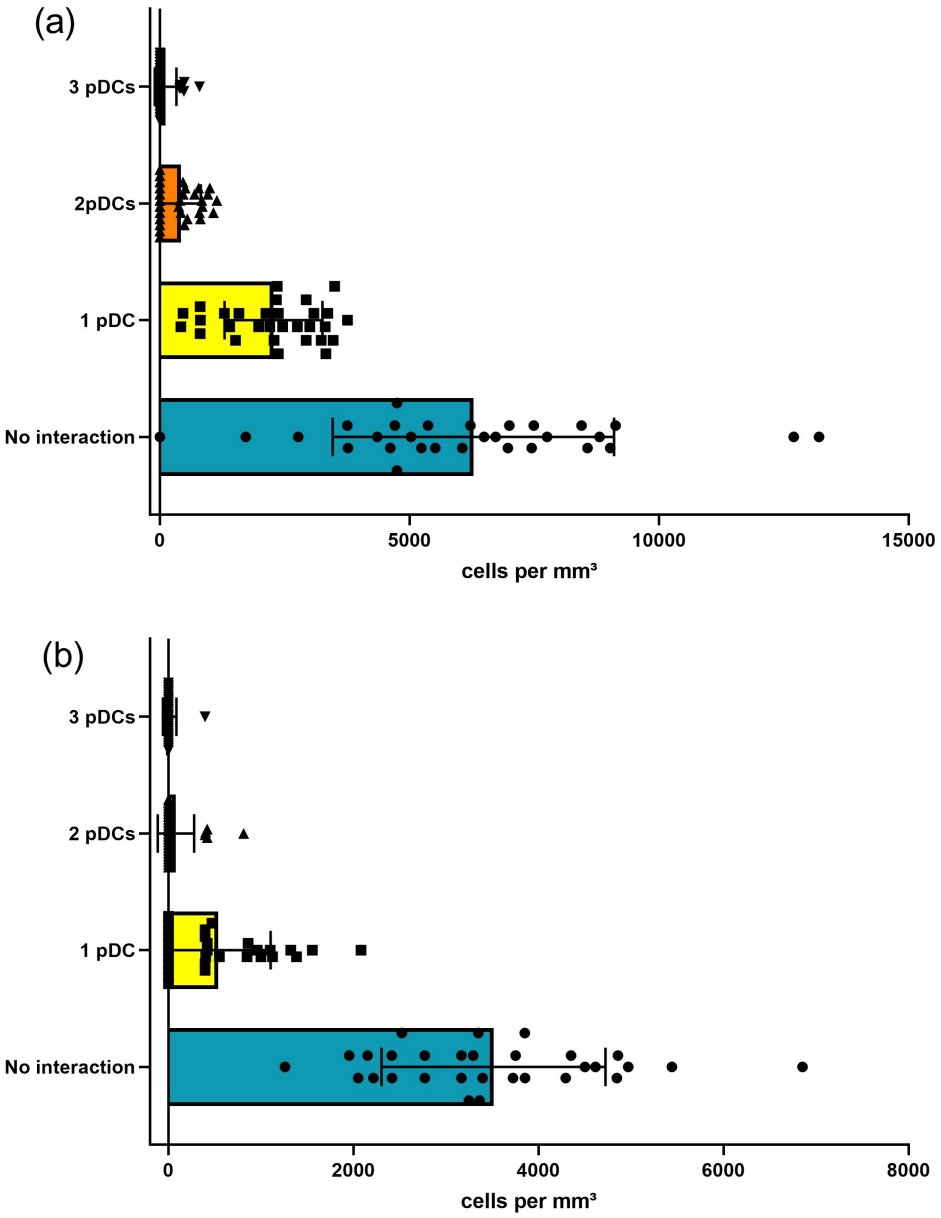

Figure 5.3: **Number of MK and MKP interacting with pDCs in steady state.** The blue bar shows the number of MK with no interaction with pDCs. The yellow bar shows the number of MK interacting with 1 pDC. The orange bar shows the number of MK interacting with 2 pDCs. The black bar shows the number of MK interacting with 3 pDCs. (a) Number of MKs interacting with 1,2 or 3 pDCs (b) Number of MKPs interacting with 1,2, or 3 pDCs. The data is pooled from 3 mice, and obtained by manual quantification of whole mount bone marrow stainings, with 10 pictures per mouse. Mean + SD.

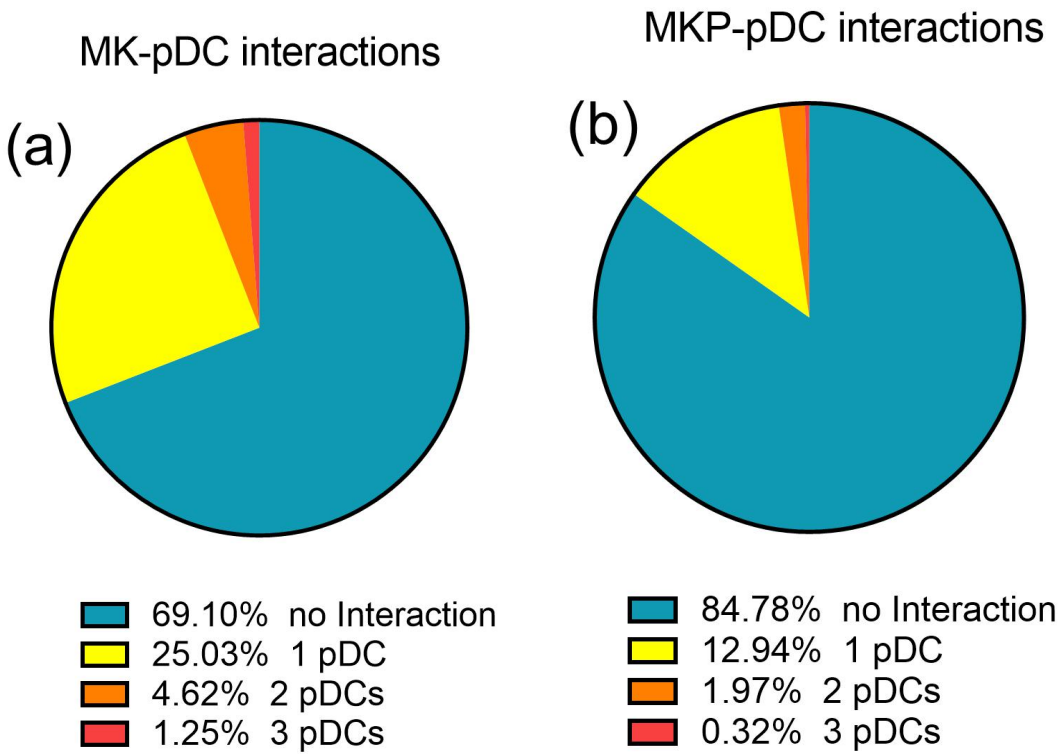

Figure 5.4: **Percentage of MKs and MKPs interacting with pDCs in steady state.** The blue section corresponds to no MK or MKP/pDC interaction, the yellow section corresponds to MK/MKP interactions with 1 pDC, the orange section corresponds to MK/MKP interactions with 2 pDCs and the red section corresponds to MK/MKP interactions with 3 pDCs. (a) Percentage of MKs interacting with 1,2 or 3 pDCs. (b) Percentage of MKPs interacting with 1,2 or 3 pDCs. The data is pooled from 3 mice, and obtained by manual quantification of whole mount bone marrow stainings, with 10 pictures per mouse.

5.2 MK and MKPs numbers under pDC depletion

A pDC-DTR mouse strain was used to study the numbers and distribution of MKs and MKPs after pDC depletion. pDCs were depleted by treating the mice with diphtheria toxin for 3 consecutive days. pDC depletion led to a decrease of MKs and MKP numbers in the bone marrow in the FACS analysis, however not significant in the unpaired t-test. The FACS results are displayed in Tab. 5.1 and plotted in Fig. 5.5. The experiment was repeated and the cell countings obtained manually with whole mount bone marrow immunofluorescence stainings with CD41 and CD42d. There was a significant decrease in

both MK and MKP numbers after pDC depletion in the manual cell countings. The results of the cell countings are displayed in Fig. 5.6.

Table 5.1: **Quantification of MK and MKP frequency in steady state and under pDC depletion.** The data is obtained by FACS analysis, using the gating strategy described in Materials and methods, see "4.4 FACS" CD41-FITC and CD42d-APC were used to label MKs (CD41+/CD42high) and MKPs (CD41+/CD42low).

Sample	Single cells	CD41+ (%)	CD41+ CD42high (%)	CD41+ CD42low (%)
Control 1	835763	0,10	0,09	0,01
Control 2	843586	0,29	0,23	0,06
Control 3	882990	0,12	0,11	0,01
pDC depletion 1	746676	0,07	0,04	0,03
pDC depletion 2	871717	0,09	0,08	0,01
pDC depletion 3	646372	0,04	0,03	0,01

When looking at the ploidy of the MKs, the FACS data showed that the MKs have a higher ploidy after pDC depletion than in steady state, see Fig. 5.7. As MKs become polyploid during their process of maturation, a cell with a high ploidy can be considered older that a cell with a low ploidy. Altogether, the experiments show that under pDC depletion, the MK number decreases, but the remaining MKs are older than under steady state.

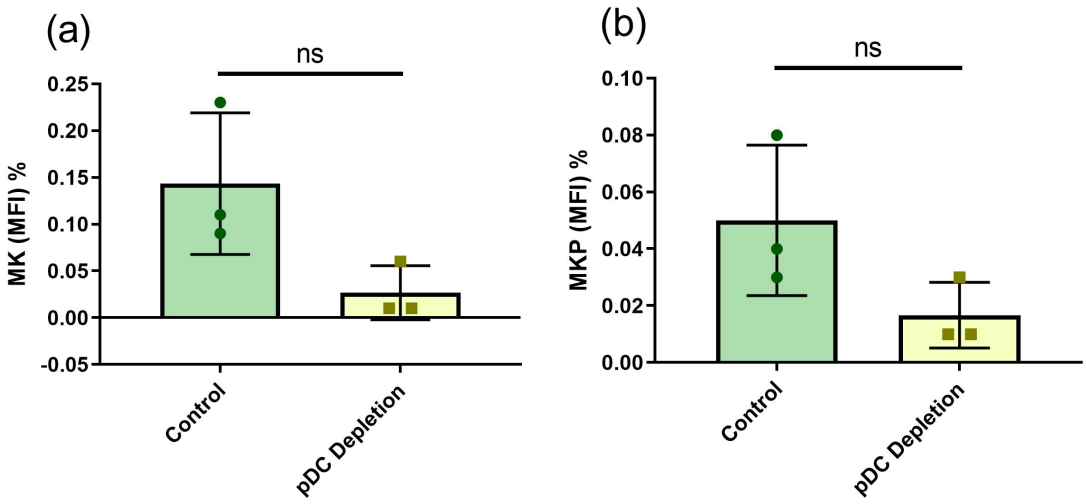

Figure 5.5: **Comparison of MK and MKP frequency under steady state and under pDC depletion.** (a) Percentage of CD41+ CD42 high cells, equivalent to MKs (b) Percentage of CD41+ CD42 low cells, equivalent to MKPs. Data was obtained by FACS analysis with anti-CD41- and anti-CD42d antibodies, as described in Table 5.1. Statistics were done using the unpaired t-test. Mean + SD.

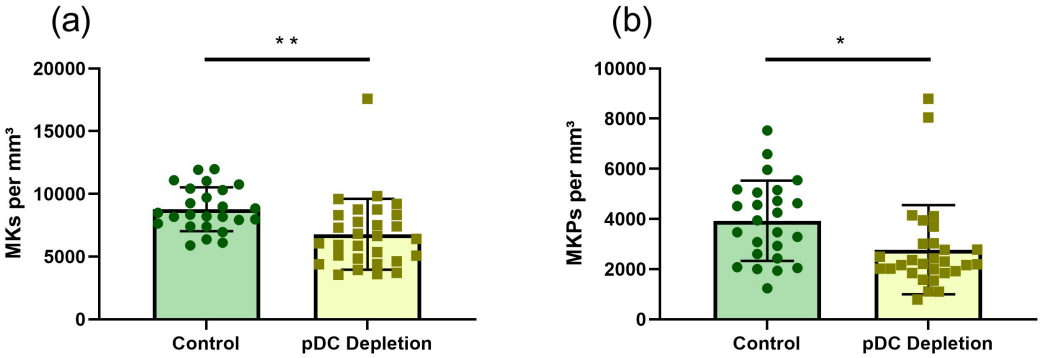

Figure 5.6: **Comparison of MK and MKP numbers in steady state and after pDC Depletion.** (a) Number of MKs. (b) Number of MKPs. Data obtained by manual quantification of whole mount bone marrow stainings with anti-CD41- and anti-CD42d-antibodies. The data is pooled from 3 mice with 10 images each. Statistics were done using the unpaired t-test. The p-value is 0.0035 for the MKs (**) and 0.0160 for MKPs (*). Mean + SD

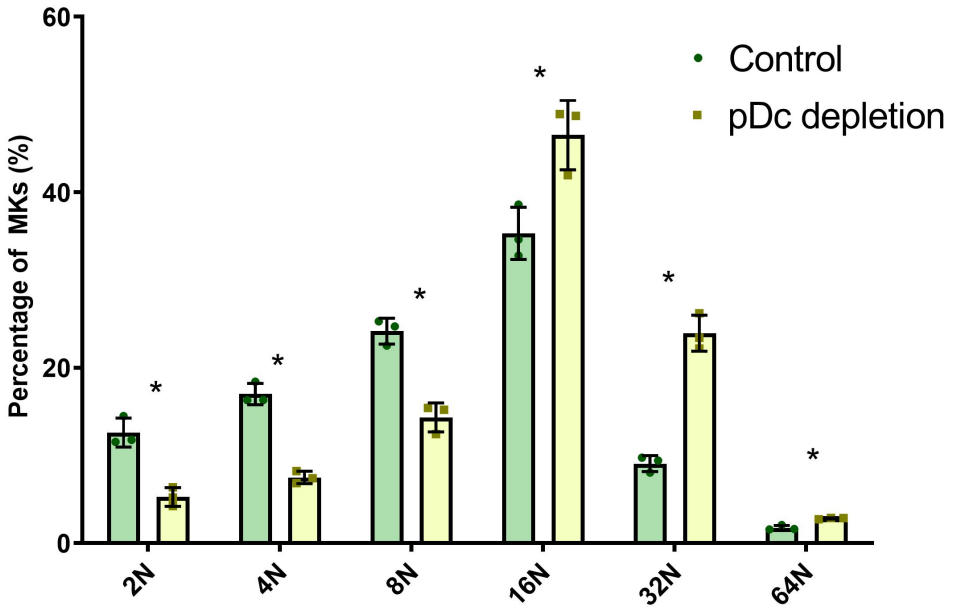

Figure 5.7: **MK ploidy in steady state and after pDC depletion.** The data was obtained by FACS analysis from 3 control and 3 pDC depleted mice, stained with anti-CD41- and anti-PI-antibodies. Statistics were done using multiple t-tests. The p-values were 0.002922 for 2N, 0.000291 for 4N, 0.001546 for 8N, 0.017327 for 16N, 0.000327 for 32N and 0.003058 for 64N. . Mean + SD.

5.3 Distribution of MKs and MKPs under pDC depletion

In the bone marrow, most MKs are situated close to the vasculature, where they release (pro)platelets into the vessels. The whole mount bone marrow stainings showed that 65.6% of the MKs and 54.1% of the MKPs were situated at a distance of 0-5 µm from the vessels. The data was analyzed using multiple t-tests. After pDC depletion, the numbers of MKs and MKPs in close proximity to the vasculature decreased significantly. The fraction of the MKs situated 0-5 µm from the vessels decreased from 65.6% to 55.1%. The fraction of the MKPs situated 0-5 µm from the vessels did not change significantly, as displayed in Fig. 5.8 and Fig. 5.9.

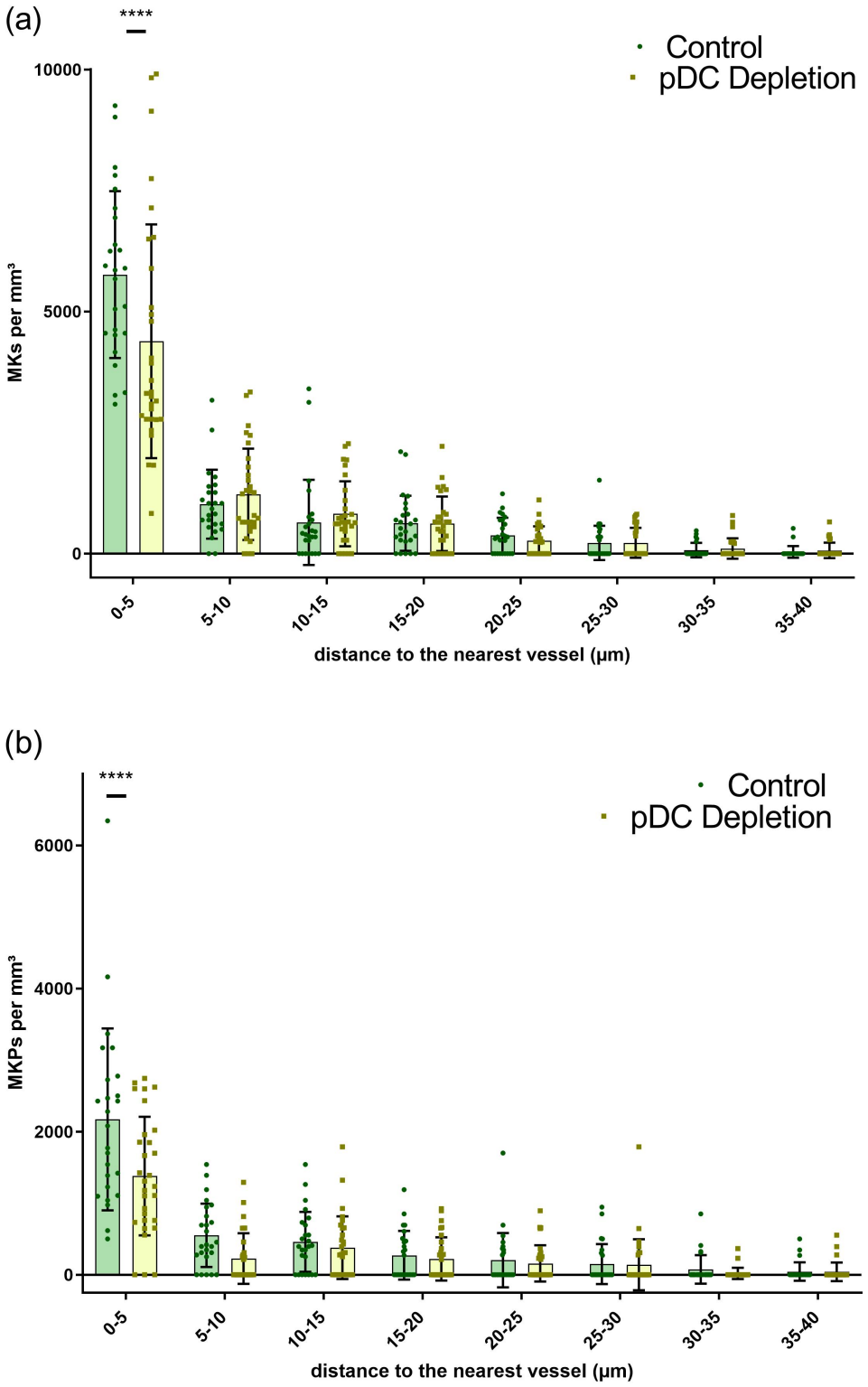

Figure 5.8: **Distances of MKs and MKPs to the vasculature in steady state and after 3d pDC depletion.** (a) MK distances to the vasculature. (b) MKP distances to the vasculature. Data are drawn from 3 C57Bl/6J and 3 pDC-DTR mice and obtained by cell counting from whole mount bone marrow staining with anti-CD41-, anti-CD42d-, and anti-CD144-antibodies. Statistics were done using the unpaired t-test. The p-values were <0.0001 for both MKs and MKPs. Mean + SD

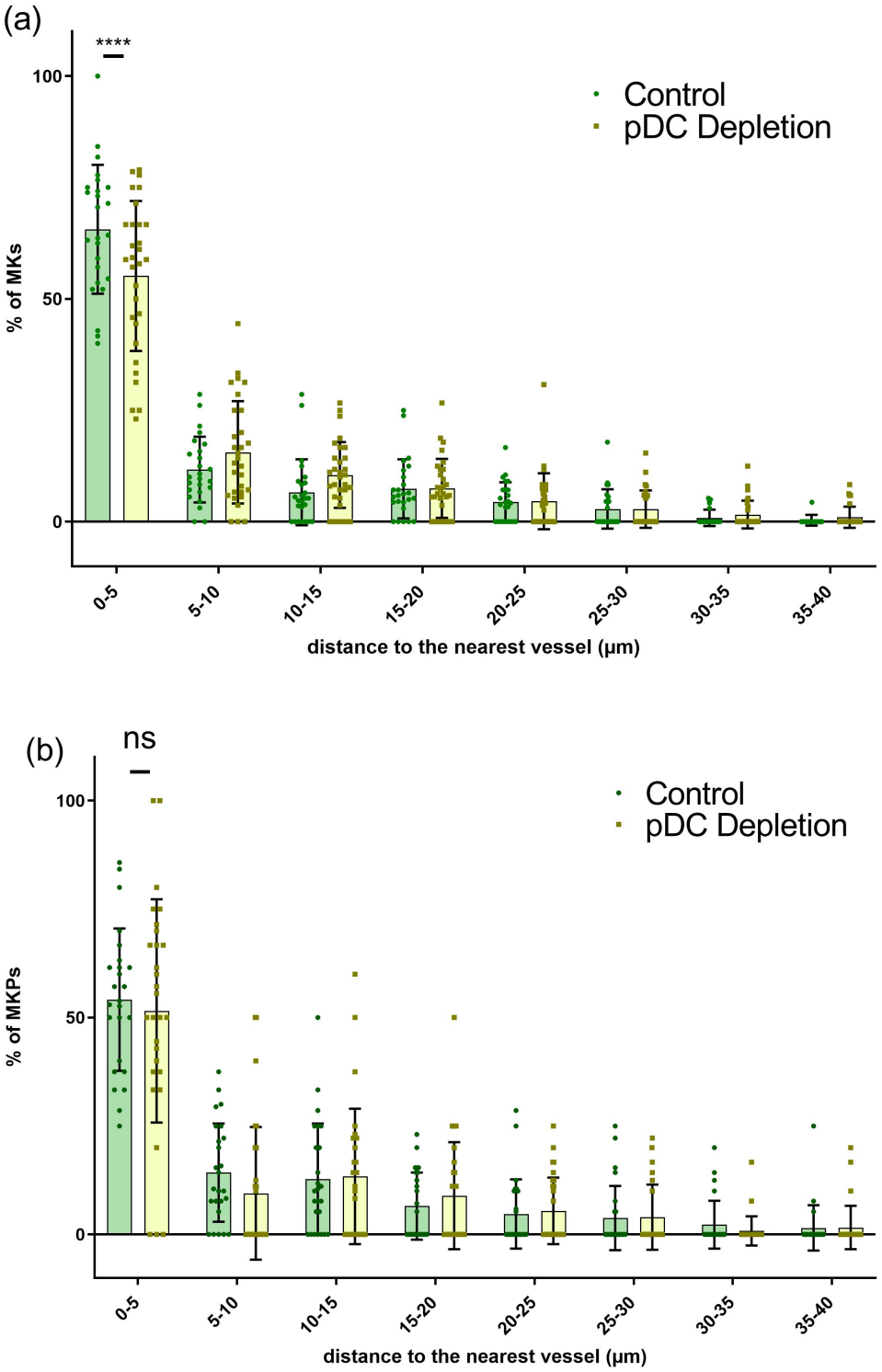

Figure 5.9: **Distances of MKs and MKPs to the vasculature in steady state and after 3d pDC depletion in percent.** (a) MK distances to the vasculature in percent. (b) MKP distances to the vasculature in percent. Data are drawn from 3 C57Bl/6J and 3 pDC-DTR mice and obtained by cell counting from whole mount bone marrow staining with anti-CD42d-, and anti-CD144-antibodies. Statistics were done using the unpaired t-test. The p-value was <0.0001 for MKs. Mean + SD

These data show that the absence of pDCs has an impact on the differentiation of MKs, as well as their recruitment to the vasculature. As pDCs' main function is the secretion of IFNα upon activation [43], cell countings were also performed after IFN treatment in C57B6J mice.

5.4 Distribution of MKs and MKPs under IFNα treatment

The influence of IFNα on megakaryopoiesis has been controversially discussed. While some authors describe an inhibition of megakaryopoiesis following IFNα treatment, others claim that IFNα in fact has a stimulatory effect on megakaryopoiesis. For instance, Wang *et al.* described in 2000 that IFNα inhibits TPO-induced murine MK growth in vitro by inhibiting the phosphorylation of TPO-induced signaling proteins in mice [76]. Eight years later, Yamane *et al.* performed some CFU-assays with human MKs and observed that IFNα inhibits the maturation of the demarcation membrane system and thus inhibits platelet production, but did not affect endomitosis [77]. In this study, IFNα seems to affect mostly mature MKs, suppressing mRNA expression of transcription factors which regulate the late-stage megakaryopoiesis. Another study looked at the stem-like megakaryocyte-committed progenitors (SL-MKPs) and how inflammation induced emergency megakaryopoiesis results from the fast cellular maturation of SL-MKPs after mimicking infection with polyinosinic:polycytidylic acid (pI:C) treatment. More precisely, the administration of pI:C led to the upregulation of MK proteins in HSCs. The administration of IFNα had the same effect as the administration of pI:C [1]. This study supports the findings of Essers *et al.*, who observed that IFNα leads to an activation of dormant HSCs *in vivo.* [78]. However, expression of IFNαR was not required for this effect. Another study came to a similar conclusion, claiming that IFNα can cause exhaustion of HSCs, whereas interferon regulatory factor-2 (IRF-2) suppresses IFNα-signaling and therefore preserves the HSC capacity [79]. In summary, IFNα seems to have an inhibitory effect on MKs, but a stimulatory effect on the stem cells, like SL-MKPs.

To investigate if IFNα affects the distribution of MKs and MKPs in the bone marrow, 3 C57BL/6J mice were treated for 4 hours with IFNα. When looking at the numbers and the distribution of MKs and MKPs compared to mice without such treatment, neither the numbers of MKs and MKPs, nor their distance to the vasculature showed any statistically

significant difference, see Fig. 5.10 and Fig. 5.11.

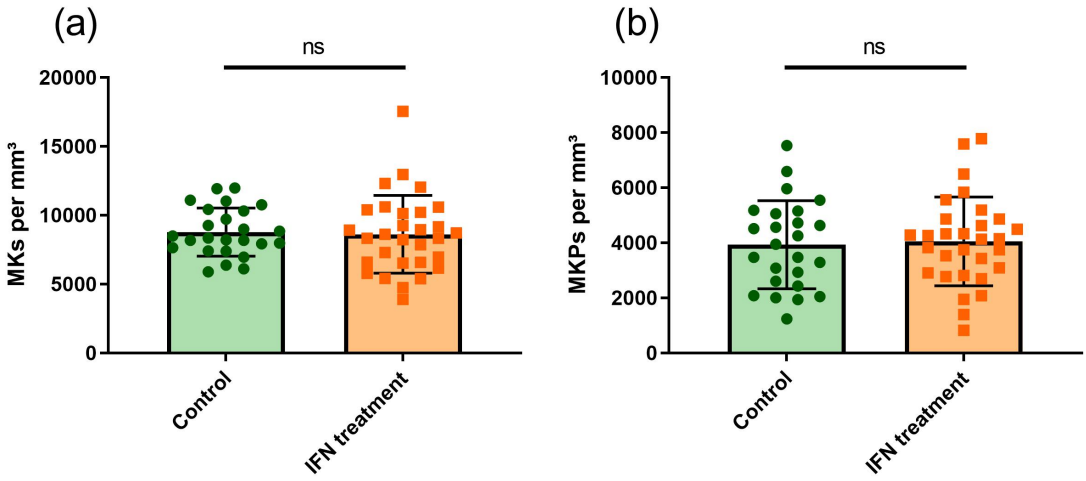

Figure 5.10: **Number of MKs and MKPs in steady state and after 4h IFNα treatment.** (a) Number of MKs. (b) Number of MKPs. The data is pooled from 3 control and 3 treated mice, with 10 images per bone, and obtained by cell counting from whole mount bone marrow stainings with anti-CD41 and anti-CD42d antibodies. Statistics were done using the unpaired t-test. Mean + SD.

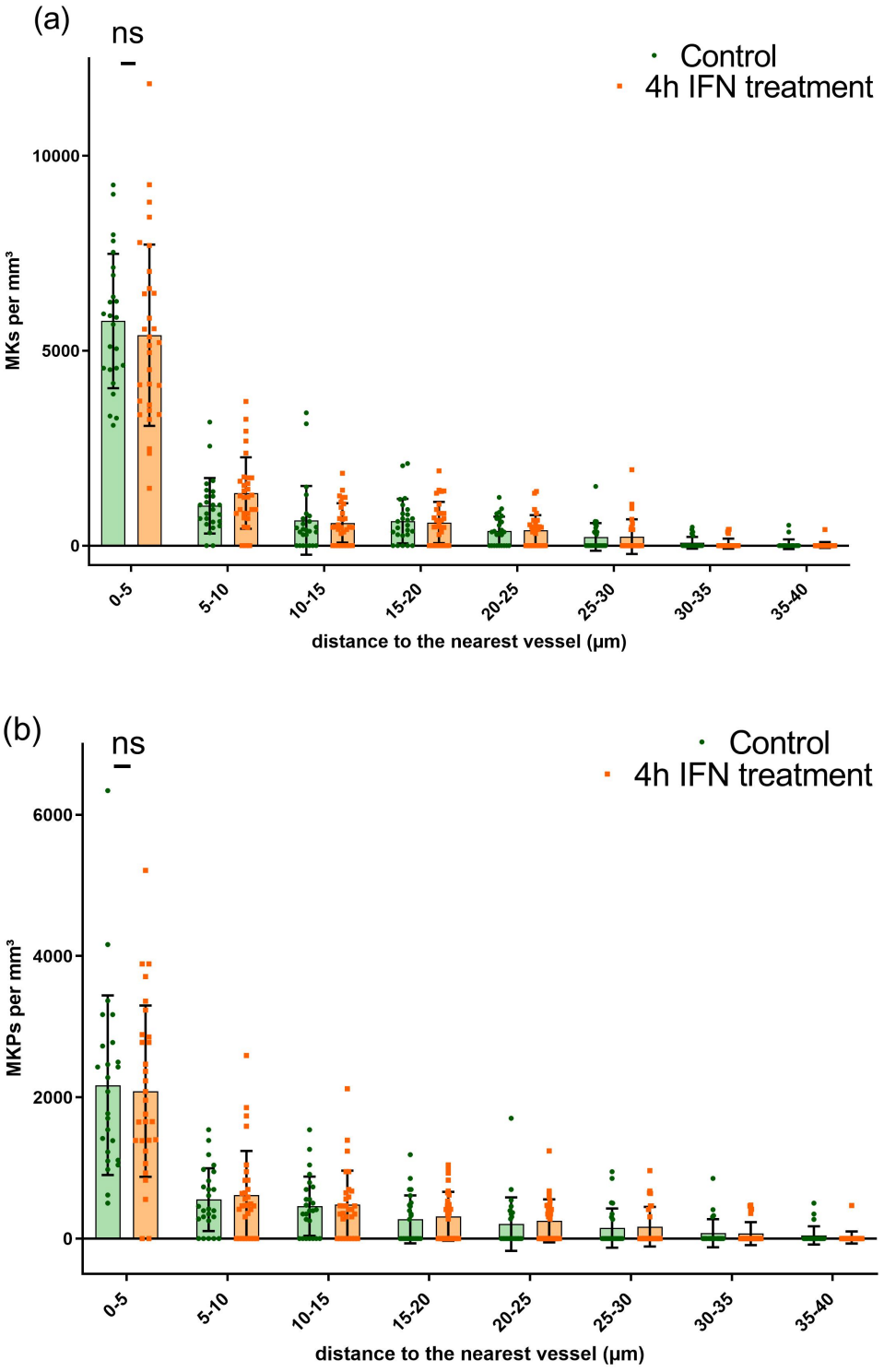

Figure 5.11: **Distance of MKs and MKPs to the vasculature in steady state and after 4h IFNα treatment.** (a) MK distances to the vasculature. (b) MKP distances to the vasculature. The data is pooled from 3 control and 3 treated mice and obtained by cell counting from whole mount bone marrow stainings with anti-CD41, CD42d, and CD144 antibodies. Statistics were done using the unpaired t-test. Mean + SD.

6 Discussion

6.1 Résumé

In this study, the interaction of pDCs with MKs/MKPs and its influence on the distribution of MKs/MKPs and megakaryopoiesis was investigated. For this purpose, FACS analysis and immunohistology were used to quantify the numbers of cells, interactions and spatial distribution of the cells in the bone marrow. Under pDC depletion however, the population of MK decreases and the remaining MKs are older than under steady state, translated by an increase in the ploidy of the MKs. In addition, the recruitment of MKs towards the vasculature was impaired in the absence of pDCs. However, the spatial distribution of MKs and MKPs did not change under IFNα treatment.

6.2 Discussion of the results

Our study showed that in average, 30% of MKs are in close contact with at least one pDC. The pDCs sometimes do not only co-localize, but are indulged in the cytoplasm of MKs. This event is called emperipolesis, which describes the situation when a living cell penetrates another living cell. MKs are known to engulf hematopoietic cells, yet this is only described within hematolymphoid disorders [80]. While it is well known that pDCs detect viral DNA and RNA, they also seem to be able to detect self DNA and RNA during apoptosis, which then leads to low IFNα responses [2]. Programmed cell death or apoptosis of MKs is well described [81]. Some authors even claim that apoptosis is necessary for proplatelet production [38], while others argument that platelet production can also occur independently of the apoptotic pathways [40]. Together with our finding of co-localization of pDCs and MKs, this indicates that pDCs in the bone marrow are likely to recognize apoptotic MKs.

A recent study by Lee-Sundlov *et al.* also focused on the interaction between pDCs and desialylated MKs. They demonstrated the recognition of desialylated MKs by pDC-like

immune cells in an ex-vivo coculture study[82]. Desialysation describes the loss of a sialic acid on cells which occurs, e.g., under acute infections and is associated with thrombocytopenia. In their experiments, pDCs did inhibit thrombopoiesis via IFN-signaling. Sialic acids bind to Siglec Receptors (sialic acid binding immunoglobulin-like lectins), such as Siglec H on pDCs, to suppress immune cell activation [83]. Zhang *et al.* described an association between apoptotic platelets and GPIbα desialylation [84]. GPIbα is part of the GPIb-IX-V complex (CD42) and originates in mature MKs [85]. The binding of the Siglec H receptors on pDCs to unsialylated proteins in MKs, such as GPIbα on the verge of apoptosis could be another possible explanation for the observed interactions in our study.

TPO is undoubtedly the main regulator of megakaryopoiesis [21]. However, a recent study in review showed that TPO does not influence the spatial distribution of MKPs [86]. When counting MKs and MKPs in whole mount bone marrow stainings after 3 days pDC depletion, a significant decrease in MK and MKP numbers was observed. This data could not be confirmed by FACS analyses, which can be explained by the low number of experiments done (n=3). Still, MKs were fewer in numbers and older after pDC depletion than in steady state. This indicates that pDCs are involved in the regulation of megakaryopoiesis. Additionally, the spatial distribution of MKs was impaired after pDC depletion. It is therefore likely that pDCs are a link between apoptotic MKs and replenishment of MKs in the vascular niche by regulating the recruitment of MKPs towards the vasculature. Thus, we hypothesize that when pDCs would encounter apoptotic MKs, they would release a signal which triggers the movement from MKPs towards the vasculature, where they would differentiate into MKs and replace their precursors. However, further studies which focus on apoptotic MKs are needed to prove this hypothesis.

In our study, we used four hours of systemic IFNα treatment to investigate the role of IFNα in the regulation of megakaryopoiesis. The time line of IFNα treatment here showed no significant change in the distribution of MKs and MKPs. However, IFNα is released locally by pDCs in the bone marrow. This locally specific distribution of IFNα is necessary to balance both the stimulative and inhibitory effects of IFNα on HSCs and MKs (see section 5.4). Here, we suggested a regulatory functions of pDCs in megakaryopoiesis through the release of IFNα. Further studies need to elucidate the role of IFNα on the spatial distribution of MKs.

Plasmacytoid dendritic cells are a type of immune cells which show an inflammatory response when encountering pathogens [2]. Thrombocytes are not only necessary for thrombus formation, but they also possess proinflammatory properties. For instance, they play

an important role in leucocyte recruitment and immunothrombosis [87]. Therefore, pDC activation stimulating megakaryopoiesis is important from an immunogenic perspective. Indeed, platelet counts do increase under acute inflammation [88]. However, chronic pDCs activation leads to chronic inflammation with is associated with thrombocytopenia [89]. Chronic elevated levels of IFNs have been shown to exhaust HSCs and therefore leading to MK replenishment [79].

Our study brings significant evidence that pDCs are regulators of platelet production by controlling megakaryopoiesis. Thus, our findings can contribute to the development of a treatment against thrombocytopenia, which is a often found side effect of various treatments, e.g., of chemotherapy. The more regulators of megakaryopoiesis are being identified and the more target options for treatments are possible. In addition, the role of pDCs in megakaryopoiesis is crucial to understanding and preventing side effects from possible treatment options of systemic lupus erythematosus (SLE), like the depletion of pDCs [72].

6.3 Limitations of the study

This study identified pDCs as regulators for megakaryopoiesis. However, we only assumed that pDCs interact preferably with apoptotic MKs, based on the literature. Further studies will have to prove this hypothesis by using, e.g., the tunel essay or caspase 3 to label apoptotic cells. Due to the time consuming manual cell counting, the experiments were performed with only 3 mice per group. A higher number of experiments might be necessary to detect significant changes in MK or MKP numbers, e.g., after treatment with IFNα. Additionally, as mentioned above, systemic treatment with IFNα may not mimic the local secretion if IFNα by pDCs. Specific activation of pDCs could be a better way to determine whether IFNα has an effect on the spatial distribution of MKs/MKPs or not. The mice in our experiment were treated for 4h with IFNα. While there was no change in the distribution of MKs/MKPs after 4h, our study design did not detect the changes after a longer period of time. In further studies, it would be interesting to repeat the experiment after, e.g., 8 and 12 hours of IFNα treatment.

In our experiments, we used whole mount bone marrow staining for cell counting. The differentiation between MKs and MKPs was difficult sometimes, as the staining (CD42d-low vs. CD42d-high) failed to be 100% specific. For this reason, we also included other parameters like the size, the shape of the cell and the number of nuclei to differentiate

between MKs and MKPs. With a more specific and more consistent staining, the quantification of the results could have been more effective and less time consuming.

7 Summary and Outlook

7.1 Summary

This study focuses on the interplay between plasmacytoid dendritic cells (pDCs) and megakaryocytes (MKs). MKs are rare and large cells in the bone marrow with the ability to produce platelets. The process of formation and differentiation of megakaryocyte progenitors (MKPs) is called megakaryopoiesis and is crucial to maintaining platelet homeostasis in the blood. During megakaryopoisis, MKs undergo endomitosis and become polyploid. In the process of maturation, MKPs migrate from the osteoblastic niche towards the vascular niche in the bone marrow, where they release platelets into the blood stream. pDCs are a type of dendritic cells which main role on the secretion of IFNα upon detection of viral DNA or RNA. This study aims to investigate the role of pDCs in megakaryopoiesis, more precisely on the spatial distribution of MKs and MKPs.

To achieve this goal, whole mount bone marrrow stainings of genetically modified mice were used to observe not only the numbers of MKs and MKPs under different conditions, but also their spatial distribution. The whole mount bone marrow stainings allowed us to preserve the morphology of the bone marrow and thus measure precisely the distance between the MKs/MKPs and the vasculature. The spatial distribution on MKs and MKPs was measured under different conditions, e.g., under pDC depletion or under IFNα treatment. Fluorescence activated cell sorting (FACS) was used to confirm the countings of MKs/MKPs and to measure the ploidy of MKs.

The whole mount bone marrow stainings in steady state showed that approximately 31% of MKs and 15% of MKPs are co-localizing with at least one pDC. Under pDC depletion, both MK and MKP numbers decreased significantly. Additionally, the MK ploidy increased under pDC depletion, which indicates that MKs were in average older under pDC depletion than in steady state. Under pDC depletion, the spatial distribution of MKs and MKPs was impaired: the distance of MKs and MKPs towards the vasculature increased. Although the main role of pDCs is the secretion of IFNα, the numbers of MKs and MKPs,

as well as their spatial distribution was not impaired after IFNα treatment.
The co-localization of pDCs and MKs can be explained by either the recognition of apoptotic MKs, more precisely their DNA and RNA, by pDCs. Another possible explanation is the recognition of unsialylated proteins, such as GPIbα on MKs by pDCs. Desialysation occurs, e.g., under acute infection. Megakarypoiesis is mainly regulated by thrombopoietin (TPO). However, as previously mentioned in the discussion, TPO does not influence the spatial distribution of MKPs. After pDC depletion, MKs were fewer in number, more polyploid and located in a greater distance to the vasculature than in steady state. This indicates that pDCs are involved in the regulation of megakaryopoiesis. Together with the knowledge that pDCs could recognize apoptotic MKs, it is likely that pDCs trigger resplenishment of MKPs from the osteoblastic niche towards the vasculature upon detection of DNA or desialysation. However, systemic treatment with IFNα did not influence the numbers or spatial distribution of MKs/MKPs. However, IFNα is known to have both stimulatory and inhibitory effects of haematopoietic stem cells (HSCs). A systemic treatment might not reflect the locally specific distribution of IFNα necessary to balance these two effects. Thus, future studies are necessary to elucidate the signaling between pDCs and MKPs.

7.2 Outlook

Although the influence of pDCs on megakaryocytes has been demonstrated, the exact mechanisms of signaling remain to be unclear. IFNα could be involved, however its secretion by pDCs is very time- and space-limited so more precise experiments should be conducted to investigate its effetcs on megakaryopoiesis.
As pDCs are involved in various chronic diseases, not last to mention Covid-19, which is associated with microthrombosis, it is crucial to understand the exact mechanisms in order to identify potential therapeutic targets. Concerning Covid-19, it would be interesting to see if and how the spatial distribution of MKs/MKPs changes under Covid-19 infection.

8 Zusammenfassung

Diese Arbeit befasst sich mit dem Zusammenspiel von plasmazytoiden dendritischen Zellen (pDCs) und Megakaryozyten (MKs). Megakaryozyten sind seltene und große Zellen im Knochenmark, welche Blutplättchen zu produzieren. Der Prozess der Bildung und Reifung von Megakaryozyten Vorläuferzellen (MKPs) wird als Megakaryopoese bezeichnet und ist für die Aufrechterhaltung der Thrombozytenhomöostase im Blut entscheidend. Während der Megakaryopoese durchlaufen die MKPs mehrere Endomitosen und werden polyploid, d.h. bekommen mehrere Zellkerne. Während des Reifungsprozesses wandern MKPs aus der osteoblastischen Nische in die vaskuläre Nische im Knochenmark, wo sie Blutplättchen in den Blutkreislauf abgeben. pDCs hingegen sind eine Art von dendritischen Zellen, deren Hauptaufgabe in der Sekretion von IFNα beim Nachweis von viraler DNA oder RNA besteht. Ziel dieser Studie war es, die Rolle der pDCs bei der Megakaryopoese, bzw. der räumlichen Verteilung von MKs und MKPs zu untersuchen.
Hierfür wurden gefärbte Knochenmarksschnitte von genetisch veränderten Mäusen verwendet, um nicht nur die Anzahl der MKs und MKPs unter verschiedenen Bedingungen, sondern auch ihre räumliche Verteilung zu beobachten. Die Knochenmarksschnitte ermöglichten es uns, die Morphologie des Knochenmarks zu erhalten und somit den Abstand zwischen den MKs/MKPs und dem Gefäßsystem genau zu messen. Die räumliche Verteilung von MKs und MKPs wurde unter verschiedenen Bedingungen gemessen, z. B. nach pDC-Depletion oder nach IFNα Behandlung. Fluoreszenz-aktivierte Zellsortierung (FACS) wurde zusätzlich verwendet, um die Zellzahlen zu quantifizieren und um die Ploidität der MKs zu messen.
Die gefärbten Knochenmarksschnitte ohne zuvor erfolgte Behandlung zeigten, dass etwa 31% der MKs und 15% der MKPs mit mindestens einem pDC kolokalisiert sind. Unter pDC-Depletion nahm die Anzahl sowohl der MK, als auch der MKP signifikant ab. Außerdem nahm die MK-Ploidität unter pDC-Depletion zu, was darauf hindeutet, dass die MKs unter pDC-Depletion im Durchschnitt älter waren als im Steady-State. Unter pDC-Depletion veränderte sich die räumliche Verteilung von MKs und MKPs: Der Abstand

zwischen MKs/MKPs und den Gefäßen nahm zu. Obwohl die Hauptaufgabe der pDCs in der Sekretion von IFNα besteht, änderte sich weder die Anzahl der MKs und MKPs, noch ihre räumliche Verteilung nach Behandlung mit IFNα.
Die Ko-Lokalisierung von pDCs und MKs kann entweder durch die Erkennung apoptotischer MKs, genauer gesagt ihrer DNA und RNA, durch pDCs erklärt werden. Eine andere mögliche Erklärung ist die Erkennung von unsialylierten Proteinen, wie GPIbα auf MKs durch pDCs. Die Desialysierung erfolgt z. B. bei einer akuten Infektion. Die Megakaryopoese wird hauptsächlich durch Thrombopoietin (TPO) reguliert. TPO hat jedoch keinen Einfluss auf die räumliche Verteilung der MKPs. Nach einer Depletion der pDCs waren die MKs zahlenmäßig geringer, polyploider und in größerem Abstand zum Gefäßsystem angeordnet als im stationären Zustand. Dies deutet darauf hin, dass pDCs an der Regulierung der Megakaryopoese beteiligt sind. Zusammen mit dem Wissen, dass pDCs apoptotische MKs erkennen können, ist es wahrscheinlich, dass pDCs die Wiederauffüllung von MKPs aus der osteoblastischen Nische in Richtung Gefäßsystem stimulieren, wenn sie DNA oder Desialysierung erkennen. Eine systemische Behandlung mit IFNα hatte jedoch keinen Einfluss auf die Anzahl oder die räumliche Verteilung der MKs/MKPs. IFNα hat jedoch sowohl stimulierende als auch hemmende Wirkungen auf hämatopoetische Stammzellen (HSCs). Eine systemische Behandlung spiegelt möglicherweise nicht die lokal spezifische Verteilung von IFNα wider, die notwendig ist, um diese beiden Effekte auszugleichen. Daher sind künftige Studien erforderlich, um die Signalübertragung zwischen pDCs und MKPs zu klären.

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