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Titel der Masterarbeit

The role of the AP-1 proteins c-Jun and JunB in the development and function of plasmacytoid dendritic cells.

verfasst von

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1. INTRODUCTION

1.1. Dendritic cells in immunity

The immune system comprises a complex set of specialized cells and molecules to defend the host against pathogens. The immune system can be largely divided into two parts, namely the innate and the adaptive immune system. The innate immune system is needed for a fast first defense against invading pathogens. It is non-specific and activates the adaptive immune system (Beutler & Rietschel, 2003). The adaptive immune system on the other hand confers a highly effective and long lasting protection against pathogens. To activate the adaptive immune system foreign antigen must be presented by antigen presenting cells to B cells (specialized in antibody production) and T cells (cell mediated immunity) (Dempsey, Vaidya, & Cheng, 2003). The most potent antigen presenting cells are the so called dendritic cells (DC) which were first discovered in 1973 by Ralph M. Steinmann (R M Steinman & Cohn, 1973). To capture antigen DCs are localized at areas of the body that are exposed to the environment, for example the skin, the pharynx, the vagina and the anus (Ralph M Steinman & Banchereau, 2007). Once the antigen is captured DCs undergo a functional and phenotypic maturation. In short they up-regulate expression of co-stimulatory molecules including members of the B7, Notch and Tumor necrosis factor (TNF) family and start synthesis of cytokines like interleukin (IL)-12 and Type I interferons (Type I IFN) (Ralph M Steinman & Banchereau, 2007). Mature DCs are then able to interact with T and B cells in the lymph node (LN) and memory T cells localized at other positions in the body (like the skin) (Helft, Ginhoux, Bogunovic, & Merad, 2010). Importantly some DC subsets solely reside in secondary lymphoid tissues (lymphoid tissue resident DC) while others reside at sites like the skin or gut (non-lymphnode tissue DC) (Shortman & Naik, 2007). Non lymphnode tissue DCs must therefore migrate to secondary lymphoid organs like spleen and LNs after antigen capture to interact with T and B cells (Banchereau & Steinman, 1998). Migration of DCs is mediated by a number of different

chemokine receptors and the expression of other trafficking molecules like integrin and selectin (Alvarez, Vollmann, & von Andrian, 2008). In short after non-lymphoid tissue DCs are activated they detach from the surrounding stroma and follow the afferent lymphatic vessel to the next lymphnode where they enter to interact with T and B cells (Alvarez et al., 2008). Lymphoid tissue resident DCs on the other hand don´t have to migrate and probably capture antigen directly in the secondary lymphoid organ (Helft et al., 2010).

1.1.1.Dendritic cells and lymphocytes

By presenting antigen, DCs initiate the clonal expansion of T cells in the LN. Upon expression of the surface molecule CD4 and CD8 T cells are divided into $CD4^+$ and $CD8^+$ T cells. To activate $CD4^+$ T cells DCs present foreign antigen on major histocompatibility complex II (MHC class II) molecules and reserve MHC class I molecules to activate $CD8^+$ T cells (Banchereau & Steinman, 1998). To present antigens via a MHC class II molecule the antigen must be taken up from the environment. Therefore DCs sample the environment with long branchlike projections and once found efficiently internalize any foreign antigen. For internalization DCs have a sophisticated receptor repertoire including C-type lectins (recognizes carbohydrates), Fc-ε and Fc-γ receptors and many others (Banchereau & Steinman, 1998). Once internalized the foreign antigen is dismantled and presented via MHC class II molecules on the surface of the DC (Banchereau & Steinman, 1998). On the other hand to present antigens on MHC class I molecules the foreign antigen must either reside within the cytoplasm of the DC, which is the case if the DC is infected with a virus or it must go through a process called “cross-presentation” (Banchereau & Steinman, 1998). In short “cross-presentation” involves uptake of the antigen in an endocytic vacuole and relocation of the antigen from the vacuole into the cytoplasm of the DC where it can now be loaded on MHC class I molecules (Bevan, 2006). Once activated by DCs T cells mediate many aspects of the adaptive immunity. In example $CD8^+$ T cells recognize virus infected cells and induce their subsequent lysis (Zhang & Bevan, 2011). $CD4^+$

T cells are mainly involved in enhancing the anti-microbial activity of macrophages and in their cross-talk with B cells to induce production of antibodies (Zhu & Paul, 2008). $CD4^+$ T cells can be further subdivided into Th1 and Th2 cells (T helper cell 1/2). Th1 cells are important for protection against intracellular bacteria and Th2 protect the host against parasitic worms like helminthes. DCs can drive the differentiation into Th1 and Th2 cells depending on the cytokines they secrete while interacting with naïve T cells. If a DC secretes IL-12 and interferon gamma during interaction the T cell will differentiate into a Th1 cell whereas secretion of IL-4 will drive the differentiation to Th2 cells (Banchereau & Steinman, 1998). Besides Th1 and Th2 cells DCs can also shift the differentiation of T cells to regulatory T cells (T_{reg}) and Th17 cells. To induce Th17 cells DCs secrete tumor-growth factor beta (TGF-β), IL-1, IL-6 and IL-23. T_{reg} differentiation requires the secretion of retinoic acid and TGF-β (Helft et al., 2010). Th17 cells are a T cell subset with the key cytokine IL-17 needed to recruit and activate neutrophils (Korn, Bettelli, Oukka, & Kuchroo, 2009). T_{regs} are a T cell subset that suppress the proliferation of auto-reactive T cells and therefore plays an important role in autoimmunity (Rowe, Ertelt, & Way, 2012). Besides T cells DCs are also important antigen presenting cells for B cells. These DC subset is called follicular DC and they display native antigens to B cells (Ralph M Steinman & Banchereau, 2007). B cells recognizing the native antigen can process and load it on their MHC class II molecules waiting to be activated by an antigen specific $CD4^+$ T cell (Banchereau & Steinman, 1998). Figure 1 summarizes the interaction of DCs with T and B cells.

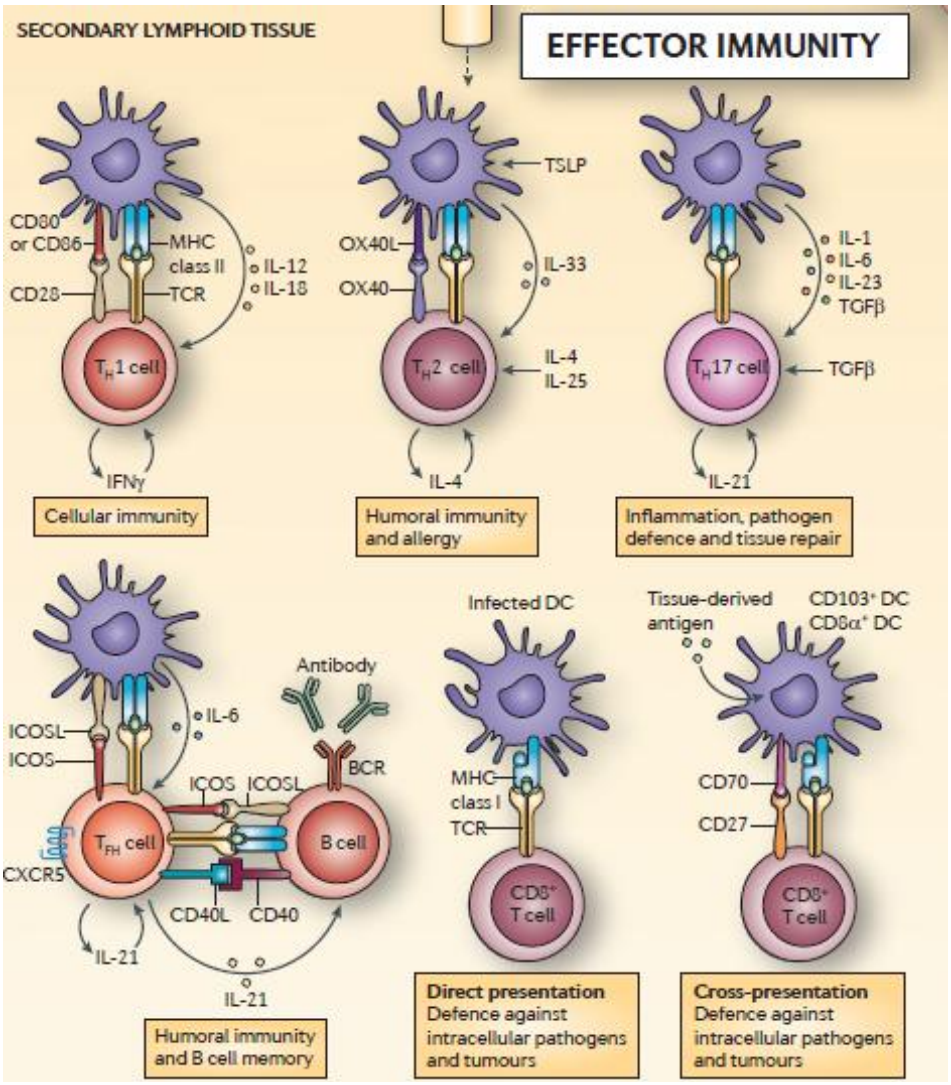

Figure 1, Dendritic cells are potent activators of naïve T and B cells.
(Adopted from a poster by Miriam Merad; Dendritic cells: controllers of adaptive immunity)

1.1.2. Murine dendritic cell subtypes

DCs can be classified according to many properties. For example some DCs migrate (non-lymphoid tissue DCs) while others remain resident in the lymphnode (lymphoid tissue resident DCs). Other properties include the activation state (immature to mature DCs), or the host stage (steady state to inflammatory) (Naik, 2008) or the expression of DC specific surface markers. In general all DCs in the mouse express CD11c, an adhesion molecule, which is a specific marker to identify DCs all over the body. Moreover surface expression like CD4, CD8-alpha or CD11b are important to further define

different DCs. It is however most convenient to categorize DCs according to their function and localization in the body. In secondary lymphoid organs (spleen, lymph node) three DC populations can be found. These are $CD8^+$, $CD8^-$ DC and pDCs. The $CD8^+$ DC subset typically localizes to the T- cell areas of the spleen and lymph node where they can cross-present self and foreign antigens to naïve T cells. $CD8^+$ DCs thereby play an important role in peripheral tolerance and in the generation of $CD8^+$ T cells (Naik, 2008). $CD8^-$ DCs (also called $CD4^+$ DC) on the other hand primarily present antigens on MHC class II molecules which results in formation of $CD4^+$ T cells (K. Liu & Nussenzweig, 2010). $CD8^+$ and $CD8^-$ DC are called classical or conventional DC (cDC) as their main function is antigen-presentation. This sets them apart from pDCs whose main function is the production of Type I IFNs. DCs in non lymphoid tissue (lung, liver, dermis, intestine and more) can be categorized according to the expression of the surface marker CD103. $CD103^+$ cells in tissue resemble $CD8^+$ cells of lymphoid organs as they are also very efficient at cross-presentation to T and B cells in the LN (K. Liu & Nussenzweig, 2010) and also have a function in peripheral tolerance. In the gut for example $CD103^+$ cells were shown to induce formation of T_{reg} cells by their secretion of retinoic acid (Helft et al., 2010). $CD103^-$ DCs comprise a much more heterogeneous population whose function is often poorly understood. $CD103^-$, $CD11b^+$ DCs (dermal DCs) in the skin for example are thought to effect $CD4^+$ effector T cells in the inflamed skin (Helft et al., 2010). In the gut $CD103^-$, $CD11b^+$, $CX3CR1^+$ DCs are closely associated with the epithelium and sample the lumen of the gut via trans-epithelial projections (Helft et al., 2010). Lastly, a highly specialized DC subtype is found in the epidermis of the skin, namely the so called langerhans cells (LC). LCs are potent antigen presenting cells that migrate to the LN after antigen uptake. LCs prominently express langerin (CD205), a lectin receptor (Merad, Ginhoux, & Collin, 2008). Figure 2 summarizes the different functions of the DC subsets and how the interact to activate the immune system.

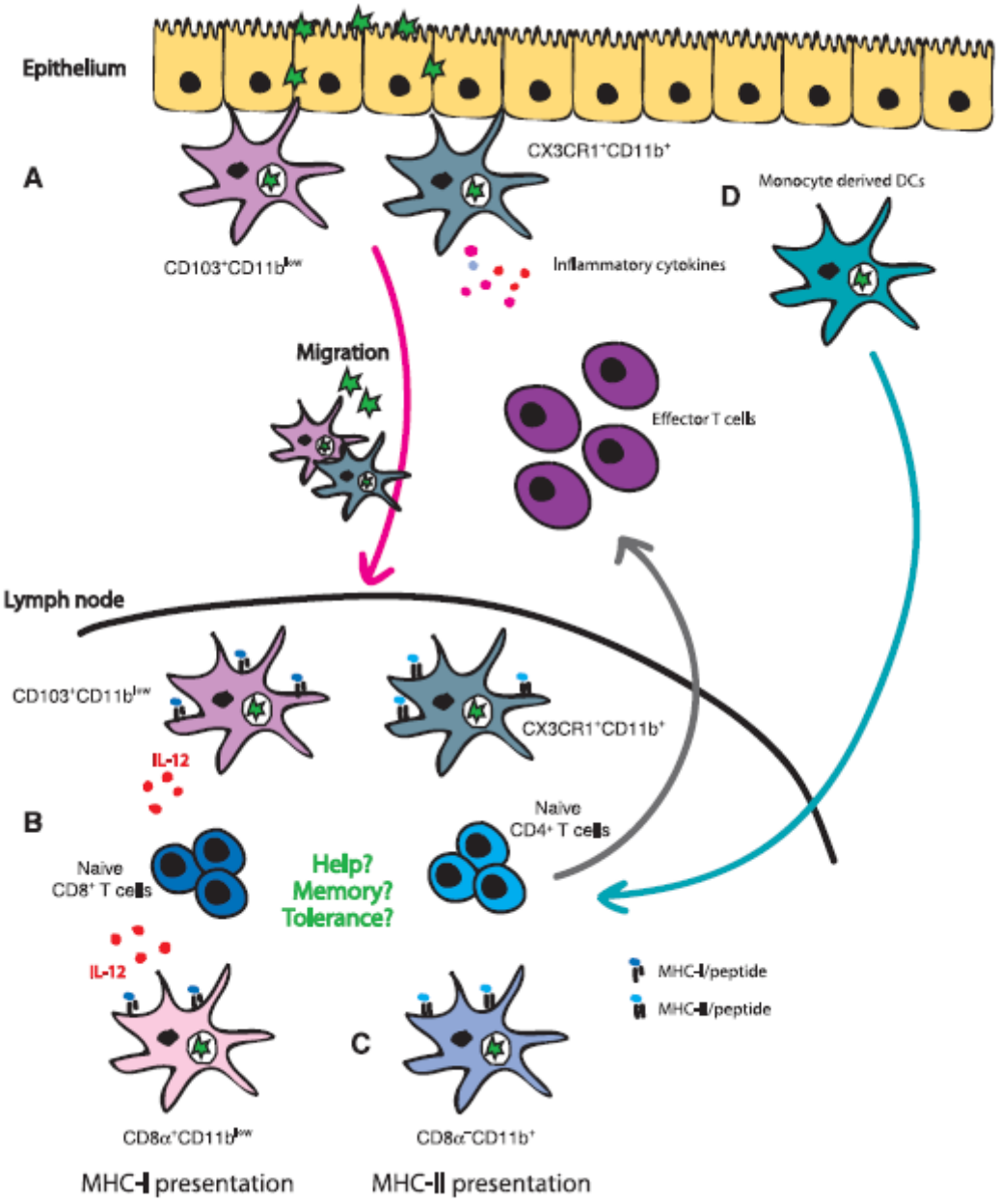

Figure 2, Dendritic subsets and their role in the immune system
Adopted from (Helft et al., 2010).

1.1.3.The complex network of dendritic cell development

All cells of the hematopoietic system arise from hematopoietic stem cells in the bone marrow (BM) (Shortman & Naik, 2007). For the development of DCs a number of key cytokines and transcription factors were identified. The most important cytokines are Fms-related tyrosine kinase 3 ligand (FLT3L) needed for steady-state cDC and pDC development (H. J. McKenna et al., 2000) and the Granulocyte macrophage colony-stimulating factor (GM-CSF), which is

needed for the formation of inflammatory DCs (Tip-DCs) from monocytes (Sallusto & Lanzavecchia, 1994). Tip-DCs are exclusively found in inflamed tissue and produce TNF and inducible nitric oxide synthase (iNOS) as their key cytokines (Bosschaerts et al., 2010). DC development has been shown to be tightly regulated by TFs. The most important transcription factors are IRF8 needed for the development of $CD8^+$ cDC and pDCs (Aliberti et al., 2003), IRF4 needed for the development of $CD4^+$ cDCs (Suzuki et al., 2004) and Spi-B needed for the development of pDCs (Shortman & Naik, 2007). DCs differentiate from hematopoietic progenitors in the BM. The most potent identified progenitor that nearly exclusively differentiates to DCs is the monocyte and DC progenitor (MDP). MDPs reside in the BM and differentiate to committed DC progenitors (CDPs) in the presence of FLT3L(K. Liu et al., 2009). CDPs can be divided upon expression of the transcription factor E2-2 into E2-2^{hi} CDPs and E-2-2^{low} CDPs (Onai et al., 2013). E2-2^{low} CDPs give rise to pre-cDCs which leave the BM and enter the blood stream to establish DC populations in lymphoid ($CD8^+$ and $CD8^-$ DCs) and non-lymphoid tissues ($CD103^+$ DCs) (K. Liu & Nussenzweig, 2010). E2-2^{high} CDPs can further differentiate to pDCs in the BM. pDCs then leave the BM and enter the bloodstream to populate the spleen, LN or the liver (Merad & Manz, 2009). pDCs are therefore also found in the blood. Other DC subsets however do not develop from BM progenitors. DCs in the thymus for example where shown to develop from a precursor giving rise to T cells and DCs (Shortman & Naik, 2007) and LCs in the epidermis are in steady-state completely independent of BM precursors but rely on a locally present LC precursor (Shortman & Naik, 2007). Only in situations of severe skin inflammation with a high efflux of LCs is the population replenished by monocytes migrating to the inflamed skin (Shortman & Naik, 2007). According to this Tip-DCs found in situations of inflammation also arise from monocytes (K. Liu & Nussenzweig, 2010). Figure 3 summarizes the development of DCs.

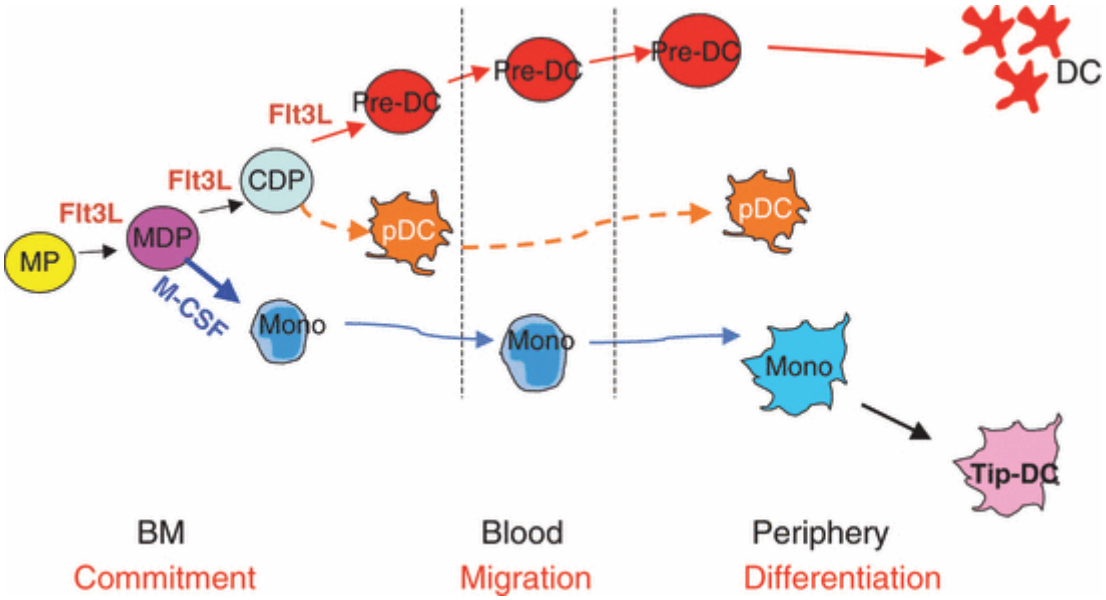

Figure 3, Differentiation of dendritic cells
Adopted from (K. Liu & Nussenzweig, 2010)

1.2. Plasmacytoid dendritic cells, a special case

pDCs were first described in 1958 as cells resembling plasma cells which lack expression of surface markers typical for B and T cells (LENNERT & REMMELE, 1958). Their precise function remained unsolved until 1999 when researchers could link these cells to the production of interferon alpha (IFN-α) (Siegal et al., 1999). Nowadays pDCs are known to be an important subtype of DCs with important functions in viral infections, in peripheral tolerance and tumor cytotoxicity (Colonna, Trinchieri, & Liu, 2004). pDCs are classified as $CD4^{+}CD123^{+}ILT3^{+}BDCA\text{-}2^{+}$ in humans (Colonna et al., 2004) and as $B220^{+}CD11c^{int}BST\text{-}2^{+}Siglec\text{-}H^{+}$ in mice (Cisse et al., 2008). pDCs enter the LN directly from the blood through high endothelial venules (HEV) by expressing trafficking molecules like C-X-C chemokine receptor type 4 (CXCR4), L-selectin and chemerin receptor 23 (ChemR23) that interact with their binding partners found on HEVs (Sozzani, Vermi, Del Prete, & Facchetti, 2010). During inflammation pDCs up-regulate the expression of other trafficking molecules like interleukin 18 receptor (IL-18R) and complement component 3 a receptor (C3aR) allowing their recruitment to inflamed skin (Sozzani et al., 2010). IL-18R is the receptor for IL-18 a cytokine released from neutrophils

(Robertson et al., 2006) and C3aR is the receptor for the anaphylatoxin C3a which is an important chemo-attractant and furthermore induces the degranulation of mast cells (Burger et al., 1987). Once recruited to the site of inflammation pDCs harbor powerful pathogen sensors allowing them to respond to DNA and RNA viruses.

1.2.1.pDCs as viral sensors

pDCs express members of the Toll-Like receptor (TLR) family to sense pathogens. In detail pDCs express TLR 7 and TLR 9 (Colonna et al., 2004) TLRs are a family of pathogen recognition receptors (PRR) consisting of 12 (TLR1-9 and TLR 11-13) members in mice each recognizing a conserved microbial motive (Takeuchi & Akira, 2010). In pDCs, TLR7 allows pDCs to recognize viral single stranded RNA (ssRNA) present in viruses like influenza and vesiculostomatitis virus (Colonna et al., 2004). TLR9 is a sensor for CpG DNA which gives pDCs the opportunity to respond to DNA viruses like herpes simplex virus and mouse cytomegalovirus (Colonna et al., 2004). Binding of agonists to TR7/9 initiates a complex signaling pathway ultimately leading to the production of Type I IFNs and other pro-inflammatory cytokines like interleukin (Il)-6 and tumor necrosis factor alpha (TNF-α). Figure 4 summarizes the signal transduction via TLR7/9 in pDCs. In short upon encounter of a virus TLR7/9 are re-localized from the endoplasmatic reticulum (ER) to endosomes via Unc-93 homolog B1 (UNC93B1). In the endosomes TLR7/9 mediated signaling is strictly dependent on association with the myeloid differentiation primary response gene 88 protein (MyD88) which allows assembly of a signaling complex including TNF receptor associated factor (TRAF 3/6), interleukin-1 receptor-associated kinase 4 (IRAK1/4), IκB kinase (IKK–α) and Interferon regulatory factor 7 (IRF-7). In this complex IRF-7 gets active through ubiquitylation by TRAF-6 and phosphorylation by IRAK-4. IRF-7 now translocates to the nucleus to induce transcription of IFN-α and interferon beta (IFN-β). TRAF-6 furthermore ubiquityltes TGF-beta activated kinase 1 (TAK-1)

which is a potent activator of mitogen activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) signaling.

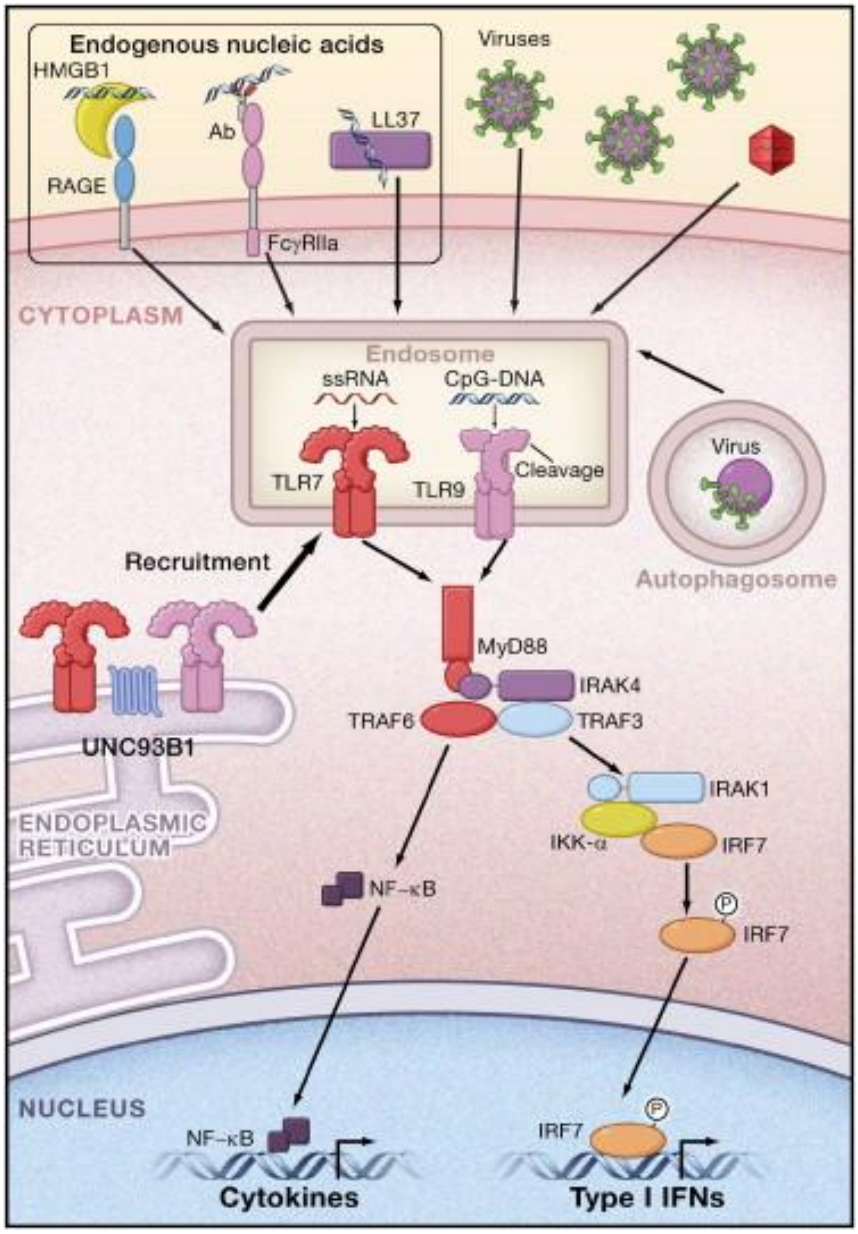

Figure 4, TLR7/9 signaling cascade in pDCs
Adopted from (Takeuchi & Akira, 2010)

1.2.2.Maturation of TLR7/9 activated pDCs

Binding of agonist to TLR7/9 induces a rapid and robust production of IFN α/β in pDCs. IFN-α and IFN-β are both Type I IFNs and their division was due to their different reactivity to antisera (Runkel, 1998). IFN-α and IFN-β are structurally and functionally closely related. Both are of helical structure with 5

α-helices joined by loops and both induce an anti-proliferative state in cells. The anti-proliferative function is linked to the induction of the *2', 5'-oligoadenylate synthetase 1* gene which ultimately initiates the degradation of RNA in virus infected cells (Platanias, 2005). Although similar in function differences between IFN-α and β are also documented. IFN-β for example has a markedly higher anti-proliferative capacity in certain cell types like melanocytes (Runkel, 1998). Signaling of IFN-α and β is mediated by the interferon alpha receptor 1 and 2 (IFNAR 1 or 2). Both of them are needed for biological activity as loss of one abrogates Type I IFN mediated signaling (Runkel, 1998). Figure 5 summarizes the signaling cascade via IFNAR. First, IFN-α/β binds to IFNAR, resulting in receptor dimerization and phosphorylation of the associated adaptor molecules tyrosine kinase 2 (Tyk2) and Janus kinase1 (Jak1). Interestingly, IFN-β but not IFN-α induces dimerization of phosphorylated IFNAR 1 and 2 highlighting a further difference between these two molecules (Runkel, 1998). Tyk2 and Jak1 then phosphorylate signal transducers and activators of transcription 1 and 2 (STAT1 and STAT2). The phosphorylated STATs then interact with IRF9 to form the interferon-stimulated gene factor-3 (ISGF3) complex. The ISGF-3 complex translocates to the nucleus mediating the transcription of interferon stimulated genes (ISGs) ultimately leading to the up-regulation of co-stimulatory molecules like CD40, CD80/86 and MHC class II found on matured pDCs (K. McKenna, Beignon, & Bhardwaj, 2005). Type I IFN signaling is therefore critical for the functional and phenotypic maturation of pDCs. As pDCs release massive amounts of Type I IFNs after induction, TLR7/9 signaling must be strictly controlled. pDCs where therefore shown to possess a variety of surface receptors like blood DC antigen 2 (BDCA-2) or immunoglobulin like transcript 7 (ILT -7) that potentially suppress TLR7/9 signaling resulting in significantly reduced expression of Type I IFNs and IL-6 (Gilliet, Cao, & Liu, 2008).

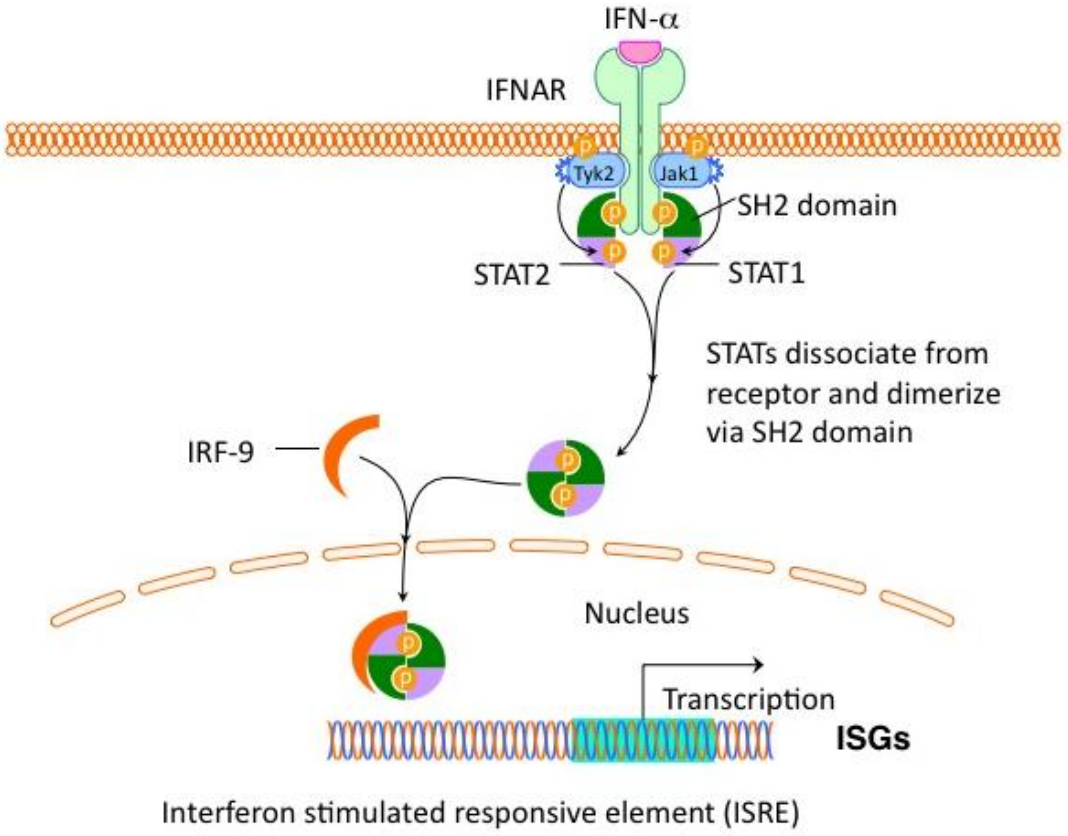

Figure 5, Type I interferon mediated JAK/STAT signaling.
Adopted from (Masumi, 2013)

1.2.3. Immunstimulatory functions of pDCs

As activated pDCs secrete Type I IFN and pro-inflammatory cytokines (IL-6, IL-12 and TNF-α) they have a number of immune-stimulatory functions. For example pDCs stimulate NK and $CD8^+$ effector T cell cytotoxicity through their secretion of INF-α and IL-12 (Colonna et al., 2004). Furthermore the stimulate myeloid dendritic cells (mDCs) to produce IL-12 through secretion of Type I IFN which enables mDCs to induce Th1 differentiation (Colonna et al., 2004). In addition secretion of IL-6 and Type I IFNs enables pDCs to differentiate B cells into plasma cells (Jego et al., 2003). Besides pro-inflammatory cytokines and IFN α/β pDCs also secrete a number of chemoattractants. Most importantly the secrete C-X-C chemokine ligand 10 (CXCL10) which recruits Th1 cells and CC chemokine ligand 4 (CCL4) which recruits NK cells (H. J. McKenna et al., 2000). Highly debated on the other hand is the role of pDCs in antigen presentation. pDCs are thought to be efficient at inducing

memory T cells and inefficient at inducing naïve T cells (K. McKenna et al., 2005). Priming of naïve T cells seems therefore to be mediated by mDCs. Once primed pDCs could amplify and direct T cell activation to Th1 cells through their secretion of IL-12 and Type I IFNs (K. McKenna et al., 2005). pDC can also crosstalk with T cells as they express CD40 and the receptor for IL-3 upon activation (Reizis, Colonna, Trinchieri, Barrat, & Gilliet, 2011). Interestingly under steady-state conditions pDCs seem to be important for peripheral tolerance as they express ICOS ligand needed for the expansion of a certain regulatory T cell subtype namely $FOXP3^{+}$ T_{reg} cells (Reizis et al., 2011). Further evidence of a tolerogenic role for pDCs was shown in studies implicating pDCs in oral tolerance (Goubier et al., 2008) and tolerance mediated to vascularized grafts (Ochando et al., 2006).Figure 6 sums up the different functions of pDCs in the immune system.

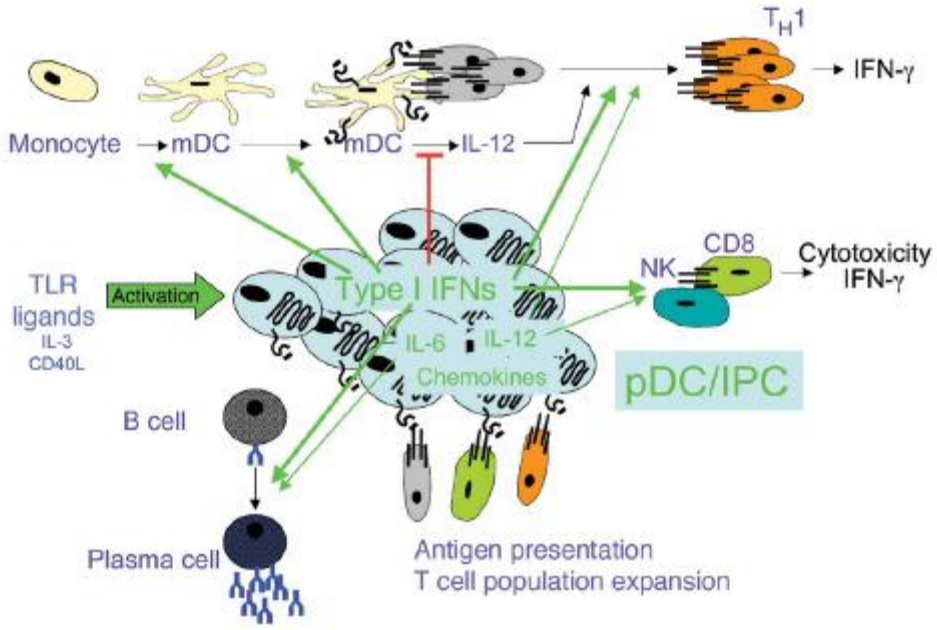

Figure 6, pDCs are activators of the immune system
Adopted from (K. McKenna et al., 2005)

1.2.4.pDCs in autoimmunity and cancer

pDCs are potent sensors for ssRNA and CpG DNA making them prone to recognize self-DNA and self-RNA as well. Indeed it was shown that in autoimmune diseases like psoriasis and systemic lupus erythematosus (SLE)

pDCs are activated after recognizing self DNA-protein complexes (Gilliet et al., 2008). In healthy individuals self DNA is not recognized by pDCs as it is rapidly degraded by DNases in the extracellular space. In psoriasis binding of cathelicidin (LL37) and in SLE binding of auto-reactive antibodies to self DNA inhibits DNase mediated degradation. LL37 is an antimicrobial peptide released from epithelial cells in the inflamed skin (Zasloff, 2002). In addition to autoimmunity pDCs also play a prominent role in cancers since they are found in tumors of the head and neck, in melanomas and in ovarian cancer (K. McKenna et al., 2005). As pDCs release large amounts of Type I IFN which activates the immune system it was thought that pDCs would contribute to tumor regression. However, some studies also show that the tumor microenvironment efficiently blocks INF-α/β production of pDCs and turns them into T_{reg} promoting immune suppressive cells (Zou et al., 2001). This effect can be overcome by re-stimulation of the pDCs with TLR7/9 specific drugs. For example treatment of basal-cell carcinoma with Aldara (patient-applied cream, containing TLR7 agonist Imiquimod) results in tumor regression (Palamara et al., 2004). Aldara acts as an immune modulator and was used to treat genital warts and human papillomavirus infections before the anti-tumor capacity was discovered. Topical treatment of mice with Aldara results in splenomegaly and an inflammation of the skin (thickening of epidermis). In recent studies it was shown, that pDCs are not just bystanders, which help to initiate anti-tumor immune responses, but that they also have a tumor-killing capacity on their own in human and mice (Stary et al., 2009)(Drobits et al., 2012). Figure 7 gives an overview of this tumor killing effect in a mouse melanoma model (Drobits et al., 2012). In short treatment of melanomas with Aldara resulted in a C-C chemokine ligand 2 (CCL2) dependent recruitment of pDCs, where they mediate tumor killing by secretion of cytolytic molecules like Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and granzyme B (Gzmb). TRAIL and Gzmb are formed after pDCs are activated in an autocrine manner through binding of IFN-α/β to the IFNAR. TRAIL induces apoptosis in cells expressing the death receptor DR4 or DR5 (Wang & El-Deiry, 2003). Binding of TRAIL to DR4/5 induces the

cleavage of caspase-8. Caspase-8 then activates caspase 3 and 7 leading to apoptosis (Tait & Green, 2010). Gzmb initiates apoptosis in cells marked by perforin. Perforin forms a hole in target cells allowing the entry of Gzmb where it cleaves pro-caspase 3 to induce apoptosis (Lord, Rajotte, Korbutt, & Bleackley, 2003). Further work in this master thesis focused if two transcription factors, namely c-Jun and JunB are important for the development and function of pDCs. Therefore we also tried to understand if c-Jun and JunB are important for the tumor killing ability of pDCs. Ultimately our goal was to contribute to a better understanding of pDC mediated tumor killing which might in the future lead to better therapies of certain cancers.

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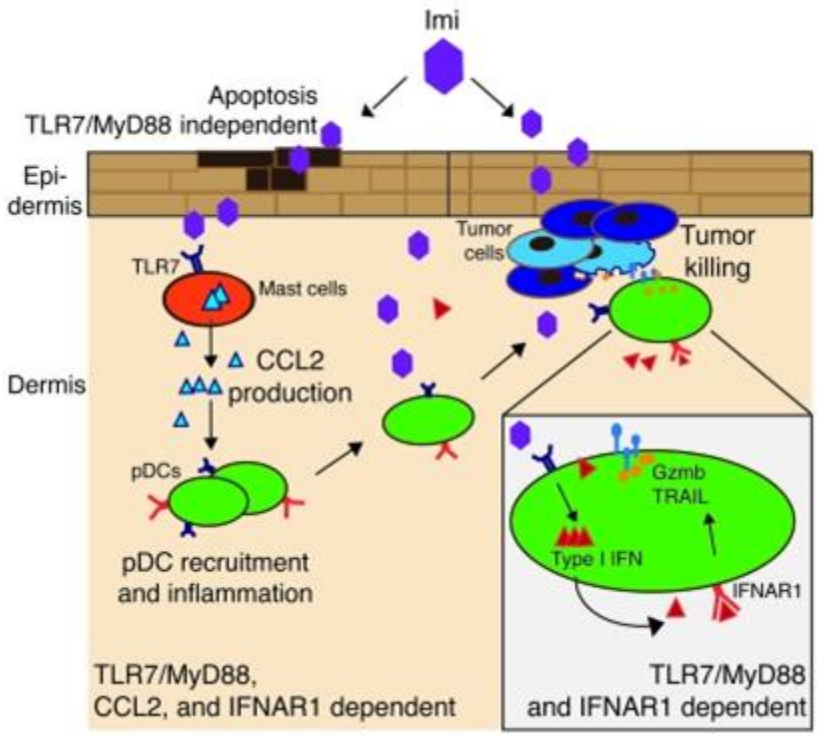

Figure 7, pDC mediate tumor regression
Adopted from (Drobits et al., 2012)

1.3. The AP-1 family of transcription factors

The activating protein 1(AP-1) family summarizes members of transcription factors sharing an evolutionary conserved bZIP (basic DNA-binding domain) domain (Hess, Angel, & Schorpp-Kistner, 2004). Group members include the Jun family (c-Jun, JunB, and JunD), the Fos family (c-Fos, FosB, Fra-1 and Fra-2), the ATF family (ATFa, ATF-2 and ATF-3), the Maf family (MafB, MafK, MafG and MafF) and the JDP family (JDP-1 and JDP-2) (Zenz et al., 2008). Structurally and functionally related, all of them bind to genes containing the TPA responsive element (TRE, with the consensus sequence 5′-TGAGCTCA-3′) (Hess et al., 2004). Binding to DNA is achieved through a positively charged basic region that interacts with the negatively charged DNA phosphate backbone (Landschulz, Johnson, & McKnight, 1988). For dimerization AP-1 proteins harbor the bZIP domain which contains a hydrophobic leucine zipper region (Landschulz et al., 1988). The different AP-1 proteins can either form homo- or heterodimers. In particular, Jun family members can form both, homo and heterodimers whereas Fos proteins can only build heterodimers (Hess et al., 2004). Lastly, AP-1 proteins contain a transactivation domain needed to initiate transcriptional processes (Hess et al., 2004).

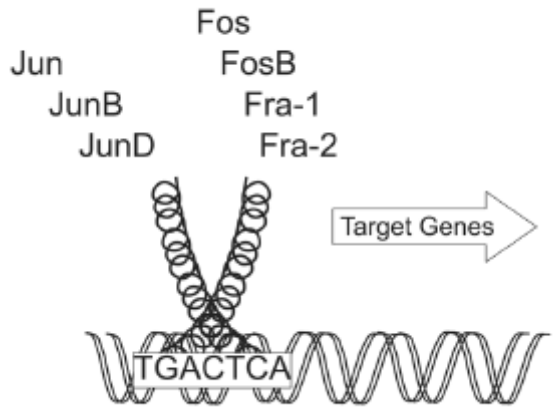

Figure 8, Binding of AP-1 proteins to DNA.
Adopted from (Zenz et al., 2008)

Activation of AP-1 proteins can be induced by a variety of different extracellular signals like growth and differentiation factors, inflammatory molecules like cytokines or stress signals. Different signal transduction pathways translate these extracellular stimuli to induce phosphorylation of AP-1 family members. Best understood is the activation of c-Jun through the MAPK signal transduction pathway. The MAPK signaling cascade activates Jun N-terminal kinase (JNK) proteins which translocate to the nucleus to phosphorylate c-Jun in its transactivation domain (Hess et al., 2004) and c-Jun can form dimers with different AP-1 family members to exert a variety of different functions.

1.3.1. The multiple faces of c-Jun

c-Jun is a member of the AP-1 family implicated in the inflammatory response, in apoptosis, cellular survival and tumor formation. Its transcription is regulated in an autocrine manner by a c-Jun/ATF-2 dimer binding to the c-Jun TRE element present in the promoter of the *c-Jun* gene (Shaulian & Karin, 2002). The activity of c-Jun is regulated by JNK which phosphorylates c-Jun on serine 73 and serine 63 (Schreiber et al., 1999). This phosphorylation increases the interaction of c-Jun with the transcriptional coactivator CREB binding protein (CBP) resulting in enhanced transcription on c-Jun target genes (Bannister, Oehler, Wilhelm, Angel, & Kouzarides, 1995). A first attempt to understand the molecular function of c-Jun involved the generation of a c-Jun knock out (*c-Jun*$^{-/-}$) mouse model. Interestingly, these mice are embryonic lethal and pathology of the dead embryos showed an impaired hepatogenisis. The importance for c-Jun in liver development was further highlighted by the fact that c-Jun deficient embryonic stem cells cannot be differentiated into hepatocytes (Hilberg, Aguzzi, Howells, & Wagner, 1993). Later it was shown that c-Jun is also needed in the liver in processes like liver regeneration (Stepniak et al., 2006) and liver tumor formation (Eferl et al., 2003). c-Jun was also one of the first transcription factors discovered to be a proto-oncogene (Vogt, 2002) with transforming activity (Schütte et al., 1989)

which immediately placed it as a potential regulator of the cell cycle. Moreover, c-Jun was shown to regulate proliferation in a variety of cell types like keratinocytes and fibroblasts. For example in keratinocytes loss of c-Jun leads to down regulation of the epidermal growth factor receptor (EGFR) resulting in reduced proliferation of primary keratinocytes (Zenz et al., 2003). On the other hand in fibroblasts loss of c-Jun results in elevated levels of p53 and p21 (Schreiber et al., 1999) which markedly reduces the proliferative capacity of fibroblasts. In addition to its role in the cell cycle c-Jun has a very prominent role in inflammatory processes. For example one of the first genes were a c-Jun binding site was identified was the *IL-2* gene which has an important role in T cell proliferation (Arai et al., 1990). However, other cytokines like IL-3 (Foletta, Segal, & Cohen, 1998) or IL-5 (Foletta et al., 1998) were also shown to be regulated by c-Jun. Lastly, also the macrophage inflammatory protein 1 beta (MIP-1β) was shown to be regulated by c-Jun (Proffitt et al., 1995). Taken together, several reports demonstrate an important role of c-Jun in the immune system including T cell proliferation, cytokine gene regulation and the production of chemoattractants. However the role of c-Jun in cells of the innate immune system like pDCs is much less understood.

1.3.2.The role of JunB in the cell cyle and inflammation

JunB is another member of the AP-1 family and structurally closely related to c-Jun. However, in contrast to c-Jun JunB cannot be phosphorylated by JNK (Kallunki, Deng, Hibi, & Karin, 1996). Transcription of the *JunB* gene can be achieved by integration of various extracellular signals like growth factors and cytokines. One well documented example is the induction of JunB through protein kinase A (PKA) mediated signaling. Here PKA phosphorylates cAMP response element-binding protein (CREB) which as a transcription factor induces JunB expression (Amato, 1996). JunB was proposed to be antagonistic to c-Jun as co-expression of JunB with c-Jun reduces the transforming activity of c-Jun (Schütte et al., 1989). This antagonizing effect is probably due to the formation of growth inhibitory c-Jun/JunB dimers, whereas

JunB alone might not have anti-proliferative effects (Shaulian & Karin, 2002). Despite this JunB was shown to induce expression of p16, a cell cycle inhibitor, in fibroblasts which prevents proliferation (Shaulian & Karin, 2002). In line with the theory that JunB has an anti-proliferative function on its own it was shown that loss of JunB in the myeloid lineage results in a myeloproliferative disease in mice (Passegué, Jochum, Schorpp-Kistner, Möhle-Steinlein, & Wagner, 2001) which is due to the increased proliferation of granulocyte progenitors. In detail, loss of JunB affects protein levels of p16 and anti-apoptotic proteins like B-cell lymphoma 2 (Bcl-2) in granulocyte progenitors resulting in a dramatic increase in the number of granulocytes. Interestingly loss of JunB does not only disrupt granulopoiesis it also affects bone formation. In detail JunB was conditionally deleted in epiblast cells of the embryo using MORE-Cre *JunB*$^{f/f}$ mice which resulted in reduced bone formation and osteopenia (reduced bone mineral density)(Kenner et al., 2004). Conditional knock-out strategies must be applied because *JunB* $^{-/-}$ mice are embryonic lethal with altered angiogenesis in the extra-embryonic tissue (Zenz et al., 2008). To make it even more complex JunB also plays an important role in immunity. Firstly, JunB was shown to regulate the differentiation of naïve T cells into Th2 cells. JunB guides differentiation to the Th2 subset as it is a positive regulator of IL-4 (Zenz et al., 2008), a cytokine which was shown to drive differentiation to Th2 cells (Z. Liu et al., 2005). Secondly, it was shown that JunB in conjunction with c-Jun plays a very important role in psoriasis as skin specific deletion of c-Jun and JunB in mice recapitulates the hallmarks of this disease (Zenz et al., 2005). Psoriasis is an autoimmune disorder where the immune cells misinterpret skin cells as foreign leading to skin inflammation (Zenz et al., 2005). Lastly, it was shown that skin specific deletion of JunB affects hematopoiesis and bone formation due to enhanced G-CSF secretion from keratinocytes (Meixner et al., 2008). Given the promiscuous role of JunB in development and inflammation we were interested to elucidate its role in pDC function and development as little is known up today.

2. GOALS OF THE THESIS

AP-1 proteins are a family of transcription factors involved in a multitude of molecular biology processes like development, immunity, cell cycle and cancer. In my master thesis I wanted to investigate how the loss of two AP-1 protein family members, namely c-Jun and JunB affects the development and function of pDCs as up to now little is known. Among the AP-1 proteins JunB has a prominent role in the formation of granulocytes a myeloid cell type whose development is closely related to that of DCs. Intrigued by this fact I wanted to determine if JunB affects the development of pDCs *in vitro.* c- Jun on the other hand is an important regulator of the cell cycle and has a well known role in the induction of many pro-inflammatory cytokines. I therefore wanted to determine if c-Jun has a similar role in pDCs. Furthermore a loss of c-Jun and JunB in mice results in severe skin inflammation resembling psoriasis a disease where pDCs play a prominent role. My goal was to investigate if loss of c-Jun and JunB affects the differentiation and cytokine secretion of pDCs. Lastly, pDCs have a profound role in anti-tumor immunity. Given that c-Jun and JunB could regulate genes mediating this anti-tumor response I also wanted to investigate if a loss of c-Jun/JunB renders the ability of pDCs to kill tumor cells *in vitro*. Taken together, I wanted to determine if a loss of the AP-1 proteins c-Jun and or JunB affects the development, maturation, cytokine secretion and anti-tumor capacity of pDCs. I believe that a better understanding of the role of AP-1 proteins, c-Jun and JunB, in pDCs could lead to the development of new drugs offering a better treatment for diseases like psoriasis or help patients with certain cancers.

3. RESULTS

3.1. Deletion of c-Jun or JunB in pDCs using the Mx cre system

To investigate the role of c-Jun and JunB in the development and function of pDCs, *c-Jun*$^{f/f}$ (Behrens et al., 2002) and *JunB*$^{f/f}$ (Kenner et al., 2004) transgenic mice were crossed to mx cre (Kühn, Schwenk, Aguet, & Rajewsky, 1995) mice to delete these AP-1 proteins in the hematopoietic compartment. These transgenic mice have the Cre recombinase under control of the Mx1 promoter. The Mx1 promoter can be induced by INF-α or by the synthetic compound poly I:C (Kühn et al., 1995). Activation of Mx1 leads to induction of cre which recombines loxP flanked alleles like *c-Jun*$^{f/f}$ or *JunB*$^{f/f}$ out of the genome. Deletion occurs in BM cells but also in other organs like the liver (Kühn et al., 1995). To delete *c-Jun* or *JunB* in the BM, 7 to 8 weeks old transgenic mice were injected twice within 5 days with poly I: C. Deleted mice are now further referred to as as *c-Jun*$^{\Delta mx}$ and *JunB*$^{\Delta mx}$ in the text and figures. After the second injection mice were left for 12 days until they were taken for experiments (see Figure 9). A 12 day rest time was necessary to resolve the poly I: C provoked inflammation in mice. To analyze the development and function of pDCs BM cells of these mice were cultured for 7 days in the presence of FLT3L (see 5.6 for details). After 7 days of culture pDCs were purified by positive selection for B220^{+} cells using the IMag Cell Separation system (see 5.7 for details). The purity of pDCs was above 80 %. In a first step, efficient deletion of *c-Jun* and *JunB* was proven in BM cells or in purified pDCs on the level of DNA, RNA and protein. Firstly, DNA was isolated from BM cells of mutant and control mice and a polymerase chain reaction (PCR) reaction was performed. Cre mediated recombination yielded a 600 bp PCR product in *c-Jun*$^{\Delta mx}$ and a 380 bp PCR product in *JunB*$^{\Delta mx}$ BM cells (see Figure 9 B). Secondly deletion of *Jun* in purified pDCs was proven on RNA level by a real time polymerase chain reaction (RT-PCR). Using Primers specific for

c-Jun or *JunB* cDNA amplification was successful in control, however, not in pDCs from knockout mice (see Figure 9 C). Lastly, deletion was proven on protein levels by Western Blot. Protein extracts were obtained from purified pDCs and a Western Blot was performed with antibodies against c-Jun or JunB. Protein levels of c-Jun or JunB were detectable in control, but were completely absent in pDCs from knockout mice (see Figure 9 D). In summary I show that the mx cre transgenic mouse model can be used to efficiently delete *c-Jun* or *JunB* in pDCs

3.2. Combined deletion of c-Jun/JunB in pDCs using the Mx cre system

Individual AP-1 protein family members are often able to form heterodimers with other family members which can drastically alter their function. For example c-Jun can form a heterodimer with JunB which antagonizes the cell proliferative capacity of c-Jun (Shaulian & Karin, 2002). It is therefore necessary to investigate if a combined loss of c-Jun and JunB results in the same phenotype as an individual loss of c-Jun or JunB.

In a second approach I therefore wanted to delete *c-Jun* and *JunB* in a mx cre mouse model. For that I used *c-Jun*/*JunB*$^{fl/fl}$ mx cre mice. As described above deletion can be accomplished by injection of poly I: C. However, the strategy described to delete *c-Jun* or *JunB* individually (see 3.1) cannot be applied as c-*Jun*/*JunB*$^{f/f}$ mx cre mice develop a severe skin phenotype after the first injection and die shortly later (unpublished data). To delete *c-Jun* and *JunB* mice were therefore injected only once with poly I: C and taken for experiments 5 days after the first injection (see Figure 10 A). These mice are referred to as *c-Jun/JunB*$^{\Delta mx}$*.* Successful deletion of *c-Jun/JunB* using this experiment model was again proven on the level of DNA (Figure 10 B), RNA (Figure 10 C) and protein (Figure 10 D) as described in 3.1.

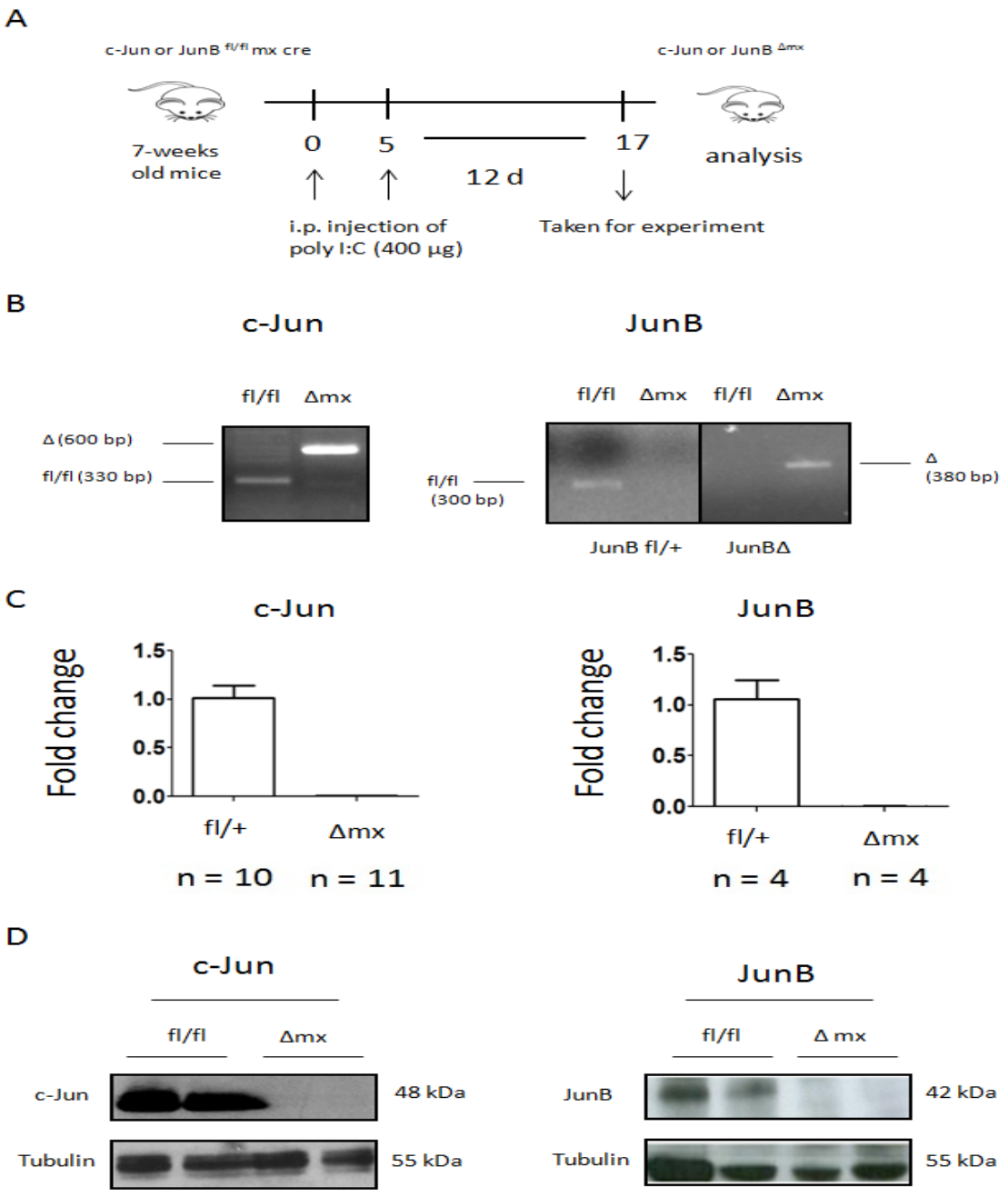

Figure 9: Deletion of *c-Jun* or *JunB* in pDCs using the Mx cre system.

(A) Experiment outline: 7-8 week old *c-Jun*$^{f/f}$ *or JunB*$^{f/f}$ mx cre mice were injected twice with poly I: C (400 µg) within 5 days. Before starting each experiment mice were left for 12 days after the second injection. (B) PCR showing efficient deletion of *c-Jun* or *JunB*. DNA was isolated from BM cells. (C) RT-PCR showing successful deletion at the level of mRNA. RNA was isolated from purified pDCs after 7 days of FLT3L supplemented BM culture. (D) Western blot showing successful deletion of c-Jun or JunB. Proteins were extracted from purified pDCs. Abbreviations: fl/fl = loxP flanked alleles, f/+ =loxP flanked or/and wild type allele, Δmx = deletion of loxP flanked alleles using the mx cre system. These abbreviations are also used in the following figures.

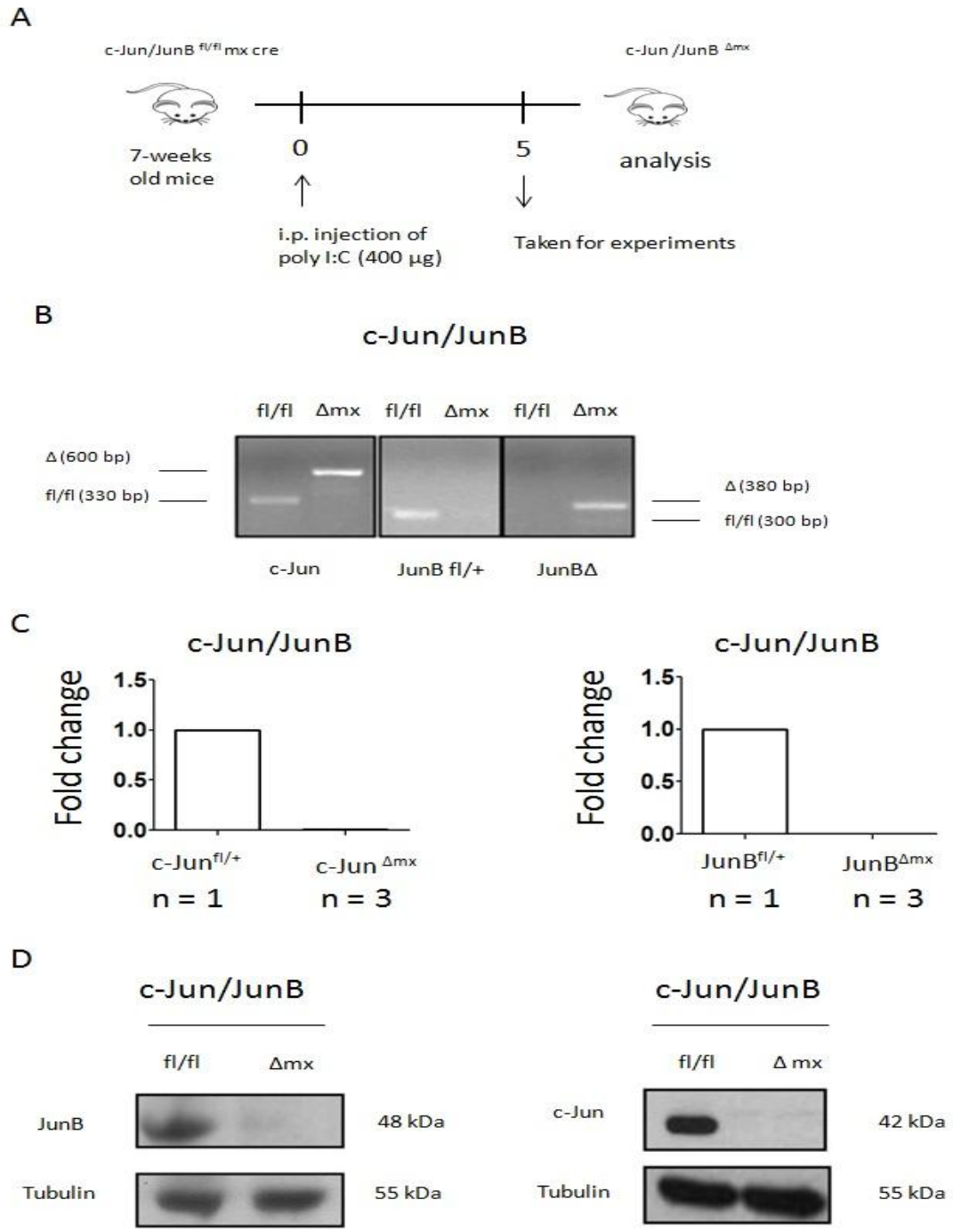

Figure 10: Combined deletion of c-Jun and JunB in pDCs is efficient in Mx cre mice.
(A) Experiment outline: Combined deletion of *c-Jun/JunB* is obtained by injection of poly I:C (400 µg) into 7-8 weeks old mice. (B) PCR analysis confirms deletion of *c-Jun and JunB* in *c-Jun/JunB*$^{\Delta mx}$ BM cells. DNA was extracted from BM cells. (C) RT-PCR of RNA isolated from purified pDCs shows successful deletion. (D) Western blot from protein extracts of purified pDCs proves deletion of *c-Jun and JunB*.

3.3. DCs progenitors (MDP-CDP) are increased in the absence of JunB and c-Jun/JunB in FLT3L supplemented BM cultures.

pDCs develop from dendritic progenitors in the bone marrow (Naik, 2008). The best characterized dendritic progenitors are the monocyte and dendritic cell progenitor (MDP) and the common dendritic progenitor (CDP) which are both positive for the expression of the cell surface marker CD115 (Colony stimulating factor 1 receptor; CSFR1). *In vitro* differentiation of pDCs from dendritic progenitors can be recapitulated by addition of FLT3L to BM cells. To test if a loss of the AP-1 proteins c-Jun and/or JunB affects the number of dendritic progenitors FLT3L supplemented BM cultures were analyzed. After 6, 7 or 8 days of culture cells were taken to perform a fluorescent activated cell sorting (FACS) analysis on dendritic progenitors (CD115+ cells; MDP-CDPs). MDP-CDPs were defined as CD115+CD11c-CD11b-. Figure 11(A-F) shows representative FACS blots of the progenitor population in control and knock-out mice on day 6, 7 and 8 of FLT3L supplemented BM culture. Interestingly, in the absence of JunB and c-Jun/JunB *in vitro* differentiation resulted in significantly increased numbers of MDP/CDPs on day 6 and 8 (see Figure 11 G, H) indicating a role of JunB in the differentiation of DCs. A loss of c-Jun on the other hand did not change the numbers of MDP/CDPs in vitro (see Figure 11 G, H). In conclusion specific deletion of JunB in BM cells increases the number of MDP-CDPs in vitro.

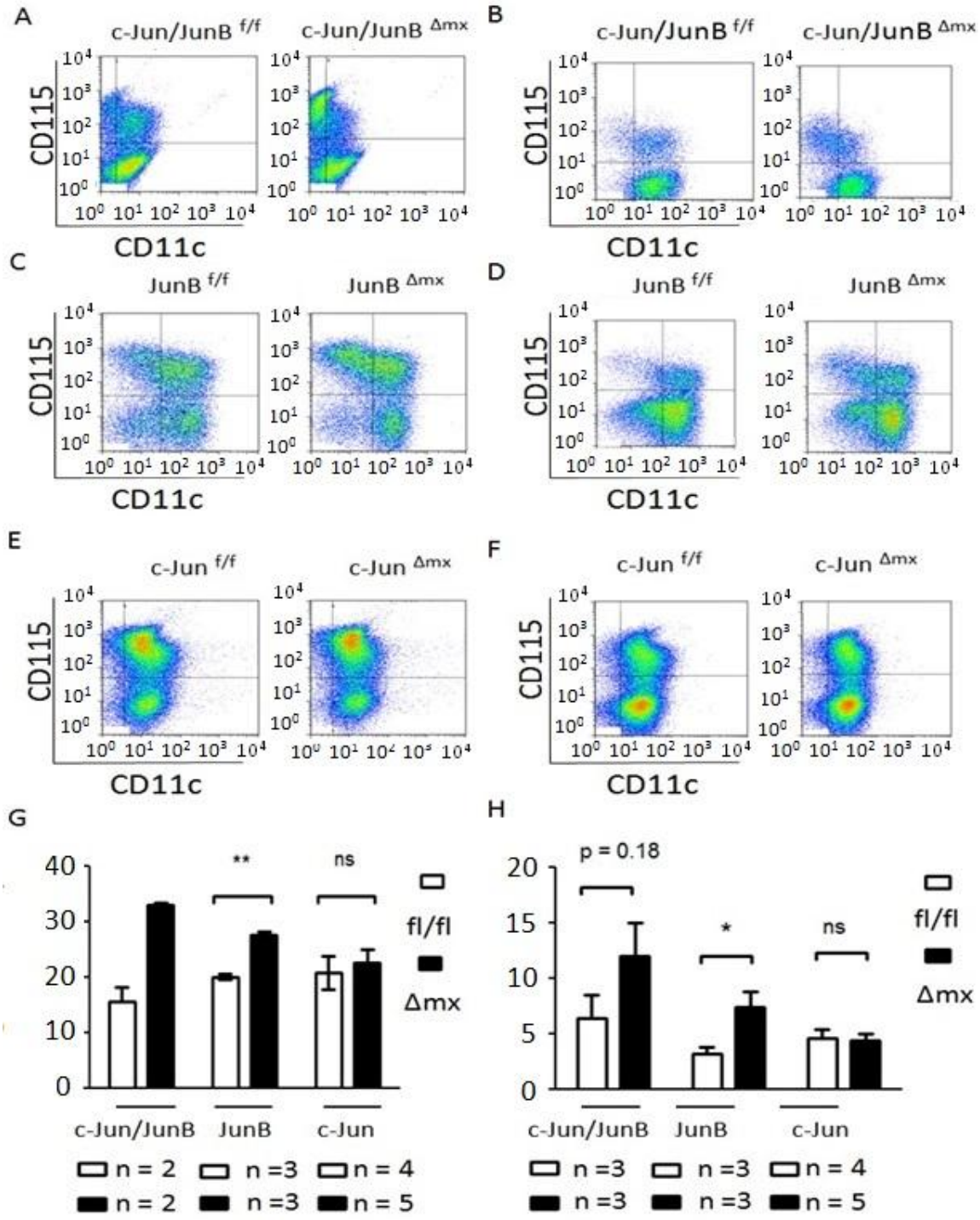

Figure 11, JunB regulates the numbers of dendritic progenitors (CD115^{+}) *in vitro.*
(A-F) Representative dot plots of dendritic progenitors (CD115^{+}) gated on living cells after 6 (A, C, E), 7 (F) and 8 (B, D) days of FLT3L supplemented BM cultures. (G, H) shows frequencies of dendritic progenitors (CD115^{+}) on day 6 (G), 7 (H; c-Jun) and 8 (H, c-Jun/JunB, JunB) of FLT3L supplemented BM cultures. As indicated in the figures BM cells are either *c-Jun* $^{f/lf}$, *JunB* $^{f/lf}$, *c-Jun/JunB* $^{f/lf}$ or *c-Jun, JunB, c-Jun/JunB* $^{\Delta mx}$. Two independent experiments were performed. Results are shown as mean ± SEM; * $p < 0.05$, ** $p<0.005$.

3.4. c-Jun and JunB are dispensable for pDC development in FLT3L supplemented BM cultures.

In the last years an increasing number of transcription factors were found to regulate the development of pDCs. For example it was shown that pDCs don´t develop from myeloid progenitors in the absence of spleen focus-forming virus integration site (Spi-B) (Cisse et al., 2008). To test if the transcription factors c-Jun and JunB influence the development of pDCs, BM cells were isolated and differentiated *in vitro* in the presence of FLT3L. At day 6, 7 or 8 a FACS analysis was done to determine the percentages of pDCs in culture. pDCs were defined as $B220^{+}CD11c^{+}CD11b^{-}$ and Figure 12 (A-F) shows representative FACS blots of pDC cultures of control and AP-1 knock-out mice on day 6, 7 and 8. Interestingly, the number of pDCs is unchanged in the absence of c-Jun, JunB or c-Jun/JunB on day 6 and day 7 or 8 of culture (see Figure 12 G, H). Therefore I conclude that c- Jun and JunB are dispensable for the *in vitro* development of pDCs in FLT3L supplemented BM cultures.

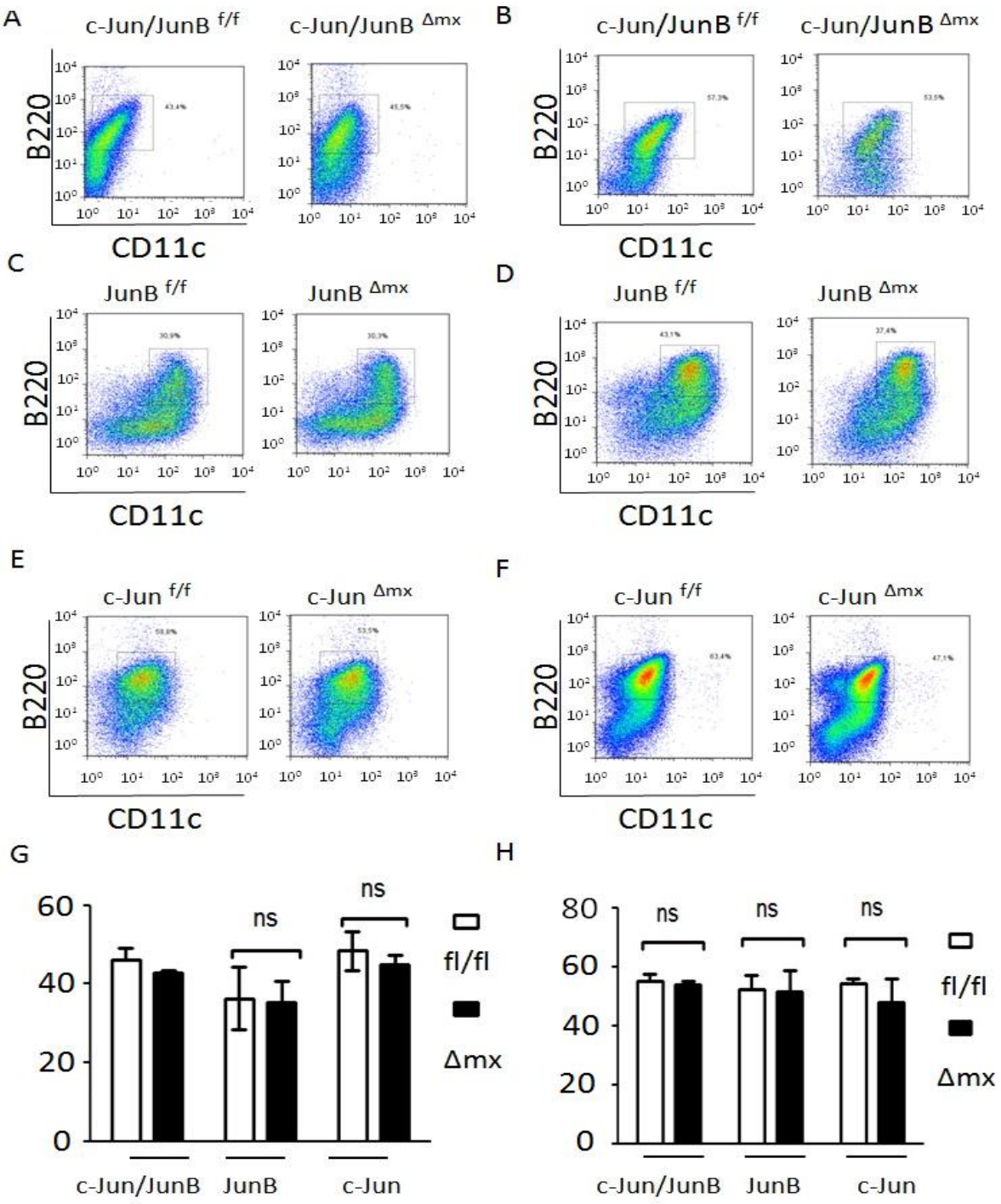

Figure 12, c-Jun and JunB are dispensable for the development of pDCs *in vitro.*

(A-F) Representative dot plots of pDCs ($B220^+CD11c^+$) gated on living cells after 6 (A,C,E), 7 (F) and 8 (B,D) days of FLT3L supplemented BM cultures (G, H) Frequency of pDCs ($B220^+CD11c^+$) on day 6 (G), 7 (H; c-Jun) and 8 (H, c-Jun/JunB, JunB) of FLT3L supplemented BM cultures. As indicated in the figures BM cells are either *c-Jun, JunB, c-Jun/JunB* $^{f/f}$ or *c-Jun, JunB, c-Jun/JunB* $^{\Delta mx}$. Two independent experiments were performed. Results are shown as mean ± SEM.

3.5. c- Jun and JunB are induced after stimulation of pDCs with TLR7 agonist Imiquimod.

Upon stimulation many resting cells rapidly change the expression of various genes. The genes most prone for expression after stimulation are called immediate-early genes. *c-Jun* and *JunB* are two prominent members of this gene family(Pelto-Huikko et al., 1995). As little is known if c-Jun and JunB are also rapidly induced in pDCs I stimulated purified pDCs with the TLR7 agonist Imiquimod (Imi, 2.5mg/ml) for 4h. After 4h RNA was isolated and a RT-PCR was performed on *c-Jun* and *JunB* cDNA. Figure 13 (A-F) shows the RNA levels of *c-Jun* and *JunB* in BM-derived pDCs of mutant and control mice. Interestingly, *c-Jun* mRNA is induced ~ 15 fold after pDCs were stimulated with Imi for 4h (Figure 13 A, C and E) compared to LAL treated control pDCs. Additionally *JunB* mRNA is induced ~3 fold after 4 h stimulation of pDCs with Imi (Figure 13 B, D and F). Moreover I could show that loss of JunB does not affect the induction of c-Jun (Figure 13 C) and vice versa (Figure 13 F). The strong induction of c-Jun could further be proven on protein level after 8h of TLR7 stimulation. (Figure 13 G). In summary I could show that stimulation with the TLR7 agonist Imi leads to a strong induction of *c-Jun* and *JunB* mRNA in pDCs, indicating an important role of these AP-1 family members in stimulated pDCs. Lastly, I could show that lack of c-Jun or JunB alone does not affect the induction of the other remaining AP-1 family member (c-Jun or JunB).

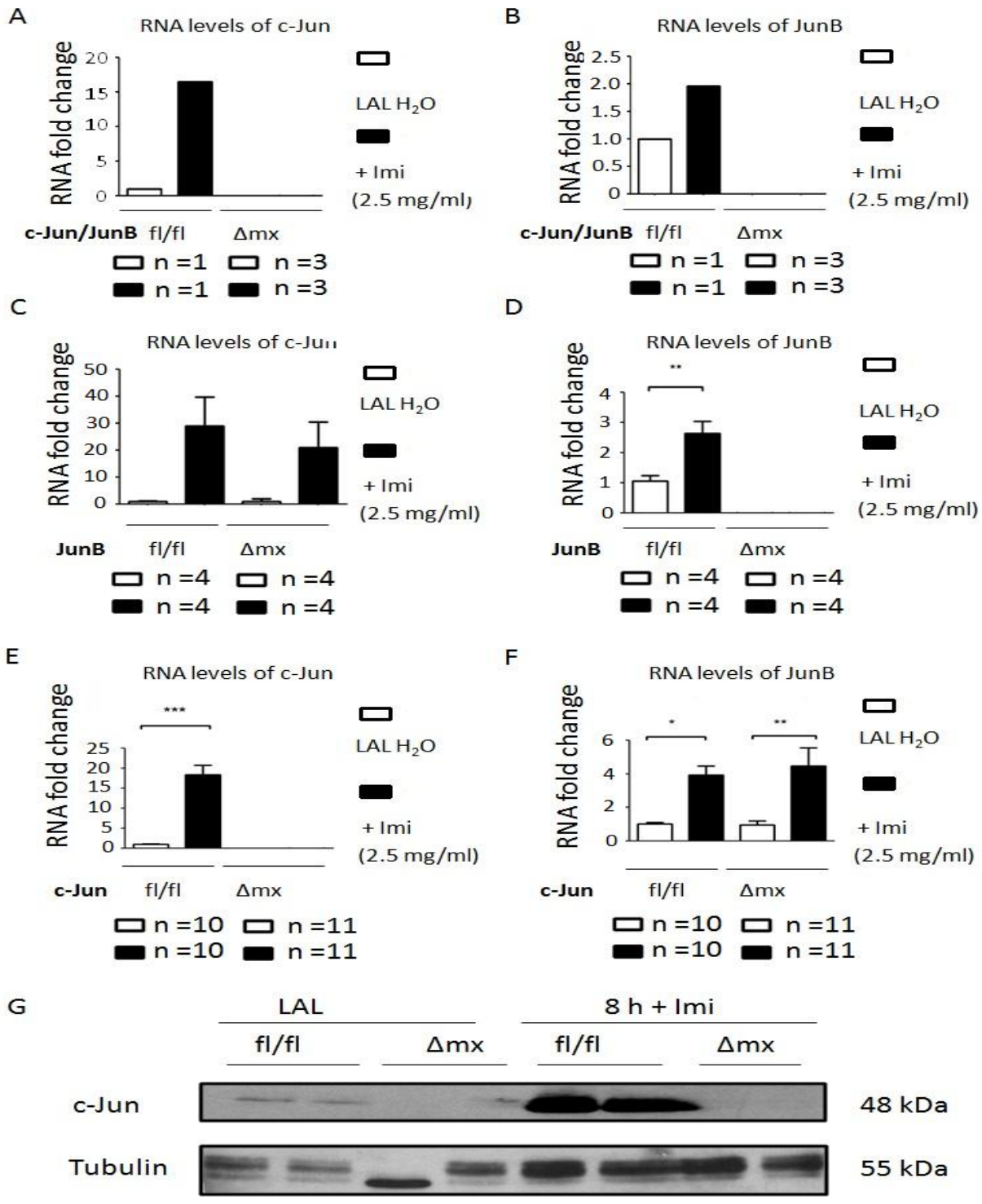

Figure 13, c-Jun and JunB are induced in pDCs when stimulated with Imi.

(A-F) RNA levels of *c-Jun* and *JunB* in purified pDCs (purity > 80 %) stimulated with Imi (2.5 mg/ml) for 4 h or LAL water as a control. *c-Jun/JunB* (A, B), *JunB* (C, D) and *c-Jun* (E, F) $^{fl/fl}$ or $^{\Delta mx}$ pDCs were analyzed. Results are shown as mean ± SEM from at least two independent experiments (A, B are results from only one batch); p: * $p < 0.05$, ** $p<0.005$, *** $p<0.0005$. (H) Western blot of protein extracts from *c-Jun* $^{f/f}$ or $^{\Delta mx}$ pDCs (purity >80%) stimulated with Imi (2.5 mg/ml) for 8 h or LAL water as a control.

3.6. Loss of c-Jun reduces the RNA levels of *Il-6* and *Infb* in pDCs stimulated with Imi.

Upon stimulation pDCs induce the expression of a variety of immune-stimulatory genes like interferon alpha/beta (*Ifna/b*) or interleukin-6 (*Il-6)* and tumor necrosis factor alpha (*Tnfa)* (K. McKenna et al., 2005). Transcription factors involved in the expression of these genes are IRF-7 as a master regulator of Type-I IFNs (Honda et al., 2005) and NF-κB as a regulator of IL-6 and IL-12 (O'Keeffe et al., 2005). Much less is known if AP-1 family members like c-Jun or JunB influence the expression of these immune-stimulatory genes. To address this question the RNA levels of *Ifnb* and *Il-6* were determined by RT-PCR after purified pDCs were stimulated with Imi for 4 h *in vitro*. Interestingly, in absence of c-Jun (*c-Jun/junB*$^{\Delta mx}$ and *c-Jun*$^{\Delta mx}$ pDCs) the RNA levels of *Il-6* and *Ifnb* are reduced after Imi stimulation (Figure 14 A-D). Loss of JunB on the other hand does not affect the RNA levels of *Il-6* and *Infb* (see Figure 14 E, F). Since I observed similar results in c-Jun single and c-Jun/JunB double knock out pDCs I conclude that c-Jun alone plays an important role for the induction of the pro-inflammatory cytokine IL-6 and of IFN-β. Since one hallmark of pDCs is there ability to produce large amounts of IFN-α, I also determined the RNA levels of interferon alpha 1 (*Ifna1)* in pDCs stimulated with Imi for 4 h. *Ifna1* is one of the 14 IFN-α subtypes and its RNA levels correlate to the other family members (K. McKenna et al., 2005). Surprisingly, I found that loss of c-Jun does not affect the RNA levels of *Ifna1* (Figure 15 E). Furthermore the RNA levels of the Mx1 gene were determined. Expression of the myxovirus resistence 1 (*Mx1*) gene correlates to an active JAK/STAT signalling cascade which is induced by Type I IFN. Interestingly, loss of c-Jun does not affect the RNA levels of *Mx1* (Figure 15 F). I therefore conclude that c-Jun is dispendsable for the induction of IFN-α in pDCs stimulated with Imi.

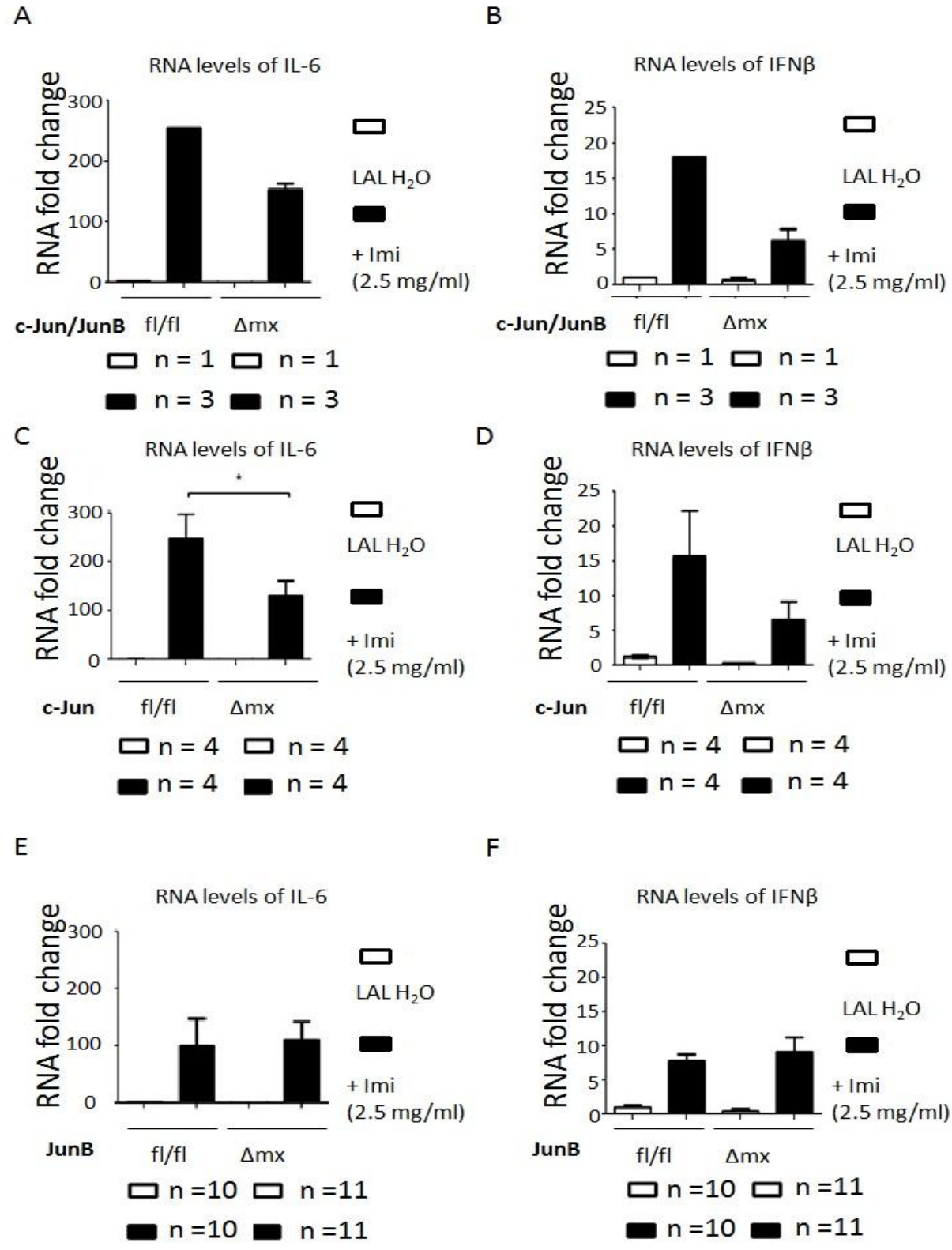

Figure 14, c-Jun regulates the expression of the *Ifnb* and *Il-6* gene in pDCs.

RNA levels of *Il-6* (A, C, E) and *Ifnb* (B, D, F) were analyzed in BM-derived pDCs stimulated with Imi for 4 h or LAL water as a control. pDCs were either *c-Jun, JunB, c-Jun/JunB* $^{f/lf}$ or *c-Jun, JunB, c-Jun/JunB* $^{\Delta mx}$. Results are shown as mean ± SEM of two independent experiments (A, B are results from only one batch); * $p<0.05$. Purity of pDCs was 80 %.

3.7. Loss of c-Jun reduces the protein levels of IL-6 and IFN β in Imi stimulated pDCs.

As described above loss of c-Jun reduces the mRNA levels of *Il-6* and *Ifnb*. I was therefore interested if the protein levels of IL-6 and IFN β are also changed in absence of c-jun. Therefore *c-Jun*$^{f/f}$ and *c-Jun*$^{\Delta mx}$ pDCs were stimulated for different timepoints (8 and 16 h) with the TLR7 agonist Imi and the supernatants were used to perform an ELISA against IL-6 and IFN β. Strikingly, I found that in the absence of c-Jun protein levels of IL-6 are decreased as early as 8h after induction (Figure 15 A). After 16 h the decrease is even more pronounced. Furhtermore I analyzed the protein levels of IFN β and found that it was also decreased in the absence of c-Jun at 8 h and 16 h (Figure 15 B). In contrast to IL-6 and IFN β loss of c-Jun did not affect the production of TNF-α (Figure 15 C, D). *Tnfa* RNA as well as TNF-α protein levels were not altered after 8 and 16 h of Imi stimulation (Figure 15 C,D). I therefore conclude that c-Jun is a specific regulator of IL-6 and IFN-β, but not of TNF-α in Imi stimulated pDCs *in vitro*.

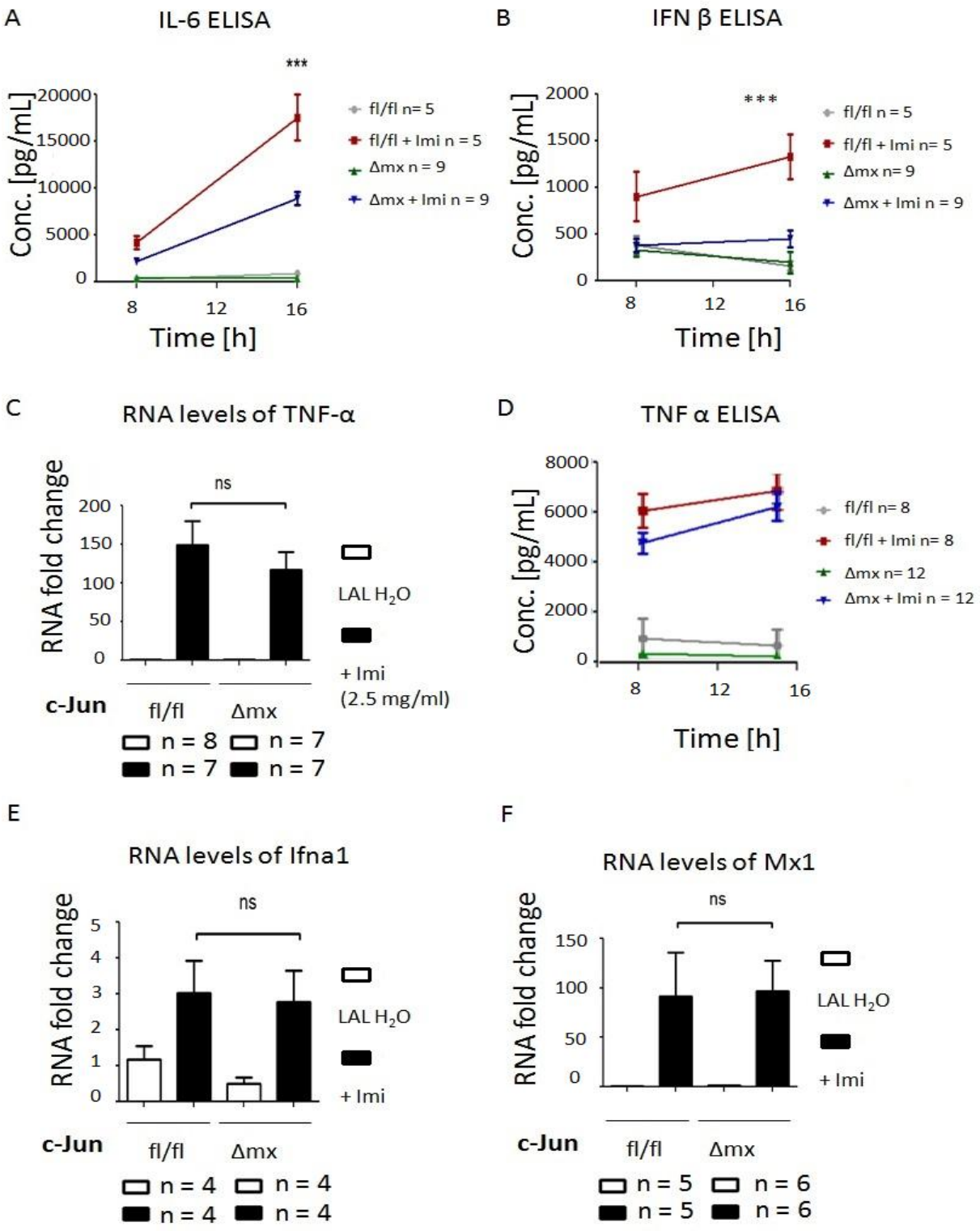

Figure 15, The role of c-Jun in the expression of cytokines in Imi stimulated pDCs. *c-Jun*$^{fl/fl}$ and *c-Jun*$^{\Delta mx}$ pDCs were stimulated with Imi for 8 and 16 h and their supernatants were taken for an IL-6 (A), IFN β (B) and TNF-α ELISA (D). RNA levels of *Tnfa* (C), *Ifna1* (E) and *Mx1* (F) were determined by RT-PCR after stimulation of *c-Jun*$^{fl/fl}$ and *c-Jun*$^{\Delta mx}$ pDCs for 4h with Imi. Results are shown as mean ± SEM of two (C, E and F) and three (A, B, D) independent experiments. ** p<0.005, *** p <0.0005

3.8. Loss of c-Jun does not alter the expression of pDC characteristic surface markers *in vitro*

pDCs are characterized by their expression of various surface markers like B220, BST-2, CD11c and Siglec-H (Cisse et al., 2008). To test if c-Jun also regulates the expression of these surface markers FLT3L supplemented BM cultures were generated. On day 7 the cells were harvested, purified and analyzed by FACS for the expression of B220, BST-2 and Siglec-H. Loss of c-Jun does not alter the expression of B220, BST-2 and Siglec-H in pDCs *in vitro* under steady state conditions (Figure 16 A-C). Furthermore c-Jun is known to regulate the proliferation in keratinocytes and fibroblasts (Zenz et al., 2008). The cell number per ml in FLT3L supplemented BM cultures was therefore determined by CASY Count. However in pDCs a loss of c-Jun does not affect the cell number per ml on day 7 of culture (Figure 16 D).

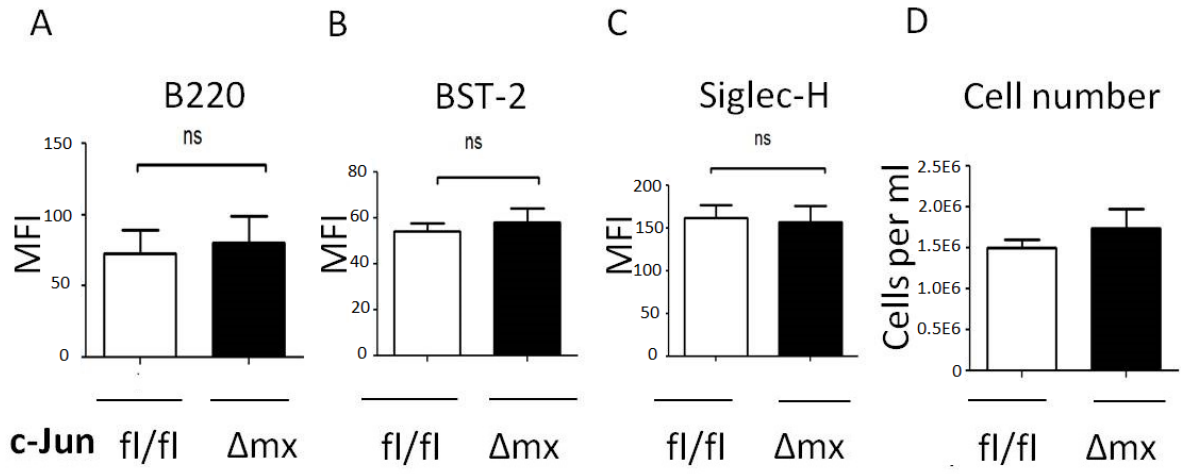

Figure 16, c-Jun is dispensable for the expression of pDC specific surface markers.
(A-C) Graphs show mean fluorescence intensity (MIF) of B220 (A), BST-2 (B) and Siglec-H (C) on day 7 of FLT3L supplemented BM cultures gated on living, $B220^{+}CD11c^{+}$ cells. Figure (D) shows the number of cells per ml on day 7 of FLT3L supplemented BM cultures as determined by CASY count. Results are shown as mean ± SEM of two (A-C) and 5 independent experiments (D).

3.9. In the absence of c-Jun pDCs mature normally

Upon stimulation pDCs up-regulate the expression of co-stimulatory molecules like CD8-α ,CD80, CD86, MHC class II proteins and other cell surface receptors like CD40 (K. McKenna et al., 2005). To investigate if c-Jun regulates the expression of these surface markers pDCs were activated with Imi for 8 and 16 h and analyzed by FACS. pDCs were purified on day 7 from FLT3L supplemented BM cultures. Loss of c-Jun did not alter the up-regulation of CD40, CD86 and MHC II after 8 h of stimulation (Figure 17 C, E and G). Strikingly, expression of CD8- α on the cell surface was reduced after 8 h of stimulation in the absence of c-Jun (Figure 17 A). Furthermore also after 16 h of stimulation no differences of CD40, CD86 and MHC II expression were observed in pDCs lacking c-Jun (Figure 18 C, E, G). CD8-α was on the other hand no longer reduced after 16 h of stimulation (Figure 18 A). From these results I conclude that pDCs mature normally in the absence of c-Jun, however, CD8-α up regulation was delayed. Maturation of pDCs is tightly coupled to Type-I IFN signaling (Asselin-Paturel et al., 2005). Loss of c-Jun, however, does not seem to affect Type-I IFN signaling (see 3.7) which explains the normal up-regulation of CD40, CD86 and MHC II. I therefore assume that CD8- α is reduced because it is either directly regulated by c-Jun or by another Type I IFN independent signaling pathway whose signaling is affected by the loss of c-Jun.

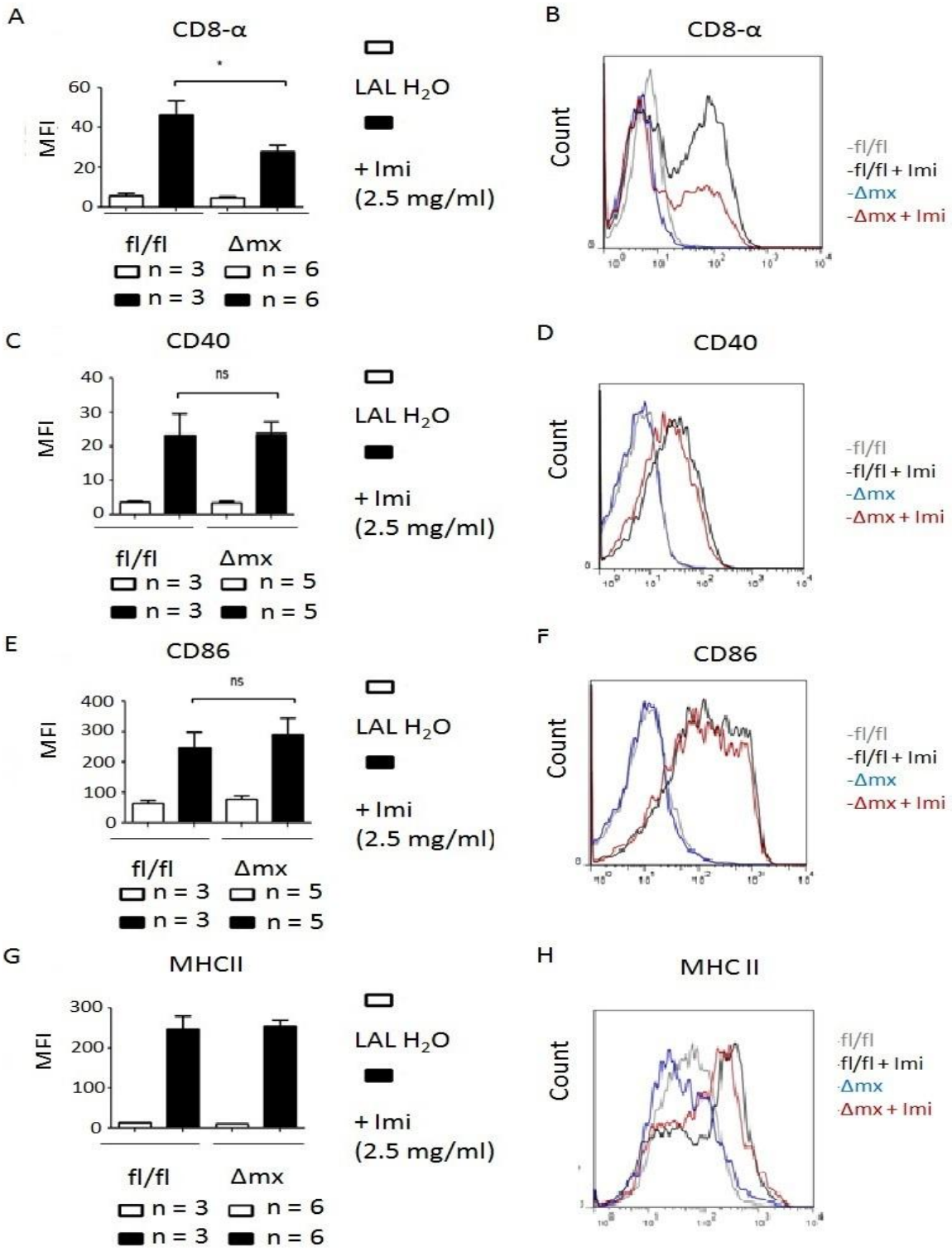

Figure 17, c-Jun is dispensable for the maturation of pDCs stimulated with Imi for 8h.
MFI of the surface markers CD40 (A), CD86 (C), MHC II (E) and CD8-α (G) as analyzed by FACS gated on pDCs ($B220^+CD11^+$) activated with Imi for 8 h. (B, D, F and H) show representative histograms of the surface markers CD40, CD86, MHCII and CD8-α as analyzed by FACS gated on pDCs ($B220^+CD11^+$) activated with Imi for 8 h. Results are shown as mean ± SEM of two independent experiments.

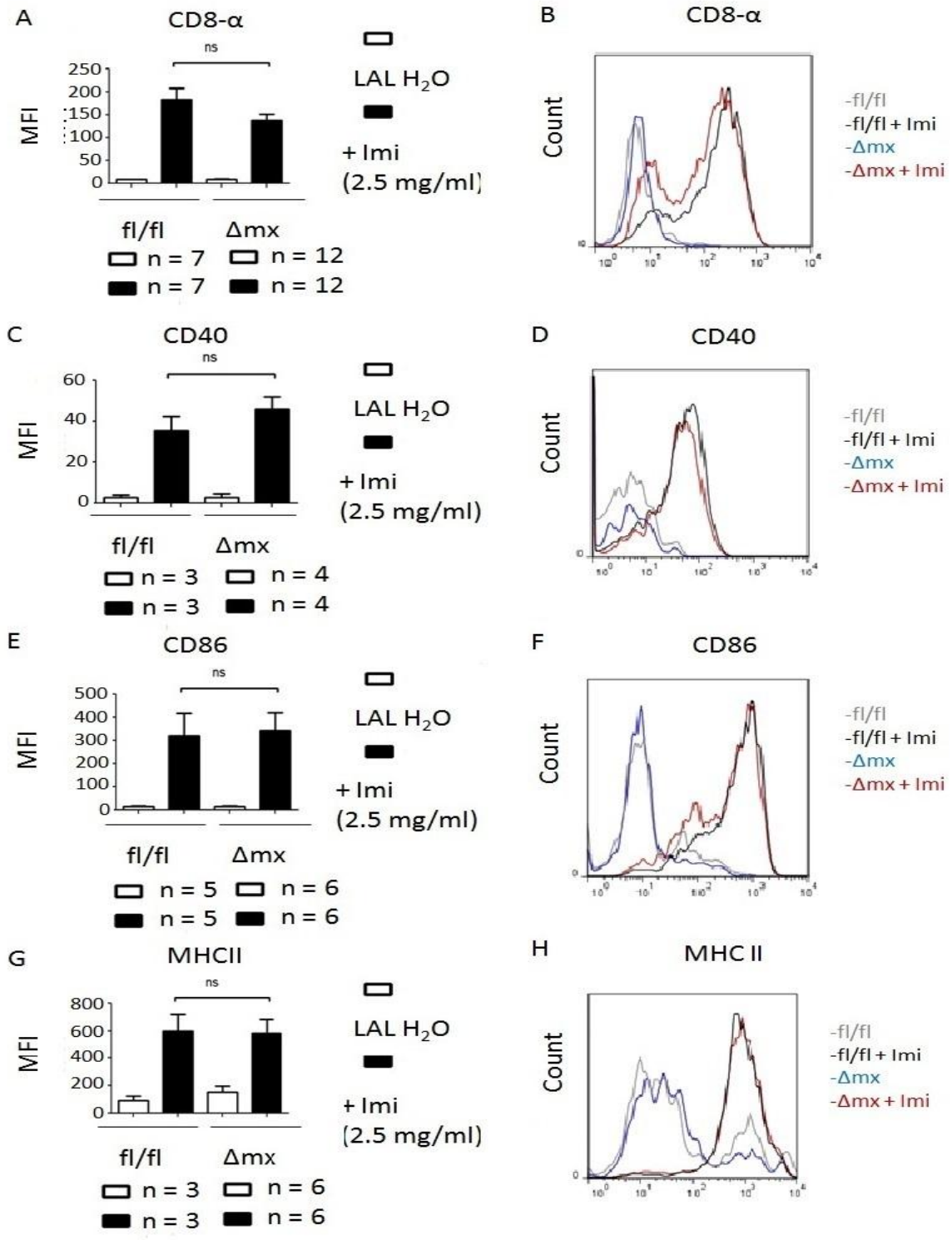

Figure 18, c-Jun is dispensable for the maturation of pDCs activated with Imi for 16 h.
MFI of the surface markers CD40 (A), CD86 (C), MHC II (E) and CD8 (G) as analyzed by FACS gated on pDCs ($B220^+CD11^+$) activated with Imi for 16 h. (B, D, F and H) show representative histograms of the surface markers CD40, CD86, MHCII and CD8-α as analyzed by FACS gated on pDCs ($B220^+CD11^+$) activated with Imi for 16 h. Results are shown as mean ± SEM of two independent experiments.

3.10. c- Jun is dispensable for the tumor killing capacity of pDCs.

Imi stimulated pDCs have been shown to kill tumor cells in the absence of adaptive immunity (Stary et al., 2009) (Drobits et al., 2012). The proposed molecular mechanism involves the cytolytic molecules TRAIL and Gzmb (Drobits et al., 2012). To test if c-Jun affects the expression of Gzmb and TRAIL BM derived pDCs were stimulated with Imi for 4 h and RNA levels of *Gzmb* and *Trail* were determined by RT-PCR. I found that loss of c-Jun does not affect the RNA levels of *Trail* (Figure 19 A). Interestingly, loss of c-Jun reduces the RNA levels of *Gzmb* (Figure 19 B). To test if reduced RNA levels of *Gzmb* result in decreased tumor killing capacity killing assays were performed. Shortly, Imi stimulated or un-stimulated pDCs were co-cultured with B16 melanoma cells for 16h. Cytotoxicity was determined using the EZ4U kit (Biomedica). The reagent EZ4U of the kit is reduced into a colored compound by intact mitochondria. This color change can be correlated to the lysis of B16 melanoma cells. Surprisingly, loss of c-Jun does not affect the tumor killing capacity *in vitro* (Figure 19 C) although reduced mRNA levels of *Gzmb* were found in Imi-stimulated pDCs in absence of c-Jun. Furthermore B16 melanoma cells were cultured in the presence of supernatant of Imi stimulated pDCs to test if produced soluble factors are able to induce tumor cell killing *in vitro*. I found that the supernatant of Imi stimulated *c-Jun*$^{\Delta mx}$ pDCs was as potent to induce lysis in B16 cells as the supernatant of Imi stimulated *c-Jun* $^{fl/fl}$ pDCs is (Figure 19 D). I therefore conclude that c-Jun is dispensable for the tumor killing in *vitro.* Ultimately *in vivo* tumor killing experiments must be performed to answer if c-Jun is involved in the tumor killing capacity of Imi-stimulated pDCs.

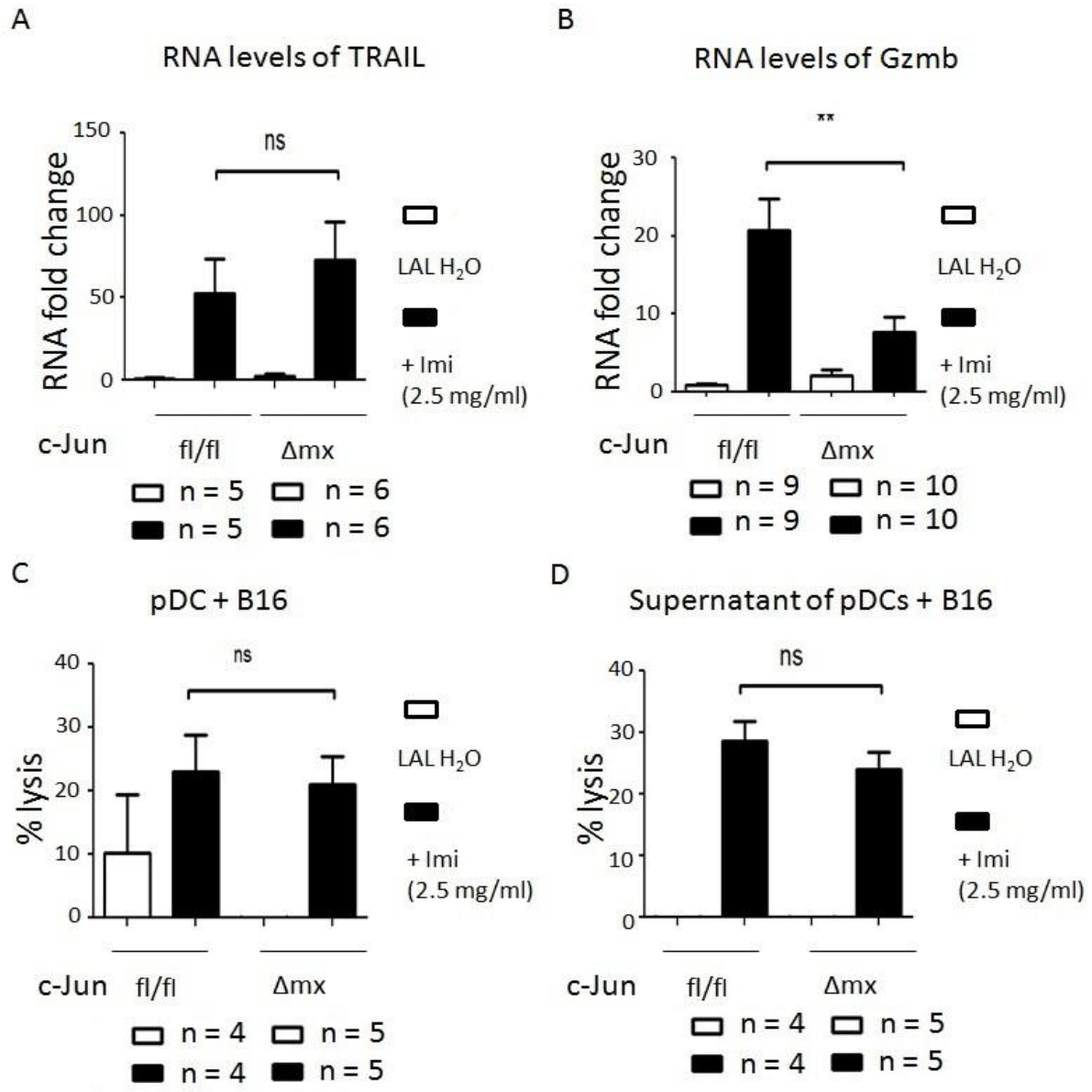

Figure 19, c- Jun is dispensable for the tumor killing capacity of pDCs.

RNA levels of *Trail* (A) and *Gzmb* (B) were determined in BM derived *c-Jun*$^{f/fl}$ or *c-Jun*$^{\Delta mx}$ pDCs stimulated with Imi for 4 h. (C) Imi (4h) stimulated pDCs were co-cultured with B16 cells at an E:T ratio of 10:1 for 16h. Lysis was determined using the EZ4U proliferation kit. (D) Alternatively the supernatant of Imi (4h) stimulated pDCs was taken to determine the lysis of B16 cells as described above. Imi was given at a concentration of 2.5 mg/ml. Results are shown as mean ± SEM of two (B, C and D) and five (A) independent experiments. ** $p<0.005$.

3.11. Effect of Imiquimod on the skin of c-Jun$^{\Delta mx}$ mice

Topical treatment of mouse back skin with Aldara, a cream containing 5% of Imi has been shown to induce strong inflammation accompanied with the recruitment of pDCs to the skin as well as epidermal thickening and splenomegaly (Palamara et al., 2004)(Drobits et al., 2012). So far I could show that, in the absence of c-Jun pDCs are impaired in their ability to produce IL-6 and IFN β upon Imi stimulation *in vitro*. To investigate if the absence of c-Jun in pDCs results in a phenotype *in vivo,* I treated mice topically with Aldara on waxed back skin and mice were analyzed for epidermal thickening and splenomegaly on day 7 of treatment. Mice were scarified and spleen weight was determined. Interestingly, splenomegaly was also present in *c-Jun*$^{\Delta mx}$ mice and the spleen/body weight ratio was comparable between mutants and controls (Figure 19A). Moreover, H&E stainings of sectioned skin of Aldara (un)-treated wild type mice (control) were compared to mice lacking c-Jun in the hematopietic system (*c-Jun*$^{\Delta mx}$ mice). *c-Jun*$^{\Delta mx}$ mice treated with Aldara showed similar signs of skin inflammation including redness and epidermal thickening as treated wild type mice (Figure 19B, C). Taken together preliminary results indicate that Aldara induced inflammation in the skin is comparable in *c-Jun*$^{\Delta mx}$ mice and *c-Jun* $^{fl/fl}$ mice. However, further experiments are needed to investigate if a lack of c-Jun in pDCs alters Aldara induced inflammation or Aldara induced anti-tumor effects.

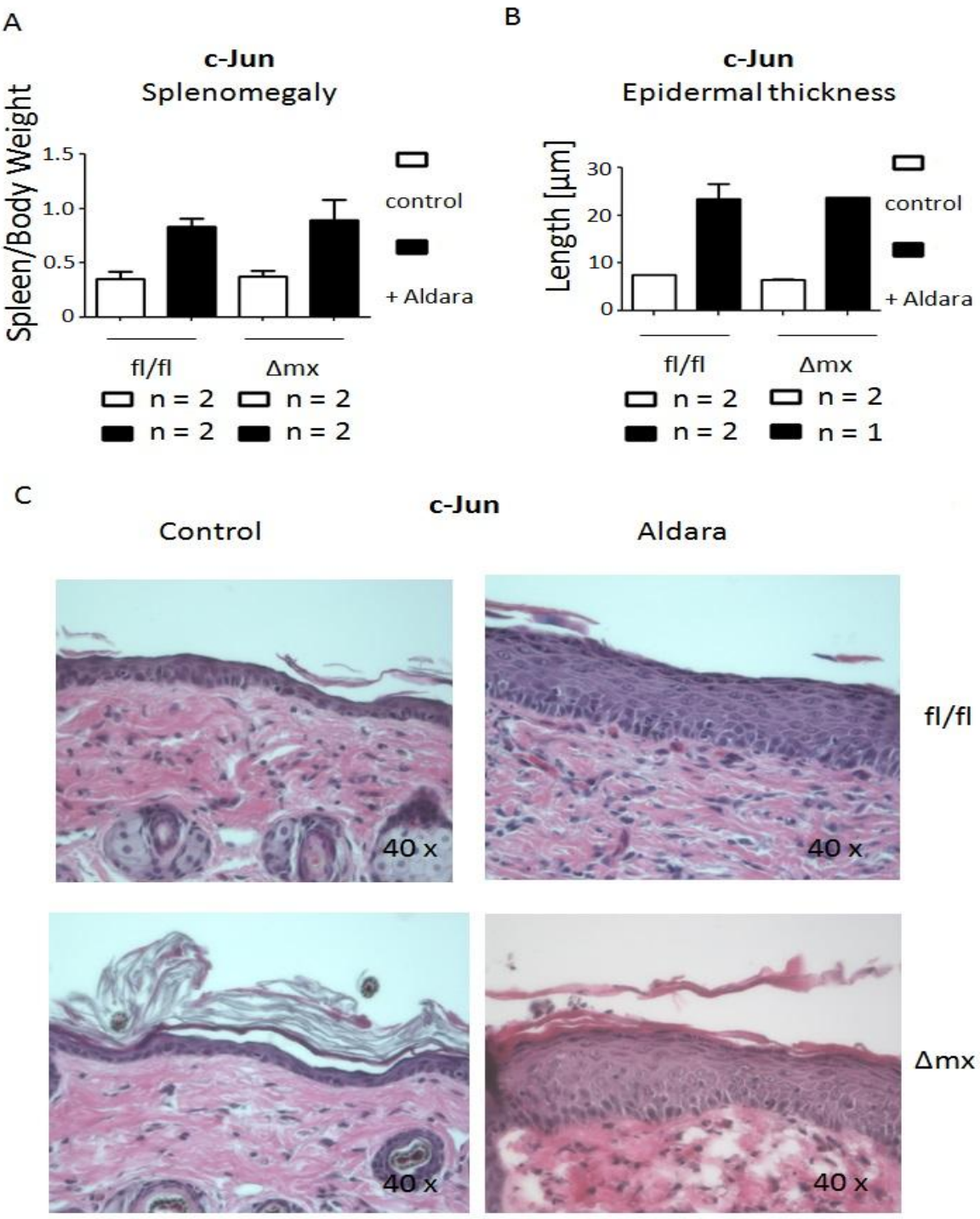

Figure 20, Epidermal thickening and splenomegaly in Aldara treated *c-Jun*$^{\Delta mx}$ mice.
(A) Splenomegaly shown as spleen to body weight ratio present in mice treated with Aldara for 7 days. (B) Epidermal thickening present in Aldara creamed mice. Thickness of epidermis was determined by measuring the length of the epidermis under the light microscope (C) H&E staining of the skin of *c-Jun*$^{\Delta mx}$ and *c-Jun* $^{fl/fl}$ mice at a magnification of 40x.

4. DISCUSSION

The aim of my master thesis was to determine the role of the AP-1 proteins c-Jun and JunB in the development and function of pDCs. c-Jun and JunB have been implicated in various biological processes like proliferation, cancer and immunity. Previous studies have highlighted a role of c-Jun and JunB in the development of different immune cells. For example c-Jun was shown to regulate the differentiation of α β and γ ∂ T cells in the thymus of mice (Riera-Sans & Behrens, 2007). On the other hand JunB is an important regulator of granulopoiesis as loss of JunB dramatically increases the numbers of granulocyte/macrophage progenitors (GMP) leading to a myeloproliferative disease in mice (Passegué et al., 2001). Furthermore JunB is important for the differentiation of naïve T cells into Th1 and Th2 cells as it regulates expression of IL-4 a key cytokine for Th2 differentiation (Zenz et al., 2008). Lastly, a deletion of *JunB* in the skin was shown to affect hematopoiesis in the BM (Meixner et al., 2008). Interestingly, in the last years an increasing number of transcription factors were shown to be important for the development of DC subsets. IRF-8 (Aliberti et al., 2003) and B-ATF3 are important for CD8+ development, IRF-4 is needed for CD4+ cDC (Suzuki et al., 2004) and Spi-B is critical for pDC development (Shortman & Naik, 2007). Much less, however, is known about the function of the transcription factors c-Jun and JunB in the development of DCs. To answer these questions *in vitro* differentiation cultures of DCs from BM cells were done in the presence of FLT3L. It is well known that BM cultures supplemented with FLT3L give rise mainly to cDCs and pDCs (Naik, O'Keeffe, Proietto, Shortman, & Wu, 2010). Interestingly in the absence of JunB differentiation of DCs *in vitro* resulted in elevated levels of dendritic progenitors (MDP/CDPs) whereas loss of c-Jun had no effect. Although dendritic progenitors are known to give rise to pDCs and other dendritic cell subsets like $CD8^+$, $CD8^-$ and $CD103^+$ cells (K. Liu & Nussenzweig, 2010), the observed increased levels of MDP/CDPs did not result in elevated numbers of pDCs. Interestingly it was shown, that GMPs, which arise from the same progenitor, as MDP/CDPs, namely the common myeloid progenitor (CMP) are

increased in JunB deficient BM (Passegué et al., 2001). It is likely that the increased number of MDP/CDP found in FLT3L cultures of JunB deficient mice is due to an altered/shifted progenitor commitment. However, c-Jun and JunB seem to be have a dispensable role in the development of pDCs *in vitro* since the numbers of cells were comparable to wild type FLT3L cultures. Further experiments will be needed to analyze if other DC subsets are affected and if DCs progenitors are also altered *in vivo* under homoeostatic or pathological conditions. pDCs sense pathogens via expression of TLR7/9 (Gilliet et al., 2008). Upon TLR7/9 stimulation pDCs produce a number of cytokines like IL-6, TNF-α, IFN-α and IFN-β. Expression of these pro-inflammatory genes is regulated by distinct transcription factors like IRF-7 or NF-κB. Previous studies revealed IRF-7 as the master regulator of Type I IFNs (Honda et al., 2005) and NF-κB as critical factor for the production of IL-6 (O'Keeffe et al., 2005). In contrast to IFR-7 and NF-κB much less is known how c-Jun and JunB regulate these pro-inflammatory genes. I therefore stimulated BM derived pDCs with the TLR7 agonist Imi for various time points and determined the levels of IL-6, IFN-β and TNF-α by RT-PCR and ELISA. Interestingly, c-Jun is needed to induce robust expression of IL-6 and IFN-β on the level of RNA and protein after TLR7 stimulation of pDCs. c-Jun is, however, not needed for the induction of other pDC specific cytokines like TNF-α as loss of c-Jun does not alter the RNA or protein levels of this cytokine. Furthermore I found that loss of JunB in contrast to c-Jun does not affect the expression of IL-6 and IFN-β and it was also dispensable for the production of TNF-α. A study done by Mitchell et al. supports the role of c-Jun in the induction of IL-6 as they show that inhibition of JNK (c-Jun N terminal kinase) by a drug significantly decreases IL-6 production after TLR7/9 stimulation in BM derived DCs (Mitchell et al., 2010). JNK acts directly upstream of c-Jun and activates c-Jun by phosphorylation (Ip & Davis, 1998). Regulation of IFN-β by c-Jun is also supported as (Panne, Maniatis, & Harrison, 2004) showed that a c-Jun/ATF-2 dimer binds in the promoter region of the *Ifnb* gene. Further experiments should be done to prove binding of c-Jun to the *Il-6* and *Ifnb* gene in pDCs and must also focus if c-Jun affects prominent signaling pathways in pDCs like

JAK/STAT or NF-κB signaling. Maturation of pDCs involves the up-regulation of molecules like CD8, CD40, CD86 and MHC class II (K. McKenna et al., 2005). To address if reduced levels of IFN-β and IL-6 impair the maturation of Imi stimulated pDCs *in vitro*, I analyzed the levels of these surface markers by FACS after 8 and 16 h of stimulation with Imi. Surprisingly, Imi stimulated pDCs from c-Jun deficient BM cultures matured similar to WT pDCs since no alteration were found in the surface marker expression of CD8α, CD40, CD86 and MHC class II. It is likely, that the maturation of pDCs, is coupled to IFNAR signaling, which involves binding of IFN-α and IFN-β to the IFNAR to activate the JAK/STAT signaling pathway. My results, however, indicate that IFN-α in the absence of IFN-β is sufficient for the maturation of pDCs. Support for this hypothesis is given by the fact that pDCs in contrast to other cell types are known for their ability to immediately produce large amounts of IFN-α upon stimulation. Crucially in pDCs this ability to quickly produce massive amounts of IFN-α is linked to the constitutive high expression of the most important regulator of IFN-α, namely IRF-7 (Gilliet et al., 2008). In other cells IRF-7 must first be induced after IFN-β has bound to the IFNAR (Gilliet et al., 2008). pDCs seem therefore to reflect a special case where the ability to produce massive amounts of IFN-α masks the loss of IFN-β during maturation, meaning that even though IFN-β is reduced the JAK/STAT signaling pathway should be activated normally. Since the RNA levels of *Mx1*, a gene which induction correlates to activation of the JAK/STAT are unchanged in c-Jun deficient and the loss of c-Jun does not seem to affect the production of IFN-α as the RNA levels of *Ifna1*, IFN-α subfamily member which expression correlates to the other family members, are normal, it is likely that IFN-α compensates for the reduced IFN-β levels in absence of c-Jun. Further experiments need to be performed to strengthen this hypothesis and should include an IFN-α ELISA and a Western Blot proving normal activation of the JAK/STAT pathway. In a last step I wanted to determine if a loss of c-Jun affects the tumor killing capacity of pDCs *in vitro.* Recently, Drobits et al. showed that pDCs kill tumor cells independently of adaptive immunity by up-regulation of cytolytic molecules like TRAIL and Gzmb in an IFNAR-dependant manner. I therefore

determined the RNA levels of *Trail* and *Gzmb* in pDCs stimulated with Imi for 4 h. Surprisingly, in the absence of c-Jun RNA levels of *Gzmb* are decreased. To test if this affects the tumor killing capacity of *c-Jun*$^{\Delta mx}$ pDCs I performed killing assays *in vitro*. However, loss of c-Jun did not affect the ability of pDCs to lyse B16 melanoma cells despite the reduced RNA levels of *Gzmb*. Since pDCs produce several different cytolytic molecules after Imi stimulation it is possible that the reduced Gzmb are compensated by other killing mechanism. So far it was not shown if and how Gzmb influences the cytolyltic capacities of pDCs. It is, however, known that pDCs do not produce perforin a pore forming molecule (Lord et al., 2003) which usually mediates entry of Gzmb into target cells. Further experiments will be needed to investigate these mechanisms and to find out if the observed reduced IFN-β levels are connected to the reduced Gzmb expression.

In summary I could show that c-Jun is dispensable for the development and maturation of pDCs *in vitro* but is needed for the induction of IL-6 and IFN-β. Further experiments will shed light if these results will alter Imi induced inflammation or tumor clearance *in vivo*. Preliminary results revealed that epidermal thickness or splenomegaly was not affected in *c-Jun*$^{\Delta mx}$ deficient mice treated topically with Aldara. However more experiments using mice that lack c-Jun specifically in pDCs will be necessary to investigate the role of c-Jun *in vivo.* In detail I would like to analyze the immune cell infiltrate of Imi treated skin. Moreover it will be interesting if mutant mice are able to resolve the induced skin inflammation similar to wild type mice. I would also like to further investigate the anti-tumor response of Imi in c-Jun deficient pDCs by inducing melanomas in mice and comparing the tumor growth and tumor infiltrates. It is likely that the reduced IFN-β and Gzmb levels have an impact in those mechanisms. Moreover in *c-Jun*$^{\Delta mx}$ mice c-Jun is deleted in all hematopoietic cells further experiments should employ a mouse model with a more specific deletion of c-Jun in pDCs. To understand the function and the underlying mechanism of the AP-1 proteins like c-Jun and JunB in pDCs might, help to develop new treatment strategies in diseases like psoriasis or cancer.

5. MATERIALS & METHODS

5.1. Reagents

5.1.1. Reagents for DNA isolation

- Tail buffer (50 mM Tris-HCl pH = 8; 100 mM EDTA; 100 mM NaCl; 1 % SDS; 0.5 mg/mL Proteinase K)
- 6 M NaCl, Isopropanol and 70 % ethanol
- TE (1 M Tris-HCl pH 8.0, 0.5 M EDTA)

5.1.2. Reagents to perform a Genotyping PCR

- Dimethylsulfoxide (DMSO)
- 10 x GB buffer (2 M Tris pH 8.8, 1 M $(NH_4)_2SO_4$, 0.5 M $MgCl_2$)
- 10 mM dNTP mix
- Tissue culture water & SERVA DNA stain G
- 1 kb DNA ladder (Nitrogen)

The following table lists the primers used to genotype transgenic mice

Table 1, PCR primer sequences

Primer	Sequence
c-Jun flox 2	5-CAGGGCGTTGTGTCACTGAGCT-3
c-Jun flox 5	5´-CTCATACCAGTTCGCACAGGCGC-3´
c-Jun flox 6	5´-CCGCTAGCACTCACGTTGGTAGC-3´
JunB-B1	5´ATCCTGCTGCTGGGAGC-GGGGAACTGAGGGAGG-3´
JunB-B6	5´-AGAGTCGTCGTGATAGAAAGGC-3´
JunB-B2	5´ GGGAACTGAGGGAAGCCACGCCGAGAAGC-3´
JunB-B10	5´-AAACATACAAAATACGCTGG-3´

5.1.3. Reagents needed for a FLT3L supplemented BM culture

- Fetal calf serum (FCS; PAA laboratories; Fetal calf serum for FLT3L supplemented BM cultures was tested for its usage to differentiate dendritic cells)
- Sodium pyruvate (1 mM; PAA)
- RPMI media 1640 (Invitrogen)
- L-glutamine (2 mM; PAA)
- Penicillin/streptomycin (100x, PAA)
- Non-essential amino acids (0.1 mM; PAA).
- β-Mercaptoethanol (50 mM; Gibco Life Technologies)
- FLT3L (Peprotech)
- ACK lysis buffer (155 mM NH_4Cl, $KHCO_3$ 10 mM, EDTA 0.1 mM)

5.1.4. Reagents to purify pDCs from FLT3L supplemented BM cultures

- Biotinylated mouse CD45R/B220 antibody (0.5 mg/ml)(BD biosience)
- Streptavidin coated beads (BD biosience)
- MACS buffer (1 x PBS, 2mM EDTA, BSA 0.5 %)

5.1.5. Reagents to isolate RNA

- TRIZOL (Invitrogen)
- 2-propanole
- 75 % ethanol
- RNAse free water

5.1.6. Reagents to remove DNA from RNA samples

- DNAse I (1 U/µL; Thermo Scientific)
- 10 x reaction buffer with $MgCl_2$ (100mM Tris-HCl, 1 mM $CaCl_2$, 25 mM $MgCl_2$; Thermo Scientific)
- 50 mM EDTA (Thermo Scientific)

5.1.7. Reagents for cDNA synthesis & RT-PCR

- M-MuLV Reaction buffer (50 mM Tris-HCl, 3 mM MgCl2, 75 mM KCl pH = 8.3; New England Biolabs)
- DTT (100 mM; New England Biolabs)
- dNTP mix (10 mM)
- Random Hexamer (50 µM; Invitrogen)
- M-MuLV reverse transcriptase (200.000 units per ml; New England Biolabs)
- SYBR Green (Applied Biosystems)

5.1.8. Primer sequences used for RT-PCR

IL-6 F

5´-GAG GAT ACC ACT CCC AAC AGA CC-3´

IL-6 R

5´-AAG TGC ATC ATC GTT GTT CAT ACA-3´

TNF-α F

5´-GAA CTG GCA GAA GAG GCA CT-3´

TNF-α R

5´-AGG GTC TGG GCC ATA GAA CT-3´

c-Jun F

5'-AAAACCTTGAAAGCGCAAAA-3'

c-Jun R

5'-CGCAACCAGTCAAGTTCTCA-3'

JunB F

5'-ATGTGCACGAAAATGGAACA-3'

JunB R

5'-CCTGACCCGAAAAGTAGCTG-3'

IFN-β F

5´-GACCTTTCAAATGCATGAGATTCA3´

IFN-β R

5´TCAGAATGAGTGGTGGTTGC3´

Ifna1 F

5´- AGT GAG CTG ACC CAG CAG AT-3´

Ifna1 R

5´- GGT GGA GGT CAT TGC AGA AT-3´

Mx1 F

5´-GAGCAAGTCTTCTTCAAGGATCA-3´

Mx1 R

5´-GGGAGGTGAGCTCCTCAGT-3´

TRAIL F

5´-GTGTCTGTGGCTGTGACTTACA-3´

TRAIL R

5'-AATGCCCTTTCCGAGAGGA-3´

Gzmb F

5´-ATTCCCCACCCAGACTATAATCC-3´

Gzmb R

5´-TTACTCTTCAGCTTTAGCAGCATGA-3‘

TBP F

5´GGGGAGCTGTGATGTGAAGT-3´

TBP R

5´ CCAGGAAATAATTCTGGCTCAT-3´

5.1.9. Reagents to perform a Western Blot

- RIPA buffer (150 mM NaCl, 0.1% SDS, 50 mM Tris, pH 8.0, Triton X-100, 0.5% sodium deoxycholate)
- Lysis buffer (1 mM sodium orthovanadate, 10 mM NaF, 1mM PMSF in RIPA buffer)
- Bradford mixture (1 part of Bradford reagence (Bio-RAD) diluted in 4 parts of distilled water).
- BSA bovine serum albumin
- 2 x Laemmli buffer (4% SDS, 10% mercaptoethan-2-ol, 0.04% bromo-phenol blue, 20% glycerol, 0.125 M Tris HCl and adjust pH to 6.8).
- Pre-stained Protein Ladder 10-250kDa (Thermo Scientific)
- Running buffer (25 mM Tris base, 190 mM glycine, 0.1% SDS adjust pH to 8.3).
- Ponceau red (1: 10 dilution of a stock containing 2% Ponceau S, 30% sulfo-salicylic acid and 30% tri-chloro-acetic acid).
- Transfer buffer (same as running buffer but with methanol at a concentration of 20 %).
- 10 x TBS (24.2 g Tris-HCl, 80.1 g NaCl adjust to a pH of 7.6 and bring to a final volume of 1000 ml).
- 1 x TBS-T (Dilute 10 x TBS in distilled water and add 0.01 % of Tween-20)
- Primary antibody (1:1000 dilution of c-Jun (rabbit; Cell Signaling Technology) and JunB (rabbit; Cell Signaling Technology) or 1:5000 dilution of Tubulin (mouse; Sigma Aldrich) in 5 % BSA.
- Secondary antibody (1:5000 dilution of horseradish peroxidase (HRP) with antibody specific for mouse or rabbit in 5 % milk; Jackson Laboratories)
- ECL reaction mixture (1:1 solution of ECL solution A and B from the GE healthcare ECL kit).

5.1.10. Reagents for FACS analysis

- FACS wash buffer (3 % fetal bovine serum in 1 x phosphate buffered saline).
- 10 x Fc-Block solution (1:50 dilution of Fcγ R III/II (0.5 mg per ml) blocking solution from Biolegend; Trade Name: TruStain fcX™ (anti-mouse CD16/32) Antibody).
- 10 x FACS staining mixture (Contains antibodies of the following table at a concentration of 10 µg per ml)

Table 2, Antibodies used for FACS analysis

FACS antibody	Company | Clone	Concentration
CD8 alpha-FITC	Bio Legend | 53-6.7	0.5
CD11b-Pb	Bio Legend | M1/70	0.5
CD11b-PECy7	Bio Legend | M1/70	0.2
CD11c-APC	Bio Legend | N418	0.5
CD11c-PE	Bio Legend | N418	0.2
CD11c-BD 605	Bio Legend | N418	0.2
CD40-PE	BD Biosciences | 3/23	0.2
CD86-PE	BD Biosciences | GL-1	0.2
CD115-PE	Bio Legend | AFS98	0.2
B220-APC	Bio Legend | RA3-6B2	0.2
B220-FITC	Bio Legend | RA3-6B2	0.5
B220-Texas Red	BD Biosciences | RA3-6B2	0.2
BST-2-PE	Bio Legend | 927	0.2
Siglec-H-FITC	Bio Legend |551	0.5
MHC-II-PECy7	Bio Legend | M5/114.15.2	0.5

5.1.11. Reagents to perform an ELISA

- Mouse IL-6 ELISA Kit from eBioscience
- Mouse TNF ELISA Kit from BD Biosciences
- Mouse IFN β ELISA Kit from Biolegend

Kits contained either stock solutions (concentrated wash buffer, coating antibody, HRP- antibody solution,) that were diluted according to the protocol sheet or contained ready to use solutions (assay diluent, substrate solution, stop solution). Standard was diluted from a standard stock solution according to the protocol sheet.

For a detailed description of the reagents look at the following links to the online protocols of the individual ELISAs performed.

IL-6: http://www.ebioscience.com/media/pdf/tds/88/88-7064.pdf

TNF: http://www.bdbiosciences.com/ptProduct.jsp?prodId=8448

IFN-β:

www.biolegend.com/media_assets/pro_detail/datasheets/439407.pdf

5.1.12. Reagents to perform a killing assay

- 0.1 % gelatine
- EZ4U Cell Proliferation Assay (Biomedica; Kit contains SUB and Activator)

5.1.13. Reagents to perform an H&E staining

- Xylol
- Ethanol (100 %, 96 %, 70 %)
- Haematoxylin
- Eosin
- Scott´s blueing solution
- Entellan

5.2. Mouse strains

c-Jun $^{f/f}$ (Behrens et al., 2002), *JunB* $^{f/f}$ (Kenner et al., 2004) and *c-Jun/JunB* $^{f/f}$ mx cre mice were used. In mx cre mice the Cre recombinase is under control of the IFN-α/β inducible Mx1 promoter (Kühn et al., 1995). Mx1 transcription can also be initiated with poly I: C. It is therefore possible to conditionally delete the gene of interest by injection of poly I: C into mice. Two different approaches were employed to delete the gene of interest. For the *c-Jun/JunB* $^{f/f}$ mx cre mice poly I: C was injected once into 7 weeks old mice. 5 days after injection the mice were used to isolate the BM. For the *c-Jun* $^{f/f}$ and *JunB* $^{f/f}$ mx cre mice poly I: C was injected twice into 7 week old mice. After the second injection the mice were left for 12 days before BM was isolated. The second injection was given 5 days after the first.

5.3. DNA isolation for genotyping

0.5 cm tail of a 5 - 12 day old mouse was given to 500 µl of Tail buffer and incubated at 55 °C o/n. On the next day 200 µl of 6 M NaCl were added. The sample was shaken and then centrifuged for 15 minutes at 13.3 rpm. After centrifugation the supernatant was taken and transferred to a new tube. 500 µl of isopropanol were added and the sample was then centrifuged for another 10 minutes at 13.3 rpm. Afterwards the pellet was washed with 1 ml of 70 % ethanol and re-suspended in 150 µL of TE. To dissolve the pellet the sample was incubated at 37 °C for 2h. For a genotyping PCR 2 µl were taken.

5.4. c- Jun/ JunB Genotyping PCR

For one PCR reaction a reaction mixture was prepared according to Table 3.

Table 3, PCR reaction mixture

Reagent	Volume
10 x GB buffer	2.5 µL
DMSO	2.5 µL
Primer 1 (1.2 µM)	2.5 µL
Primer 2 (1.2 µM)	2.5 µL
dNTP mix (10 mM)	2.5 µL
Tissue culture water	11.2 µL
Taq polymerase	0.3
Sample	2 µL

To determine the genotype of *c-Jun*$^{f/f}$ and *c-Jun*$^{\Delta mx}$ mice c-Jun flox2, c-Jun flox5 and c-Jun flox6 primers were used (see Table 3). As this PCR reaction includes 3 primer pairs the amount of water was reduced from 11.2 µl (see Table 3) to 8.7 µL. To differ between *JunB*$^{f/f}$ and wild type mice JunB-B1 and JunB-B6 primers (see Table 3) were used. Lastly, to differ between *JunB*$^{f/f}$ and *JunB*$^{\Delta mx}$ mice JunB-B2 and JunB-B10 (seeTable 3) were used as primers. The thermal cycler conditions were 94°C for 2 min, 40 x (94 °C- 30 sec, 60 °C- 40 sec, 65 °C- 1 min 30 sec), 65 °C for 5 minutes and 4 °C forever. The PCR product was run on a 2.5 % agarose gel, stained with SERVA (5 µL/100 ml).

5.5. B16 cell line

B16 melanoma cells were cultured in a B16 cell culture medium (see Table 4) at 37 °C and 5 % CO_2. The B16 cell line is derived from the skin of a mouse with a C57BL/6 background.

Table 4, DC and B16 culture medium composition

Reagent	Concentration
RPMI media 1640	84.5 %
Fetal calf serum	10 %
Sodium pyruvate (1 mM)	1 %
Penicillin/streptomycin (100x)	1 %
L-glutamine (2mM)	1 %
Non-essential amino acids (0.1 mM)	1 %
β-Mercaptoethanol (50 mM)	0.5%

5.6. Isolation of BM cells and culture in FLT3L supplemented BM culture

The tibia and femur of mice were taken. The ends of the bones were cut and BM cells were washed out with DC cell culture medium (see Table 4) in a 10 cm² dish. The cell suspension was pipetted through a 70 µm filter and centrifuged at 4 °C for 7 min at 1.200 rpm. The pellet was re-suspended in 2 ml ACK lysis buffer for 1 minute to remove red blood cells. The reaction was stopped with 8 ml DC cell culture medium. 50 µL were taken to determine the concentration of cells/ml with a CASY COUNT. Cells were then centrifuged for 7 minutes at 1.200 rpm and re-suspended in a DC cell culture medium at a concentration of 7.5 x 10^6 cells/ ml. Cells were seeded at a concentration of 3 x 10^6 cells per ml in a total volume of 3 ml with the addition of FLT3L at a concentration of 80 ng per ml.

5.7. Isolation of pDCs from FLT3L supplemented BM cultures

BM cells were cultured for 6, 7 and 8 days in an FLT3L supplemented BM culture. On day 6, 7 or 8 cells were harvested, pelleted (1.200 rpm; 7

minutes) and re-suspended in 300 µL of 3 % FCS/PBS. 3 µl (1:100) of a biotin conjugated B220 antibody were added. An incubation time of 20 minutes followed (on ice). The incubation was stopped with 5 ml MACS buffer. Cells were then pelleted (7 min, 1.200 rpm) again. The supernatant was discarded and cells were re-suspended in 100 µL of streptavidin coated beads. An incubation of 30 minutes at 6°-12°C followed. Incubation was stopped by the addition of 1mL MACS buffer. pDCs were now sorted in a positive selection ($B220^+$ cells). For that the tube containing the cells was placed in a BD IMagnet unit and incubated sequentially for 6, 3 and 3 minutes. After the first and second time-point the supernatant was aspirated and cells were re-suspended in 1 ml MACS buffer. Following the last incubation time-point cells were re-suspended in 1ml DC cell culture medium. 10 µL of the solution were taken to determine the concentration by CASY COUNT. Cells were peletted (7 min, 1.200 min) and re-suspended in the desired concentration. Success of sorting was evaluated by FACS analysis. pDCs were defined as $B220^+$, $CD11c^+$ and $CD11b^-$ cells. Further experiments were carried out only if the sorting yielded a purity of above 80 %.

5.8. Isolation of RNA

For RNA analysis purified pDCs were seeded at a concentration of 1×10^6 cells per 200µL in a 96 well round bottom plate. pDCs were stimulated for 4 h by adding Imi in a final concentration of 2.5 µg per ml . After the 4h passed cells were pelleted for 7 min at 1.200 rpm at 4°C. The supernatant was withdrawn and cells were washed once with ice-cold PBS. Cells were pelleted again (7 min, 1.200 rpm at 4 °C) and re-suspended in 500 µL TRIZOL. Cells were homogenized and incubated for 5 minutes at room temperature. 100 µL of chloroform were added and samples were shaken by hand for 15 seconds. An incubation time of 5 minutes followed. After that a centrifugation step (15 minutes, 11.000 rpm, 4 °C) was done. 150 µL of the clear supernatant were taken and given to 250 µL of isopropanol. An

incubation time of 5 minutes followed. After that a centrifugation step (10 minutes, 11.000 rpm, 4°C) was done. The pellet was washed twice with 75 % ethanol. Centrifugation was done at 11.000 rpm for 5 minutes at 4 °C. The pellet was re-suspended in 10 µL of RNAse free water.

5.9. Removing DNA contaminations from RNA samples

RNA concentration was determined with a Nano Droplet. 1µg of RNA were taken and given to the DNAse digestion mixture (see Table 5). The mixture was incubated for 30 minutes at 37 °C. The reaction was stopped through the addition of 1 µl 50 mM EDTA and heating up to 65 °C for 10 minutes.

Table 5, Preparation of the DNAse digestion mixture

Reagent	Volume
DNAse I	1 µl
10 x reaction buffer with $MgCl_2$	1µl
RNA	1µg
RNase free water	To 10 µl

5.10. Making cDNA from RNA samples

500 ng of RNA were used for cDNA synthesis according to Table 6.

Table 6, Preparation of the reaction mixture for cDNA synthesis

Reagent	Volume
M-MuLV Reaction buffer	4µl
DTT	2 µl
Random hexamer	2 µl
10 mM dNTP mix	1 µl
M-MuLV Reverse transcriptase	0.75 µl
RNase free water	To 20 µl

The reaction mixture was then incubated at 10 minutes at RT, for 50 minutes at 42 °C and at 10 minutes at 70 °C. After that 30 µL of RNAse free water were added giving a total volume of 50 µL. A RT-PCR was carried out according to Table 7. Data were acquired on an ABI7500 (Applied Biosystem).

Table 7, Reaction mixture used to perform a RT-PCR.

Reagent	Volume
Sample	1.5 µl
Forward Primer 5 µM	1µl
Reverse Primer 5 µm	1 µl
SYBR Green	10 µl
Tissue culture H2O	To 20 µl

5.11. Performing a Western Blot from protein extracts

To get protein extracts cells were lysed with lysis buffer for 10 minutes on ice. After the 10 minutes a centrifugation step followed (5 minutes, 13.3 rpm, 4°C). The supernatant was taken and the pellet containing the cell debris was discarded. To determine the protein concentration a Bradford assay was performed. For that 1 µl of the supernatant was given to 200 µL of Bradford mixture. The color change was determined by a Mithras Plate Reader (measuring wavelength: 595 nm). BSA was used as a standard. After the protein concentration was determined 25-40 µg of protein were diluted 1:1 with a 2 x Laemmli buffer and incubated at 95°C for 5 minutes to denature the protein. The sample was then loaded onto a 7.5 or 10 % SDS-PAGE gel. As a molecular weight marker a pre-stained protein marker (10-250 kDa) was taken. First the gel was run at 80 V (in stacking gel) and then at 140 V (separating gel) until the end of the gel was reached. Protein bands were then transferred from the gel onto a PVDF membrane in a western blot

chamber (run at 400 mA for 2 hours). Success of transfer was checked with Ponceau red. The membrane was then blocked in a 5 % BSA solution for 30 minutes. The primary antibody was then added to the membrane (o/n at 4 °C). Three wash steps with TBS-T (10 minutes each) were then performed to wash away unspecific bound antibody. Afterwards the secondary antibody (HRP-mouse/rat) was given to the membrane for an incubation time of 1 hour. Again three wash steps with TBS-T followed (10 minutes each). Lastly, protein bands were made visible with an ECL reaction mixture (membrane was incubated for 1 minute) resulting in a chemiluminiscent reaction that darkened an x-ray film.

5.12. FACS analysis of cell surface markers

For FACS analysis cells were first washed with FACS wash buffer. Cells were then pelleted (7 min, 300g, 4 °C) and re-suspended in 45 µl FACS wash buffer and 5 µl of an Fc-Block solution was added. After a 10 minute incubation time 5 µl of a FACS staining mixture was added. After 30 minutes the cells were washed twice with FACS wash buffer as described above. Stained cells were analyzed with an LSR II flow cytometer (BD Biosciences). FlowJo (Treestar) was used to analyze the data.

5.13. ELISA of IL-6, TNF-α and IFN-β

5 x 10^5 purified pDCs were seeded in a 96-well plate in 200 µL of DC culture medium and stimulated (or not) with Imi for 8 or 16 h. The supernatant of these cells were taken for an ELISA against IL-6, IFN-β and TNF-α. Prior to use the supernatant of 8 h stimulated pDCs was diluted 1:20 and the supernatant of 16 h stimulated pDCs was diluted 1:40 with assay diluent.

IL-6 and TNF-alpha

For a detailed description follow the links to the online protocols.
IL-6: http://www.ebioscience.com/media/pdf/tds/88/88-7064.pdf
TNF: http://www.bdbiosciences.com/ptProduct.jsp?prodId=8448
In short a 96 well corning costar plate was coated o/n with coating buffer. A wash step followed after which the wells were blocked with assay diluent for 1h at room temperature. Then 100 µl of sample or standard were added to the wells and an incubation time of 2 h followed. After a wash step 100 µl of Avidin-HRP solution was added. An incubation time of 30 minutes followed. After a wash step 100 µl of substrate solution was given to the wells. Another 15 minutes later 50 µl of stop solution were added. The color change was determined with a Mithras Plate Reader (measuring wavelength: 450 nm; reference wavelength: 570 nm).

Interferon beta

For a detailed description follow the link to the online protocol. IFN-β: www.biolegend.com/media_assets/pro_detail/datasheets/439407.pdf
In short a pre-coated plate is washed and 100 µl of sample or standard are added to the wells. An incubation at RT for 2h followed. After another wash step 100 µl Avidin-HRP solution were added and the plate was then incubated at RT for 30 minutes. A last wash step was done after which 100 µl of substrate solution was added. The reaction was stopped after 15 minutes by the addition of 100 µl stop solution. The color change was determined with a Mithras Plate Reader (measuring wavelength: 450 nm; reference wavelength: 570 nm).

5.14. Killing assay

A 96-well plate was coated with 0.1 % gelatin for 1 h. B16 melanoma cells were seeded on the coated plate at a concentration of 1 x 10^5 cells per ml for 4 h (0.5 x 10^4 cells per 50 µL per well) before pDCs were added. pDCs

were purified on day 7 of FLT3L supplemented BM culture and stimulated with Imi (2.5 mg/ml) for 16 h. pDCs were then harvested and centrifuged for 5 minutes at 2.220 rpm. The supernatant was collected and the pDC cell pellet was re-suspended at a concentration of 1 x 10^6 cells per ml. 50 µl of supernatant or of the re-suspended pDCs were given to B16 cells according to Table 8. Controls were also done for the assay (see Table 8). The final volume was brought to 200 µl with DC culture medium and an incubation time of 20h followed. After the 20 h of incubation cell lysis was determined with the EZ4U nonradioactive cell proliferation assay (Biomedica). In this assay the nonradioactive dye (SUB) is dissolved in 2.5 ml ACT solution (activator). 20 µL of this solution were given to 200 µL sample and left for incubation for 2h. After the incubation time passed the color change of the nonradioactive dye was determined with a Mithras Plate Reader (measuring wavelength: 450 nm and reference wavelength was 630 nm). Cell lysis of B16 cells was calculated according to the formula in Table 9.

Table 8, Experimental set-up to perform a killing assay

First compound	Second compound
B16 cells	Un-stimulated pDC
B16 cells	Imi stimulated pDC
B16 cells	-
B16 cells	Un-stimulated pDC supernatant
B16 cells	Imi stimulated pDC supernatant
B16 cells	Imi 2.5 µg per ml
B16 cells	Imi 5 µg per ml
-	Unstimulated pDCs
-	Imi stimulated pDCs
-	DC culture medium

Table 9, Formula to calculate B16 cell lysis

$$\%\ \text{lysis} = \left(1 - \frac{\text{Abs. cocultured cells} - \text{Abs. of effector cells}}{\text{Abs. of target cells}}\right) * 100$$

Co-cultured cells =	pDCs + B16 cells
Effector cells =	pDCs alone
Target cells =	B16 cells alone

5.15. Epidermal thickness & Splenomegaly

Mice were shaved on the back and creamed with Aldara for 7 days or left untreated (control). After these 7 days the mice and their spleen were weighed. Furthermore skin from mice was taken and fixed in 4 % para-formaldehyde. Fixed tissue was then embedded in paraffin and cut into 5 µm sections with a microtome. Cut sections were then deparaffinized. To do so, the sample was consecutively placed into xylol and ethanol solutions (100%, 100 %, 70 %, 70 %) for 1 minute. After that the sections were stained with haematoxylin (4 min), blued in Scott´s blueing solution (5 sec) and counterstained with eosin (1 min). The stained sections were then dehydrated with ethanol (70 %, 96 %, 96 %, 100 %, 100 %; one minute each). Entellan was used to mount the sections. Images of stained sections were taken with a Nikon eclipse 80i. The epidermal thickness in the images was determined with Adobe Photoshop CS6.

6. APPENDIX

6.1. List of Figures

6.2. List of Tables

6.3. References

Aliberti, J., Schulz, O., Pennington, D. J., Tsujimura, H., Reis e Sousa, C., Ozato, K., & Sher, A. (2003). Essential role for ICSBP in the in vivo development of murine CD8alpha + dendritic cells. *Blood*, *101*(1), 305–10. doi:10.1182/blood-2002-04-1088

Alvarez, D., Vollmann, E. H., & von Andrian, U. H. (2008). Mechanisms and consequences of dendritic cell migration. *Immunity*, *29*(3), 325–42. doi:10.1016/j.immuni.2008.08.006

Arai, K. I., Lee, F., Miyajima, A., Miyatake, S., Arai, N., & Yokota, T. (1990). Cytokines: coordinators of immune and inflammatory responses. *Annual review of biochemistry*, *59*, 783–836. doi:10.1146/annurev.bi.59.070190.004031

Asselin-Paturel, C., Brizard, G., Chemin, K., Boonstra, A., O'Garra, A., Vicari, A., & Trinchieri, G. (2005). Type I interferon dependence of plasmacytoid dendritic cell activation and migration. *The Journal of experimental medicine*, *201*(7), 1157–67. doi:10.1084/jem.20041930

Banchereau, J., & Steinman, R. M. (1998). Dendritic cells and the control of immunity. *Nature*, *392*(6673), 245–52. doi:10.1038/32588

Bannister, A., Oehler, T., Wilhelm, D., Angel, P., & Kouzarides, T. (1995). Stimulation of c-Jun activity by CBP: c-Jun residues Ser63/73 are required for CBP induced stimulation in vivo and CBP binding in vitro. *Oncogene*, *11*(12), 2509–2514. Retrieved from http://espace.library.uq.edu.au/view/UQ:249826

Behrens, A., Sibilia, M., David, J.-P., Möhle-Steinlein, U., Tronche, F., Schütz, G., & Wagner, E. F. (2002). Impaired postnatal hepatocyte proliferation and liver regeneration in mice lacking c-jun in the liver. *The EMBO journal*, *21*(7), 1782–90. doi:10.1093/emboj/21.7.1782

Beutler, B., & Rietschel, E. T. (2003). Innate immune sensing and its roots: the story of endotoxin. *Nature reviews. Immunology*, *3*(2), 169–76. doi:10.1038/nri1004

Bevan, M. J. (2006). Cross-priming. *Nature immunology*, *7*(4), 363–5. doi:10.1038/ni0406-363

Bosschaerts, T., Guilliams, M., Stijlemans, B., Morias, Y., Engel, D., Tacke, F., … Beschin, A. (2010). Tip-DC development during parasitic infection is regulated by IL-10 and requires CCL2/CCR2, IFN-gamma and MyD88 signaling. *PLoS pathogens*, *6*(8), e1001045. doi:10.1371/journal.ppat.1001045

Burger, R., Bader, A., Kirschfink, M., Rother, U., Schrod, L., Wörner, I., & Zilow, G. (1987). Functional analysis and quantification of the complement C3 derived anaphylatoxin C3a with a monoclonal antibody. *Clinical and experimental immunology*, *68*(3), 703–11. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1542768&tool=pmcentrez&rendertype=abstract

Cisse, B., Caton, M. L., Lehner, M., Maeda, T., Scheu, S., Locksley, R., … Reizis, B. (2008). Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell*, *135*(1), 37–48. doi:10.1016/j.cell.2008.09.016

Colonna, M., Trinchieri, G., & Liu, Y.-J. (2004). Plasmacytoid dendritic cells in immunity. *Nature immunology*, *5*(12), 1219–26. doi:10.1038/ni1141

Dempsey, P. W., Vaidya, S. A., & Cheng, G. (2003). The art of war: Innate and adaptive immune responses. *Cellular and molecular life sciences : CMLS*, *60*(12), 2604–21. doi:10.1007/s00018-003-3180-y

Drobits, B., Holcmann, M., Amberg, N., Swiecki, M., Grundtner, R., Hammer, M., … Sibilia, M. (2012). Imiquimod clears tumors in mice independent of adaptive immunity by converting pDCs into tumor-killing effector cells. *The Journal of clinical investigation*, *122*(2), 575–85. doi:10.1172/JCI61034

Eferl, R., Ricci, R., Kenner, L., Zenz, R., David, J.-P., Rath, M., & Wagner, E. F. (2003). Liver tumor development. c-Jun antagonizes the proapoptotic activity of p53. *Cell*, *112*(2), 181–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12553907

Foletta, V., Segal, D., & Cohen, D. (1998). Transcriptional regulation in the immune system: all roads lead to AP-1. *J. Leukoc. Biol.*, *63*(2), 139–152. Retrieved from http://www.jleukbio.org/content/63/2/139.abstract

Gilliet, M., Cao, W., & Liu, Y.-J. (2008). Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nature reviews. Immunology*, *8*(8), 594–606. doi:10.1038/nri2358

Goubier, A., Dubois, B., Gheit, H., Joubert, G., Villard-Truc, F., Asselin-Paturel, C., … Kaiserlian, D. (2008). Plasmacytoid dendritic cells mediate oral tolerance. *Immunity*, *29*(3), 464–75. doi:10.1016/j.immuni.2008.06.017

Helft, J., Ginhoux, F., Bogunovic, M., & Merad, M. (2010). Origin and functional heterogeneity of non-lymphoid tissue dendritic cells in mice. *Immunological reviews*, *234*(1), 55–75. doi:10.1111/j.0105-2896.2009.00885.x

Hess, J., Angel, P., & Schorpp-Kistner, M. (2004). AP-1 subunits: quarrel and harmony among siblings. *Journal of cell science*, *117*(Pt 25), 5965–73. doi:10.1242/jcs.01589

Hilberg, F., Aguzzi, A., Howells, N., & Wagner, E. F. (1993). c-jun is essential for normal mouse development and hepatogenesis. *Nature*, *365*(6442), 179–81. doi:10.1038/365179a0

Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., Mizutani, T., … Taniguchi, T. (2005). IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature*, *434*(7034), 772–7. doi:10.1038/nature03464

Ip, Y. T., & Davis, R. J. (1998). Signal transduction by the c-Jun N-terminal kinase (JNK) — from inflammation to development. *Current Opinion in Cell Biology*, *10*(2), 205–219. doi:10.1016/S0955-0674(98)80143-9

Jego, G., Palucka, A. K., Blanck, J.-P., Chalouni, C., Pascual, V., & Banchereau, J. (2003). Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity*, *19*(2), 225–34. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12932356

Kallunki, T., Deng, T., Hibi, M., & Karin, M. (1996). c-Jun Can Recruit JNK to Phosphorylate Dimerization Partners via Specific Docking Interactions. *Cell*, *87*(5), 929–939. doi:10.1016/S0092-8674(00)81999-6

Kenner, L., Hoebertz, A., Beil, F. T., Beil, T., Keon, N., Karreth, F., … Wagner, E. F. (2004). Mice lacking JunB are osteopenic due to cell-autonomous osteoblast and osteoclast defects. *The Journal of cell biology*, *164*(4), 613–23. doi:10.1083/jcb.200308155

Korn, T., Bettelli, E., Oukka, M., & Kuchroo, V. K. (2009). IL-17 and Th17 Cells. *Annual review of immunology*, *27*, 485–517. doi:10.1146/annurev.immunol.021908.132710

Kühn, R., Schwenk, F., Aguet, M., & Rajewsky, K. (1995). Inducible gene targeting in mice. *Science (New York, N.Y.)*, *269*(5229), 1427–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7660125

Landschulz, W. H., Johnson, P. F., & McKnight, S. L. (1988). The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science (New York, N.Y.)*, *240*(4860), 1759–64. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3289117

LENNERT, K., & REMMELE, W. (1958). [Karyometric research on lymph node cells in man. I. Germinoblasts, lymphoblasts & lymphocytes]. *Acta haematologica*, *19*(2), 99–113. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/13520253

Liu, K., & Nussenzweig, M. C. (2010). Origin and development of dendritic cells. *Immunological reviews*, *234*(1), 45–54. doi:10.1111/j.0105-2896.2009.00879.x

Liu, K., Victora, G. D., Schwickert, T. A., Guermonprez, P., Meredith, M. M., Yao, K., … Nussenzweig, M. (2009). In vivo analysis of dendritic cell development and homeostasis. *Science (New York, N.Y.)*, *324*(5925), 392–7. doi:10.1126/science.1170540

Liu, Z., Liu, Q., Hamed, H., Anthony, R. M., Foster, A., Finkelman, F. D., … Gause, W. C. (2005). IL-2 and autocrine IL-4 drive the in vivo development of antigen-specific Th2 T cells elicited by nematode parasites. *Journal of immunology (Baltimore, Md. : 1950)*, *174*(4), 2242–9. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1978543&tool=pmcentrez&rendertype=abstract

Lord, S. J., Rajotte, R. V, Korbutt, G. S., & Bleackley, R. C. (2003). Granzyme B: a natural born killer. *Immunological reviews*, *193*, 31–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12752668

Masumi, A. (2013). *Stem Cell Biology in Normal Life and Diseases*. (K. Alimoghaddam, Ed.). InTech. doi:10.5772/46173

McKenna, H. J., Stocking, K. L., Miller, R. E., Brasel, K., De Smedt, T., Maraskovsky, E., … Peschon, J. J. (2000). Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood*, *95*(11), 3489–3497. Retrieved from http://bloodjournal.hematologylibrary.org/content/95/11/3489.full

McKenna, K., Beignon, A.-S., & Bhardwaj, N. (2005). Plasmacytoid dendritic cells: linking innate and adaptive immunity. *Journal of virology*, *79*(1), 17–27. doi:10.1128/JVI.79.1.17-27.2005

Meixner, A., Zenz, R., Schonthaler, H. B., Kenner, L., Scheuch, H., Penninger, J. M., & Wagner, E. F. (2008). Epidermal JunB represses G-CSF transcription and affects

haematopoiesis and bone formation. *Nature cell biology*, *10*(8), 1003–11. doi:10.1038/ncb1761

Merad, M., Ginhoux, F., & Collin, M. (2008). Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nature reviews. Immunology*, *8*(12), 935–47. doi:10.1038/nri2455

Merad, M., & Manz, M. G. (2009). Dendritic cell homeostasis. *Blood*, *113*(15), 3418–27. doi:10.1182/blood-2008-12-180646

Mitchell, D., Yong, M., Schroder, W., Black, M., Tirrell, M., & Olive, C. (2010). Dual stimulation of MyD88-dependent Toll-like receptors induces synergistically enhanced production of inflammatory cytokines in murine bone marrow-derived dendritic cells. *The Journal of infectious diseases*, *202*(2), 318–29. doi:10.1086/653499

Naik, S. H. (2008). Demystifying the development of dendritic cell subtypes, a little. *Immunology and Cell Biology*, *86*(5), 439–452. doi:10.1038/icb.2008.28

Naik, S. H., O'Keeffe, M., Proietto, A., Shortman, H. H. K., & Wu, L. (2010). CD8+, CD8-, and plasmacytoid dendritic cell generation in vitro using flt3 ligand. *Methods in molecular biology (Clifton, N.J.)*, *595*, 167–76. doi:10.1007/978-1-60761-421-0_10

O'Keeffe, M., Grumont, R. J., Hochrein, H., Fuchsberger, M., Gugasyan, R., Vremec, D., … Gerondakis, S. (2005). Distinct roles for the NF-kappaB1 and c-Rel transcription factors in the differentiation and survival of plasmacytoid and conventional dendritic cells activated by TLR-9 signals. *Blood*, *106*(10), 3457–64. doi:10.1182/blood-2004-12-4965

Ochando, J. C., Homma, C., Yang, Y., Hidalgo, A., Garin, A., Tacke, F., … Bromberg, J. S. (2006). Alloantigen-presenting plasmacytoid dendritic cells mediate tolerance to vascularized grafts. *Nature immunology*, *7*(6), 652–62. doi:10.1038/ni1333

Onai, N., Kurabayashi, K., Hosoi-Amaike, M., Toyama-Sorimachi, N., Matsushima, K., Inaba, K., & Ohteki, T. (2013). A Clonogenic Progenitor with Prominent Plasmacytoid Dendritic Cell Developmental Potential. *Immunity*, *38*(5), 943–957. doi:10.1016/j.immuni.2013.04.006

Palamara, F., Meindl, S., Holcmann, M., Lührs, P., Stingl, G., & Sibilia, M. (2004). Identification and characterization of pDC-like cells in normal mouse skin and melanomas treated with imiquimod. *Journal of immunology (Baltimore, Md. : 1950)*, *173*(5), 3051–61. Retrieved from http://www.jimmunol.org/content/173/5/3051.full

Panne, D., Maniatis, T., & Harrison, S. C. (2004). Crystal structure of ATF-2/c-Jun and IRF-3 bound to the interferon-beta enhancer. *The EMBO journal*, *23*(22), 4384–93. doi:10.1038/sj.emboj.7600453

Passegué, E., Jochum, W., Schorpp-Kistner, M., Möhle-Steinlein, U., & Wagner, E. F. (2001). Chronic Myeloid Leukemia with Increased Granulocyte Progenitors in Mice Lacking JunB Expression in the Myeloid Lineage. *Cell*, *104*(1), 21–32. doi:10.1016/S0092-8674(01)00188-X

Pelto-Huikko, M., Dagerlind, A., Kononen, J., Lundberg, J. M., Villar, M., Koistinaho, J., … Hökfelt, T. (1995). Neuronal regulation of c-fos, c-jun, and junB immediate-early genes in rat adrenal medulla. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *15*(3 Pt 1), 1854–68. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7891139

Platanias, L. C. (2005). Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nature reviews. Immunology*, *5*(5), 375–86. doi:10.1038/nri1604

Proffitt, J., Crabtree, G., Grove, M., Daubersies, P., Bailleul, B., Wright, E., & Plumb, M. (1995). An ATF/CREB-binding site is essential for cell-specific and inducible transcription of the murine MIP-1 beta cytokine gene. *Gene*, *152*(2), 173–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7835696

Reizis, B., Colonna, M., Trinchieri, G., Barrat, F., & Gilliet, M. (2011). Plasmacytoid dendritic cells: one-trick ponies or workhorses of the immune system? *Nature reviews. Immunology*, *11*(8), 558–65. doi:10.1038/nri3027

Riera-Sans, L., & Behrens, A. (2007). Regulation of alphabeta/gammadelta T cell development by the activator protein 1 transcription factor c-Jun. *Journal of immunology (Baltimore, Md. : 1950)*, *178*(9), 5690–700. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17442952

Robertson, S. E., Young, J. D., Kitson, S., Pitt, A., Evans, J., Roes, J., … McInnes, I. B. (2006). Expression and alternative processing of IL-18 in human neutrophils. *European journal of immunology*, *36*(3), 722–31. doi:10.1002/eji.200535402

Rowe, J. H., Ertelt, J. M., & Way, S. S. (2012). Foxp3(+) regulatory T cells, immune stimulation and host defence against infection. *Immunology*, *136*(1), 1–10. doi:10.1111/j.1365-2567.2011.03551.x

Runkel, L. (1998). Differences in Activity between alpha and beta Type I Interferons Explored by Mutational Analysis. *Journal of Biological Chemistry*, *273*(14), 8003–8008. doi:10.1074/jbc.273.14.8003

Sallusto, F., & Lanzavecchia, A. (1994). Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *The Journal of experimental medicine*, *179*(4), 1109–18. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2191432&tool=pmcentrez&rendertype=abstract

Schreiber, M., Kolbus, A., Piu, F., Szabowski, A., Möhle-Steinlein, U., Tian, J., … Wagner, E. F. (1999). Control of cell cycle progression by c-Jun is p53 dependent. *Genes & development*, *13*(5), 607–19. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=316508&tool=pmcentrez&rendertype=abstract

Schütte, J., Viallet, J., Nau, M., Segal, S., Fedorko, J., & Minna, J. (1989). jun-B inhibits and c-fos stimulates the transforming and trans-activating activities of c-jun. *Cell*, *59*(6), 987–97. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2513129

Shaulian, E., & Karin, M. (2002). AP-1 as a regulator of cell life and death. *Nature cell biology*, *4*(5), E131–6. doi:10.1038/ncb0502-e131

Shortman, K., & Naik, S. H. (2007). Steady-state and inflammatory dendritic-cell development. *Nature reviews. Immunology*, *7*(1), 19–30. doi:10.1038/nri1996

Siegal, F. P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P. A., Shah, K., Ho, S., … Liu, Y. J. (1999). The nature of the principal type 1 interferon-producing cells in human

blood. *Science (New York, N.Y.)*, *284*(5421), 1835–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10364556

Sozzani, S., Vermi, W., Del Prete, A., & Facchetti, F. (2010). Trafficking properties of plasmacytoid dendritic cells in health and disease. *Trends in immunology*, *31*(7), 270–7. doi:10.1016/j.it.2010.05.004

Stary, G., Klein, I., Kohlhofer, S., Koszik, F., Scherzer, T., Müllauer, L., … Stingl, G. (2009). Plasmacytoid dendritic cells express TRAIL and induce CD4+ T-cell apoptosis in HIV-1 viremic patients. *Blood*, *114*(18), 3854–63. doi:10.1182/blood-2009-04-217927

Steinman, R M, & Cohn, Z. A. (1973). Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *The Journal of experimental medicine*, *137*(5), 1142–62. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2139237&tool=pmcentrez&rendertype=abstract

Steinman, Ralph M, & Banchereau, J. (2007). Taking dendritic cells into medicine. *Nature*, *449*(7161), 419–26. doi:10.1038/nature06175

Stepniak, E., Ricci, R., Eferl, R., Sumara, G., Sumara, I., Rath, M., … Wagner, E. F. (2006). c-Jun/AP-1 controls liver regeneration by repressing p53/p21 and p38 MAPK activity. *Genes & development*, *20*(16), 2306–14. doi:10.1101/gad.390506

Suzuki, S., Honma, K., Matsuyama, T., Suzuki, K., Toriyama, K., Akitoyo, I., … Kumatori, A. (2004). Critical roles of interferon regulatory factor 4 in CD11bhighCD8alpha- dendritic cell development. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(24), 8981–6. doi:10.1073/pnas.0402139101

Tait, S. W. G., & Green, D. R. (2010). Mitochondria and cell death: outer membrane permeabilization and beyond. *Nature reviews. Molecular cell biology*, *11*(9), 621–32. doi:10.1038/nrm2952

Takeuchi, O., & Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell*, *140*(6), 805–20. doi:10.1016/j.cell.2010.01.022

Vogt, P. K. (2002). Fortuitous convergences: the beginnings of JUN. *Nature reviews. Cancer*, *2*(6), 465–9. doi:10.1038/nrc818

Wang, S., & El-Deiry, W. S. (2003). TRAIL and apoptosis induction by TNF-family death receptors. *Oncogene*, *22*(53), 8628–8633. doi:10.1038/sj.onc.1207232

Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature*, *415*(6870), 389–95. doi:10.1038/415389a

Zenz, R., Eferl, R., Kenner, L., Florin, L., Hummerich, L., Mehic, D., … Wagner, E. F. (2005). Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. *Nature*, *437*(7057), 369–75. doi:10.1038/nature03963

Zenz, R., Eferl, R., Scheinecker, C., Redlich, K., Smolen, J., Schonthaler, H. B., … Wagner, E. F. (2008). Activator protein 1 (Fos/Jun) functions in inflammatory bone and skin disease. *Arthritis research & therapy*, *10*(1), 201. doi:10.1186/ar2338

Zenz, R., Scheuch, H., Martin, P., Frank, C., Eferl, R., Kenner, L., … Wagner, E. F. (2003). c-Jun regulates eyelid closure and skin tumor development through EGFR signaling. *Developmental cell*, *4*(6), 879–89. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12791272

Zhang, N., & Bevan, M. J. (2011). CD8(+) T cells: foot soldiers of the immune system. *Immunity*, *35*(2), 161–8. doi:10.1016/j.immuni.2011.07.010

Zhu, J., & Paul, W. E. (2008). CD4 T cells: fates, functions, and faults. *Blood*, *112*(5), 1557–69. doi:10.1182/blood-2008-05-078154

Zou, W., Machelon, V., Coulomb-L'Hermin, A., Borvak, J., Nome, F., Isaeva, T., … Curiel, T. J. (2001). Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nature medicine*, *7*(12), 1339–46. doi:10.1038/nm1201-1339

6.4. ZUSAMMENFASSUNG

Plasmazytoide dendritische Zellen haben eine zentrale Rolle in der Produktion von Typ I Interferonen. Die Produktion dieser Interferone in pDCs ist gekoppelt an die Erkennung von Viren mittels TLR7(ssRNA) und TRL9 (dsRNA). Die Produktion der Typ I Interferone und weiterer Zytokine, wie IL-6 und TNF-α wird in stimulierten pDCs durch Transkriptionsfaktoren vermittelt. Bekannte Vertreter sind IRF-7 als essentieller Regulator der Produktion von IFN-α/β und NF-κB als kritischer Faktor für die Produktion von IL-6. Weiters ist in den letzten Jahren die Bedeutung vieler Transkriptionsfaktoren (z.B. Spi-B, IRF-8) für die Entwicklung von pDCs bekannt geworden. In meiner Master-Arbeit wollte ich die Rolle der Transkriptionsfaktoren c-Jun und JunB, welche zur AP-1 Familie gehören, für die Entwicklung und Funktion von pDCs untersuchen. Dafür verwendete ich genetisch modifizierte Mäuse, nämlich *c-Jun*$^{f/f}$, *JunB*$^{f/f}$ und *c-Jun/JunB*$^{f/f}$ mx cre Mäuse. In diesen Mäusen kann *c-Jun*, *JunB* oder beides effizient im Knochenmark und in pDCs deletiert werden. Durch *in vitro* Differenzierung von Knochenmarkszellen konnte ich zeigen, dass c-Jun und JunB keine Rolle in der Entwicklung von pDCs spielen. Überraschenderweise fand ich aber, dass *in vitro* Kulturen mit Knochenmarkszellen ohne JunB eine höhere Anzahl dendritischer Vorläufer (CD115+) hatten. Weiters konnte ich zeigen, dass in pDCs welche mit dem TLR7 Agonisten Imi stimuliert wurden, c-Jun und JunB hochreguliert werden. Durch Imi Stimulierung der pDCs konnte ich außerdem zeigen, dass c-Jun, aber nicht JunB, wichtig ist für die Produktion von IL-6 und IFN-β. Für andere Zytokine, wie TNF-α und Interferon-α1 ist c-Jun aber erlässlich. Zusätzlich konnte ich beweisen, dass *c-Jun* $^{-/-}$ pDCs, trotz verminderter Produktion von IFN-β und IL-6 normal maturieren, nachdem man sie mit Imi für 8 oder 16 h stimuliert hat. Das konnte ich an der normalen Hochregulierung von Molekülen wie CD40, CD86 und MHCII zeigen. Am Schluss befasste ich mich mit der kürzlich endeckten Anti-Tumor Eigenschaft von pDCs. Es wurde gezeigt, dass Imi stimulierte pDCs Tumorzellen durch die Produktion von zytolytischen Proteinen wie TRAIL und Gzmb lysieren können. Ich konnte auf RNA Ebene

zeigen, dass c-Jun in pDCs wichtig ist für die Produktion von *Gzmb*, aber nicht für die Produktion von *Trail*. Durch Killing Assays fand ich aber, dass c-Jun in pDCs, trotz der Reduktion von *Gzmb* mRNA, keine Rolle für den Anti-Tumor Effekt von pDCs hat. Zusammengefasst zeigen meine Resultate, dass c-Jun wichtig ist für die Produktion von IL-6 und IFN-β und deuten auf eine Rolle von JunB in der Regulation dendritischer Vorläufer ($CD115^+$ Zellen). Mehr Experimente sind notwendig um zu zeigen, wie sich JunB auf die Entwicklung von dendritischen Zellen und deren Vorläufer auswirkt. Desweiteren ist zu klären, ob durch die verminderte Produktion von IL-6 und IFN-β die Immunantwort *in vivo* verändert ist.

6.5. SUMMARY

Plasmacytoid dendritic cells (pDCs) have a pivotal role as Type I interferon producing cells in human and mice. The production of Type I IFN is closely linked to their ability to sense viruses through the expression of TLR 7 (ssRNA) and TLR 9 (dsRNA). Different transcription factors are known to be important for the production of Type I IFN and other cytokines like IL-6 and TNF-α after pDCs have become activated. Prominently IRF-7 was shown to be the master regulator for the production of IFN-α/β and NF-κB is critical for the production of IL-6. Furthermore in the last years many different transcription factors (like Spi-B, IRF-8) were shown to be important for pDC development. In this master thesis I wanted to evaluate the role of the transcription factors c-Jun and JunB (AP-1 family members) in the development and function of pDCs as little is known up today. To answer these questions I used genetically modified mice, namely *c-Jun*$^{f/f}$, *JunB*$^{f/f}$ and *c-Jun/JunB*$^{f/f}$ mx cre mice. I demonstrate that this mouse model can be used to efficiently delete c-Jun, JunB or both in bone marrow cells and pDCs. Through *in vitro* differentiation of bone marrow cells I show that c-Jun and JunB are dispensable for the development of pDCs *in vitro*. Strikingly, however, loss of JunB increases the number of dendritic progenitors (CD115^{+} cells) in these *in vitro* differentiation cultures. Furthermore I show that c-Jun and JunB are induced after pDCs are stimulated with Imi (TLR7 agonist). Moreover through stimulation of pDCs with Imi I show that c-Jun, not JunB, is important for the production of IL-6 and IFN-β, but dispensable for the production of TNF-α and Interferon-α1. In addition I demonstrate that despite the reduced levels of IFN-β and IL-6 *c-Jun* $^{-/-}$ pDCs mature normally after stimulation with Imi for 8 and 16 h as shown by the normal up-regulation of CD40, CD86 and MHC II. Lastly, Imi stimulated pDCs are known to have a tumor killing capacity that is independent of adaptive immunity and mediated through TRAIL and Gzmb. I show that loss of c-Jun reduces the expression of RNA levels of *Gzmb*, not *Trail*, in Imi stimulated pDCs. Through killing assays I demonstrate that c-Jun is dispensable for the tumor killing capacity of pDCs *in vitro* despite the

reduction of *Gzmb* RNA. Taken together my results demonstrate that c-Jun is needed to produce IL-6 and IFN-β in Imi stimulated pDCs and point to a role of JunB in the regulation of dendritic progenitors ($CD115^{+}$). More experiments need to be carried out to analyze how JunB affects the development of dendritic cells and their progenitors. Lastly, future experiments need to shed light if the reduced levels of IL-6 and IFN-β change the immune response *in vivo*.

6.6. CURRICULUM VITAE

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2003-2008 College of Chemistry Specializing in Biochemistry, Genetic Engineering; Höhere Bundeslehr- und Versuchsanstalt für chemische Industrie (HBLVA Rosensteingasse), Abteilung: Biochemie, Bio- und Gentechnologie

2008-2009 Civilian service; Rescue service

2009-2011 University of Vienna, Bachelor in Biology – Specialization Microbiology and Genetics, Graduated with distinction
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