Influence of T cell-expressed CD83 on the regulation of immune responses

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1 Introduction

1.1 The immune system

The immune system protects the host from pathogens (e.g. bacteria, fungi, viruses, parasites), malignant cells and toxic substances. In addition to the anatomic and physiologic barrier, the immune system consists of two major parts the innate and the adaptive immune response, which act complementary in order to achieve an efficient host defense [1, 2].

1.1.1 The innate immune system

The innate immune response is the first line of defense against pathogens. It is characterized by an immediate, non-specific host defense based on the so called pattern recognition receptors (PRRs). PRRs are invariant receptors, encoded by genes in the host`s germline. They sense common structures like conserved microbial components (pathogen associated molecular patterns, PAMPs) or common metabolic components released upon inflammation and infection (damage associated molecular patterns, DAMPs) [3]. The PRRs are expressed by hematopoietic cells such as neutrophils, macrophages, dendritic cells (DCs), mast cells, basophils, eosinophils and natural killer (NK) cells as well as non-hematopoietic cells such as epithelial cells of the skin, respiratory and gastrointestinal tract. Receptor expression varies between cell types, albeit one cell type expresses the same set of receptors. Hence, different cell types are specialized for the recognition of specific structures and activate specific signaling pathways to induce a proper immune response [4].

Besides mechanisms like phagocytosis and degranulation to take up and eliminate pathogens, an important feature of innate immune cells is the release of growth factors, cytokines and chemokines. The secretion of these soluble molecules initiates an acute inflammatory immune response, which is relevant for the recruitment of further immune cells such as DCs. DCs possess a special role by bridging the innate and adaptive immune response. Recruited DCs phagocytose pathogens and present antigens via major histocompatibility complex (MHC) class I or class II molecules to T cells to initiate the adaptive immune response. In addition to DCs, macrophages and B cells can perform antigen presentation as well [5].

Innate immunity also encompasses circulating humoral compounds like proteins of the complement system, the lipopolysaccharide(LPS)-binding protein, the C-reactive binding protein and anti-microbial peptides, which assist to carry out a protective and efficient immune response [3, 4].

1.1.2 The adaptive immune system

The adaptive immune response represents the second part of an efficient immune system and is mainly based on antigen-specific receptors expressed on the surface of T and B lymphocytes. During development of T and B cells in primary lymphoid organs, germline gene elements are randomly assembled by somatic rearrangement. Hence, a broad variety of genes, which encode for antigen-specific T and B cell receptors are generated and transcribed resulting in a large diversity of receptor specificities [6].

Mature T and B cells circulate through the lymph system and accumulate in secondary lymphoid organs (SLOs), where they get activated by antigen-presenting cells (APCs) such as the aforementioned DCs. Within days antigen-specific T and B cells fully differentiate and undergo clonal expansion, followed by migration to the site of inflammation [1, 7]. At the site of inflammation T and B cells participate in the elimination of pathogens, infected or malignant cells and other harmful agents. T cells mainly exert their effector function on cellular level and via cytokine secretion, whereas B cells predominantly secrete antigen-specific immunoglobulins.

Another important feature of the adaptive immune system is the generation of long-lived memory T and B cells, so that in case of subsequent exposure to the same antigen the immunological memory provides a rapid and efficient immune response.

In contrast to the non-specific, but immediate innate immune response, the development of the adaptive immune response takes several days, but provides highly specific and efficient antigen-specific T and B cells. Nevertheless, both arms of the immune system are needed to keep up immune homeostasis and elicit an appropriate immune response in case of an acute threat [7].

1.2 Initiation and regulation of T cell responses

1.2.1 T cell development in the thymus

The lymphocyte population of the adaptive immune system is mainly composed of T and B cells. B and T cell precursors originate from a common lymphoid

hematopoietic stem cell in the bone marrow. B cells directly mature in the bone marrow, whereas T cell maturation takes place in the thymus. During T cell development in the thymus, T cell receptor (TCR) gene rearrangement as well as T cell selection takes place [8, 9].

When thymocytes enter the thymus, they do not express the coreceptor cluster of differentiation 4 or 8 (CD4, CD8) and are termed double negative (DN). The DN cells undergo different steps of maturation, characterized by specific expression of the adhesion molecule CD44 and the interleukin-2 (IL-2) receptor α-chain CD25. DN cells first rearrange the TCR β-chain. Then a pre-TCR is built by complex formation with a pre-α-chain and the CD3 subunits. During further development, expression of the CD4 and CD8 coreceptors is upregulated and thymocytes are termed double positive (DP) now. The DP cells finally rearrange the α-chain to build a functional αβ-TCR. Noteworthy, a small population of thymocytes harbors a γδ-TCR, albeit most of the thymocytes become αβ-T cells [9].

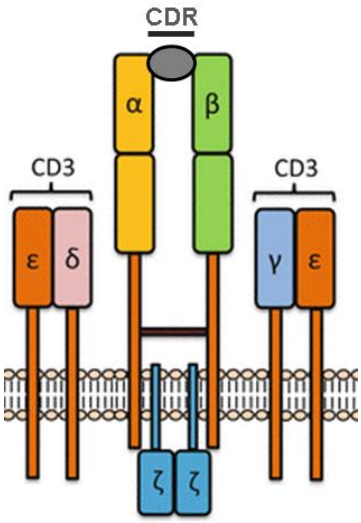

Figure 1: Schematic drawing of the TCR-CD3 complex.
The TCR comprises a αβ-heterodimer complexed with three CD3 subunits (CD3εγ, CD3εδ, CD3ζζ). The α-chain consists of V- and J-genes and the β-chain additionally harbors D-genes. TCR diversity is represented in the CDRs, which form the antigen recognition site (adapted from [10]).

Overall, the TCR consists of either αβ-chains or γδ-chains alongside with the CD3 complex, which consists of the heterodimers CD3εγ and CD3εδ and the homodimer CD3ζζ (Figure 1). The α-chain is composed of variable (V) and joining (J) genes and the β-chain additionally contains diversity (D) genes. During development a vast repertoire of TCRs is generated by gene rearrangement of the exons of the variable domains from the V-J (α-chain) and the V-D-J segments (β-chain). In addition, junctional diversity by random deletions and insertions between V, D and J regions increases the variety. However, TCR diversity is represented in the complementary

determining regions (CDRs), which form the antigen recognition site. Based on the described mechanism, T cells can recognize a large number of antigens [11].

Upon TCR rearrangement, T cells undergo two cycles of selection (Figure 2). First, positive selection of thymocytes is performed in the cortex. DP cells that bind with low affinity with their TCR to self-peptides, presented via MHCI or MHCII molecules on cortical thymic epithelial cells (cTECs), survive, whereas thymocytes with high affinity interaction die. During this process either the CD4 or CD8 coreceptor is downregulated, depending on the ability of the TCR to bind to the peptide-MHCI ($CD8^+$) or -MHCII ($CD4^+$) complex. The remaining single positive (SP) thymocytes migrate from the cortex to the medulla. Those cells, which exhibit a very strong binding of the TCR to the self-peptide-MHCI/MHCII complex on medullary thymic epithelial cells (mTECs) or conventional DCs undergo apoptosis or differentiate into regulatory T cells. This process is called negative selection and avoids the generation of self-reactive T cells as well as ensures the survival of T cells specific for foreign antigens [9].

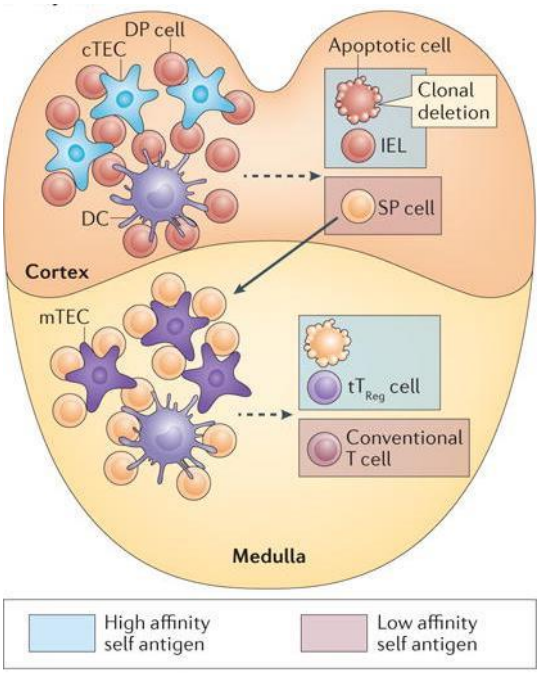

Figure 2: Overview of the T cell selection in the thymus.
During maturation in the thymus, T cells undergo two cycles of selection. In the cortex DP thymocytes that bind with low affinity with their TCR to self-peptides, presented via MHCI/MHCII molecules on cTECs, survive, whereas thymocytes with high affinity interaction die (positive selection). Upon migration into the medulla those cells, which exhibit a strong binding of the TCR to the self-peptide-MHCI/MHCII complex on mTECs or conventional DCs undergo apoptosis (negative selection) or differentiate into regulatory T cells. Hence, thymic T cell selection avoids the generation of self-reactive T cells and ensures the survival of T cells specific for foreign antigens (adapted from [12]).

Finally, naive $CD4^+$ or $CD8^+$ T cells can leave the thymus and circulate through the lymph system and peripheral lymphoid organs. In the periphery T cells sense for

peptide-MHC complexes. Upon recognition of their specific antigen, T cells get activated and differentiate into specific subtypes [8, 13]. In addition to conventional $CD4^+$ and $CD8^+$ T cells, a small population of regulatory T cells, the so called thymus-derived natural Treg cells (nTregs), exerts the thymus into circulation.

1.2.2 Dendritic cells and their role for T cell activation

DCs develop from a common hematopoietic stem cell in the bone marrow. They represent a heterogeneous cell population with different subtypes performing specific functions. DCs can be identified by their expression of CD11c. Conventional dendritic cells (cDCs) express high levels of CD11c ($CD11c^{high}$), whereas plasmacytoid dendritic cells (pDCs) express intermediate to low levels of CD11c ($CD11c^{int\text{-}low}$). Both subsets express intermediate levels of MHCII, which is increased upon DC activation. Furthermore, the different DC sublineages are supposed to develop from different precursors. In the murine lymphoid system five subtypes of cDCs can be distinguished concerning their expression of CD4, CD8, CD11b and CD205. However, all DC subpopulations act as mediators for T cell activation and induce the antigen-specific, adaptive immune response [14].

Under homeostasis immature DCs migrate through the blood, peripheral tissue, lymph and lymphoid organs. At the site of inflammation DCs internalize antigens from pathogens, infected or malignant cells and other harmful agents. The uptake can occur via different mechanisms including phagocytosis or receptor-mediated endocytosis. Moreover, DCs themselves can be infected by a pathogen [15, 16]. In addition to antigen uptake, DCs recognize PAMPs and DAMPs via their specialized, surface-expressed PRRs. PRRs expressed by DCs are divided into three major families termed Toll-like receptors (TLRs), RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs). The activation of PRRs induces the maturation of DCs, which is necessary for DCs to become professional APCs [17]. Upon maturation, antigen presentation capabilities are enhanced, expression of costimulatory molecules (CD80/B7.1, CD86/B7.2, CD40) increases and the release of proinflammatory cytokines (IL-12, IL-6, IL-1β, tumor necrosis factor alpha (TNF-α)) and type I interferons (IFNs) is triggered. Maturation is initiated in response to signaling via the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and the IFN regulatory factor (IRF). Upon alteration of adhesion molecule and chemokine

receptor expression DCs start to migrate to secondary lymphoid organs, where they present antigens to T cells [18].

For antigen presentation antigens have to be intracellularly degraded into small peptides, which are loaded onto MHCI or MHCII molecules. Based on different processes to form the peptide-MHC complexes, classically exogenous antigens are presented via MHCII, whereas endogenous antigens are presented via MHCI. Peptides loaded on MHCI are presented to $CD8^+$ T cells, whereas peptides complexed with MHCII molecules are presented to $CD4^+$ T cells [19-21]. In addition, some DCs are specialized to cross-present exogenous antigens via MHCI to $CD8^+$ T cells [22].

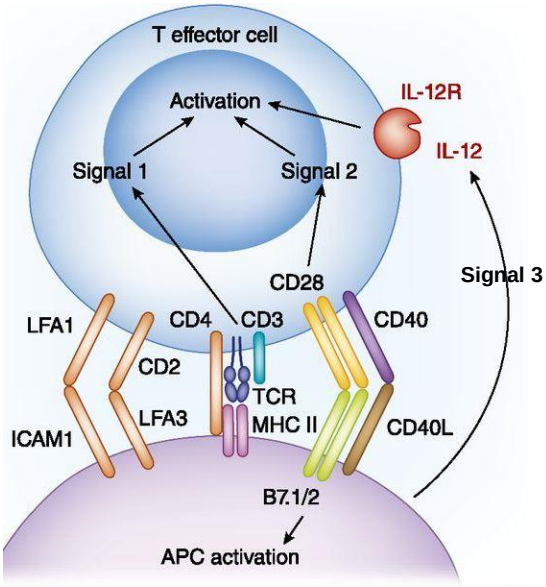

Figure 3: Overview of the immunological synapse during T cell priming.
In T cell zones mature DCs transiently bind to naive T cells by the interaction of different adhesion molecules on T cells with their DC-expressed counterparts. After recognition of its specific antigen presented via MHCI or MHCII on the surface of mature DCs, T cell activation is initiated. This process is called T cell priming and requires three essential steps. First, the entire TCR-CD3 complex with either the CD4 or CD8 coreceptor, expressed on the T cell surface, engages with the peptide-MHC complex on the APC (Signal 1). In addition, costimulatory molecules, expressed on the surface of both cells, interact with each other. An essential costimulatory receptor-ligand pair interaction for T cell activation is the binding of CD28, expressed on the T cell surface, to CD80/CD86 (B7.1/2), expressed by the APC (Signal 2). In a last step, cytokines secreted by activated DCs bind to cytokine receptors on the T cell surface, which further enhances T cell activation and influences T cell differentiation (Signal 3). The interacting molecules, expressed on DCs and T cells, form the so called immunological synapse (adapted from [23]).

In T cell zones mature DCs present antigens as peptide-loaded MHC molecules to circulating, naive T cells, which transiently bind to DCs by the interaction of different adhesion molecules on T cells (e.g. lymphocyte function-associated antigen 1 (LFA1)) with their DC-expressed counterparts (e.g. intercellular adhesion molecule 1 (ICAM1)). During this process T cells randomly patrol a huge number of presented

antigens. If a T cell recognizes its specific peptide presented via MHCI or MHCII, T cell activation is initiated. This process is called T cell priming and requires three essential steps (Figure 3). First of all, the entire TCR-CD3 complex with either the CD4 or CD8 coreceptor, expressed on the T cell surface, engages with the peptide-MHC complex on the APC. In addition, costimulatory molecules, expressed on the surface of both cells, interact with each other. An essential costimulatory receptor-ligand pair interaction for T cell activation is the binding of CD28, expressed on the T cell surface, to CD80 (B7.1)/CD86 (B7.2), expressed by the APC. In a last step, cytokines, secreted from activated DCs, bind to cytokine receptors on the T cell surface, which further enhances T cell activation and influences T cell differentiation. All these interacting molecules, expressed on DCs and T cells, form the so called immunological synapse. Beside the mentioned key mediators, other adhesion and costimulatory molecules and cytokines can participate in the process of T cell activation [24].

1.2.3 T cell activation and alterations in T cell phenotype

During acute immune responses the T cell phenotype strongly changes. Depending on the activation status T cells are defined as naive, effector and memory T cells. From one stage to the other the expression profile of the T cell is strongly altered. The surface expression of adhesion molecules, costimulatory/coinhibitory molecules, activation marker as well as cytokine, chemokine and growth factor receptors is significantly modified (Figure 4).

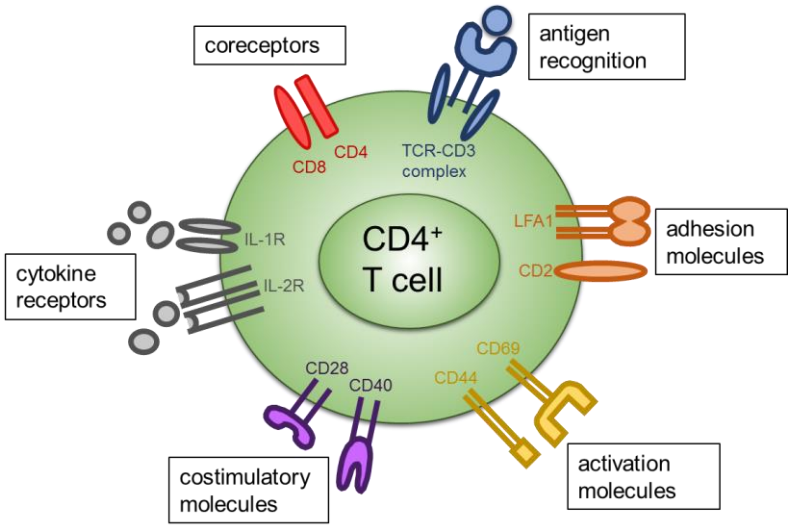

Figure 4: Overview of molecules expressed on the T cell surface.
On the surface of activated T cells serval groups of molecules with different function are expressed. These molecules include the TCR-CD3 complex, coreceptors, cytokine receptors, costimulatory molecules, activation molecules and adhesion molecules.

T cells that have not been stimulated by APCs are termed naive. Naive T cells circulate between blood and peripheral lymphoid organs. CD62 ligand (CD62L) is an integrin expressed on naive T cells. Via binding of CD62L to its receptor on high endothelial venules entrance of the T cell into lymphoid organs is mediated. Hence, CD62L expression can be used to discriminate between naive, effector and memory T cells [25].

As summarized in chapter 1.2.2, T cell activation requires several signals. Once the TCR has recognized and bound to the peptide-MHC complex on APCs, the antigen-independent costimulation takes place and naive T cells develop into effector T cells.

Costimulation is induced by receptor-ligand interactions on the cell surface of APCs and T cells. First of all CD28, constitutively expressed on the surface of naive T cells, binds to CD80 or CD86, which are expressed on APCs. By several other costimulatory molecule interactions, further activating signals are delivered to the T cell [26]. Most costimulatory molecules belong to either the immunoglobulin (Ig) or tumor necrosis factor (TNF) receptor superfamily. The afore mentioned CD28 protein belongs to the Ig superfamily and plays a very important role in T cell activation [27]. An important costimulatory molecule of the TNF receptor superfamily is CD40L. CD40L interacts with its receptor CD40 expressed on APCs, B cells and macrophages. This interaction supports full maturation of APCs including the upregulation of cytokine production, costimulatory molecules and cross-presentation, which in turn promote T cell activation [28]. In contrast to CD28 and the TCR, which are constitutively expressed on T cells, the other costimulatory molecules are only upregulated upon antigen recognition, providing a tool to distinguish naive and effector T cells.

In addition to costimulatory molecules, effector T cells express further cell surface molecules such as cytokine and chemokine receptors as well as adhesion and migration molecules. CD69, which is a membrane-bound, type II C-lectin receptor, is immediately upregulated upon TCR engagement and thus broadly used as marker for lymphocyte activation. CD69 expression enhances T cell activation and proliferation. Furthermore, CD69 is expressed at the site of inflammation in many chronic human inflammatory diseases, where its role is still discussed controversially [29]. Another molecule, which is rapidly upregulated on T cells upon activation, is CD25, the IL-2 receptor α-chain. CD25 expression enables binding of IL-2 to the T cell and promotes further T cell activation and proliferation [30]. Noteworthy, CD25 is constitutively expressed on regulatory T cells. Hence, CD25 should not be used

separately to determine T cell activation [31]. In addition, CD44 is upregulated on T cells upon activation. CD44 is a type I transmembrane glycoprotein and known as a widely expressed adhesion receptor. Interaction of CD44 with hyaluronic acid, which is expressed on vascular endothelial cells, initiates migration of effector T cells to the site of infection and inflammation. Therefore, upregulation of CD44 alongside with downregulation of CD62L identifies effector T cells [32].

Once an acute immune response declines, the expression level of CD44 further increases, so that $CD44^{high}$ expression can be used to identify memory T cells [32].

Taken together several molecules are upregulated upon T cell activation and provide a tool to distinguish naive, effector and memory T cells. The outcome and degree of the T cell response is determined by integration of all the signals induced by these molecules.

1.2.4 T cell differentiation and effector functions of different T cell subsets

During the process of T cell priming, T cells differentiate into several subtypes, which clonally expand afterwards. Differentiation is mainly influenced by signals of the innate immune system including the cytokine microenvironment, the antigen concentration, the type of APCs and costimulatory molecule expression. $CD8^+$ T cells mainly differentiate into cytotoxic T lymphocytes (CTLs) in response to antigen presentation via MHCI molecules. In contrast, $CD4^+$ T cells can either differentiate into conventional T cells (Tconv cells) or regulatory T cells (Treg cells). Thus, the induction of the specific conventional or regulatory $CD4^+$ T cell subtype ensures an appropriate immune response against the specific type of antigen.

$CD4^+$ Tconv cells include several subtypes of T helper cells (TH cells), which are characterized by the expression of specific transcription factors (TF) and cytokines. The most prominent TH cell subsets comprise TH1, TH2 and TH17 cells [33, 34] (Figure 5).

TH1 cells are induced in response to intracellular infections (bacteria, viruses, protozoan), which especially activate TLRs. TLR stimulation activates the NF-κB pathway in DCs, which in turn induces the secretion of several proinflammatory cytokines such as IL-1, IL-6, TNF-α, IL-12p40 and type I IFNs [17, 35]. Functionally active IL-12p70 and IFN-γ are the most important cytokines for the initiation of TH1-inducing signaling pathways. IL-12p70 is a heterodimer of IL-12p40 and IL-12p35. IL-

12p35 secretion is independently induced by type I IFNs and IFN-γ produced by TH1 cells, NK cells and innate lymphoid cells (ILCs) [36]. Finally, the activation of many different TFs leads to the differentiation into TH1 cells. The most important TF is the T-box-containing protein expressed in T cells (T-bet). Via activation of a specific set of genes, T-bet increases the secretion of IFN-γ and exerts an inhibitory effect on the differentiation into TH2 and TH17 cells [37, 38]. Differentiated TH1 cells mainly produce IFN-γ, lymphotoxin α (LTα) and IL-2. Upon binding to its receptor, IFN-γ fulfills its function via activation of IFN-γ responsive genes. In this manner, IFN-γ has an effect on many other immune cells. It activates mononuclear phagocytes as well as $CD8^+$ CTLs and supports B cell antibody production. In consequence, all the induced processes help to eliminate pathogens and infected cells [39]. Furthermore, TH1 cells are associated with organ-specific autoimmunity and tumor immunity [40-42].

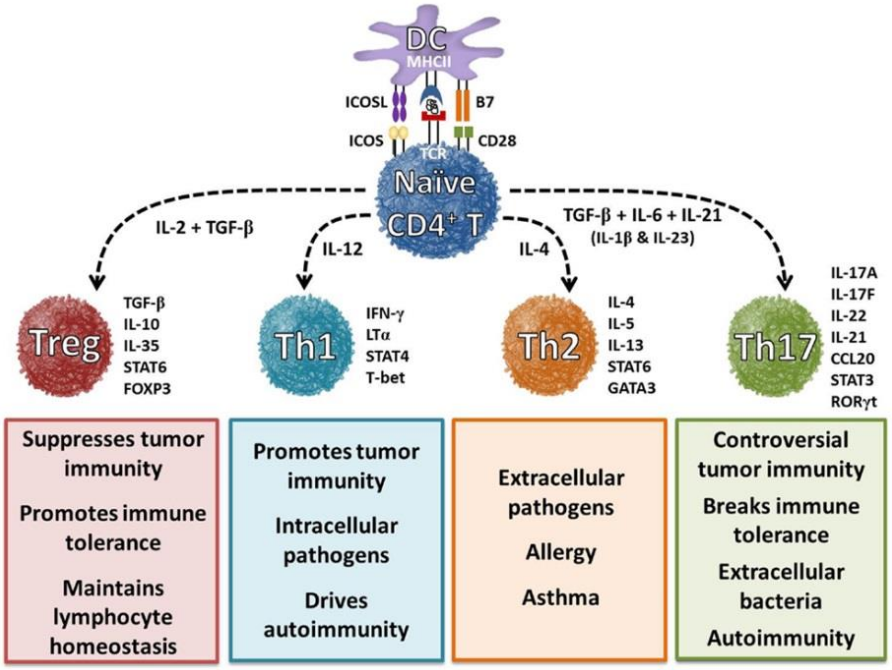

Figure 5: Overview of the different T cell subsets.
TH1 cells are induced in response to intracellular pathogens, promote tumor immunity and contribute to autoimmunity. Functionally active IL-12p70 and IFN-γ secreted by mature DCs are the major cytokines promoting TH1 cell differentiation. TH1 cells characteristically express T-bet and mainly secrete IFN-γ and LTα. TH2 cells are induced upon infection with extracellular pathogens and play a major role during allergy and asthma. IL-4 is the key cytokine to induce TH2 cell differentiation. GATA3 represents the specific TF and IL-4, IL-5 as well as IL-13 are the major cytokines expressed by TH2 cells. TH17 cells play an important role in the protective immune response against infections with extracellular bacteria, but they are also linked to autoimmunity. A combined secretion of IL-6, IL-21 and TGF-β by DCs promotes TH17 cell differentiation. RORγt is the TH17-characterizing TF and IL-17A and IL-17F are the main cytokines produced by TH17 cells. Additionally, TH17 cells produce IL-21, IL-22, GM-CSF and TNF-α. Peripheral induced Treg cells develop in response to TGF-β and IL-2. They are characterized by the expression of Foxp3 and mainly secrete IL-10, TGF-β and IL-35. Induced Treg cells dampen the effector T cell response to limit immunopathology during acute inflammation (adapted from [42]).

IL-4-producing TH2 cells are mainly induced upon infection with extracellular pathogens especially helminths [43] and play a major role in allergy and asthma [42]. Especially the activation of C-type lectin receptors (CLRs) and partly TLRs by conserved parasite-derived molecules leads to induction of TH2 cells. IL-4 and IL-2 are the key cytokines produced by DCs to induce TH2 cells [33, 34]. Subsequent activation of IL-4-induced STAT6, which promotes the activation of GATA-binding protein 3 (GATA3), is the essential step for differentiation of naive T cells into TH2 cells [44]. GATA3 promotes TH2 differentiation by increasing TH2-related cytokine production, proliferation of TH2 cells and inhibition of T-bet [45]. Differentiated TH2 cells exert their protective function via the production of IL-4, IL-5 and IL-13. These cytokines promote antibody isotype class-switching in B cells, alternative activation of macrophages and influence many non-hematopoietic cells to exert an efficient immune response against invaded parasites [43].

TH17 cells are important for the protective immune response against infections with extracellular bacteria and fungi, but are also linked to a immunopathological role during many autoimmune diseases [46]. The combined secretion of IL-6, IL-21, IL-23 and TGF-β by DCs and the surrounding microenvironment promotes TH17 cell differentiation. Among the different cytokines TGF-β secretion plays a special role during TH17 differentiation. High concentrations of TGF-β alone induces Foxp3 expression and Treg cell differentiation [47], whereas low levels of TGF-β in combination with IL-6 favors TH17 differentiation [48]. TGF-β-induced downstream signaling activates the retinoic acid receptor-related orphan receptor gamma-t (RORγt), which is the most important TF for TH17 differentiation. RORγt expression leads to the production of IL-17A and IL-17F, which are the main cytokines produced by TH17 cells [46]. In addition, TH17 cells produce IL-21, IL-22, TNF-α and the granulocyte-macrophage colony-stimulating factor (GM-CSF). In a feedback loop IL-21 secretion by differentiated TH17 cells further promotes TH17 cell differentiation [49].

In addition to the several TH cell subtypes, summarized as Tconv cells, $CD4^+$ T cells can also fulfill regulatory functions. Two major subsets of $CD4^+$ Treg cells can be discriminated. Natural thymus-derived Treg cells (nTreg cells) stably express Foxp3 and are fully functional when leaving the thymus. In contrast, peripheral induced Treg cells (iTreg cells) develop, similar to TH cells, from naive $CD4^+CD25^-$ T cells in peripheral lymphoid organs in response to antigen priming in a specific cytokine

environment. TGF-β and IL-2 are the key mediators for iTreg cell development. Upon TCR engagement, TGF-β activates downstream signaling pathways, which induce lineage-specific Foxp3 expression. In addition, IL-2 induces STAT5, which further enhances Foxp3 expression [47, 50]. One of the major tasks of nTreg cells is to mediate immunological tolerance to self-antigens and thus to inhibit the induction of autoimmunity, whereas iTreg cells dampen the effector T cell response upon pathogen elimination to limit immunopathology and restore homeostasis [51].

Both types of Treg cells mediate their function via different mechanisms. One mechanism is the secretion of inhibitory cytokines such as IL-10, TGF-β and IL-35. Especially IL-10 suppresses proinflammatory effector T cells, thus inhibiting tissue damage. In addition, Treg cells have been described to exert cytolysis via the release of granzymes and perforin-dependent killing mechanisms. They can also disrupt normal metabolism for example by expression of the high-affinity CD25 receptor, allowing efficient consumption of IL-2. Furthermore, Treg cells can influence DC maturation via e.g. lymphocyte-activation gene 3 (LAG3)-MHC-class-II interaction, which in turn blocks Tconv cell activation [52].

In summary, differentiation of naive T cells into the different TH or Treg cell subsets is a complex network influenced by many signals, which enables an appropriate T cell response against a specific type of antigen.

1.2.5 T cells and immune homeostasis

In healthy individuals immune homeostasis is an equilibrium maintained by innate and adaptive immune cells. These cells continuously sense their environment to mediate a balance between tolerance to self-antigens and an efficient response to foreign antigens [53, 54].

Initially, tolerance to self-antigens is provided by positive and negative selection of T cells during T cell maturation in the thymus (central tolerance) (see chapter 1.2.1). Due to the fact that some self-reactive T cells escape deletion and leave the thymus, different mechanisms to suppress self-reactive T cells in the periphery have been evolved (peripheral tolerance). Intrinsic mechanisms of peripheral tolerance include anergy and peripheral deletion. T cells, which recognize self-peptide-MHC complexes in the periphery, become anergic due to missing costimulatory signals or high levels of coinhibitory signals. Anergic T cells stay in an inactivated, hyporesponsive state with weak expansion and differentiation capacity. In addition,

self-reactive T cells undergo peripheral deletion due to apoptosis induced by interaction of the death receptor Fas and its ligand FasL [55]. Moreover, thymus-derived nTreg cells deliver extrinsic peripheral tolerance. In the periphery, they inhibit the expansion and function of effector T cells and other immune cells [56]. All these mechanisms provide immunological tolerance to self-antigens, thereby inhibiting the induction of autoimmunity and maintain homeostasis.

In case of an immune response against foreign antigens, activation of immune cells is a necessary step to efficiently eliminate pathogens. Besides immune cell activation, an appropriate immune response also requires regulation of activated immune cells to avoid immunopathology and/or chronic inflammation. During an ongoing immune response, Treg cells dampen the effector T cell response via different mechanisms (see chapter 1.2.4) to limit immunopathology.

In addition, negative regulators of T cell activation, expressed on Tconv as well as Treg cells, balance the immune response and restore homeostasis. Once the TCR recognizes the peptide-MHC complex on APCs, the antigen-independent costimulation induced by receptor-ligand pair interactions takes place (see chapter 1.2.2). As described above (see chapter 1.2.3), the B7 proteins, expressed on the cell surface of APCs, interact with T cell-expressed molecules of the CD28 family [27]. In addition to costimulatory molecules, the CD28 family also includes coinhibitory molecules such as the cytotoxic T-lymphocyte antigen-4 (CTLA-4) and the programmed cell death protein-1 (PD-1) [57], which negatively regulate T cell activation. CTLA-4 and PD-1 are both upregulated on activated T cells. CTLA-4 shares the receptor with CD28, but has a higher affinity to CD80/CD86 compared to CD28. In contrast to CD28, CTLA-4 dampens T cell activation by inhibiting the IL-2 production and receptor expression. Due to ligand sequestration CTLA-4 decreases T cell activation [58, 59]. PD-1, another member of the CD28 family, was originally identified on cell lines undergoing programmed cell death. The interaction of PD-1, expressed on T cells, with its ligands PD-L1 or PD-L2, expressed on APCs or other cells, regulates T cell activation by blocking TCR signaling and induction of activated T cell death [60].

Taken together, the integration of all signals provides tolerance to self-antigens and enables an appropriate immune response to foreign antigens. A defective signal delivery in one of the different compartments can have deleterious consequences leading to chronic diseases or autoimmunity.

1.3 The role of T cells during immunopathology

Defects in the innate or adaptive immune response, either under normal physiological conditions or in case of an acute threat, can lead to disease. Different types of dysregulated immune responses leading to immunopathology can be distinguished.

Hypersensitivity reactions are described as exaggerated immune responses against exogenous or endogenous antigens/allergens. There are four types of hypersensitivity reactions classified by Coombs and Gel. Type I, II and III are immediate hypersensitivity reactions (HRs) and antibody-dependent, whereas type IV responses are cell-dependent and classified as delayed HRs. Allergic contact dermatitis is a prominent skin disease belonging to the type IV HRs. It occurs within a time delay of 48 to 72 hours post exposure [61].

Loss of immune homeostasis due to defects in central and/or peripheral tolerance can cause an abnormal immune response against self-antigens termed autoimmunity. Autoimmunity is characterized by the presence of self-reactive T cells and auto-antibodies leading to inflammation. Widespread examples of autoimmune diseases are type 1 diabetes mellitus and Graves` disease [62],

Immunodeficiency is characterized by a compromised or absent immune response against harmful agents like pathogens. It can arise from genetic mutations or can be acquired, for example via an infection. The most prominent acquired immunodeficiency is the acquired immune deficiency syndrome (AIDS) caused by an infection with the human immunodeficiency virus (HIV) [63, 64].

Immunopathology can also be caused by a dysregulated inflammatory response. In general, inflammation is part of the protective host response to eliminate infections. If inflammation becomes uncontrolled, such as overproduction of inflammatory cytokines or enhanced recruitment of inflammatory cells, it leads to chronic inflammatory diseases accompanied by tissue damage and pain. Prominent examples of widespread inflammatory diseases are rheumatoid arthritis and inflammatory bowel disease [65].

1.3.1 Hypersensitivity reactions

Allergic contact dermatitis (ACD) is a very common skin disease belonging to the group of type IV delayed HRs. The murine contact hypersensitivity (CHS) model is commonly used to study ACD. In this model the initial treatment (sensitization phase)

and reexposure (elicitation phase) of the skin with a hapten induces an immune response against modified self-proteins leading to a local CHS reaction at the site of reexposure [66, 67].

Haptens are small chemical compounds with a molecular mass < 500 kDa, which are able to cross the skin barrier. In general, haptens are too small to elicit an immune response, but at the site of exposure they complex with self-proteins. The tight binding to self-proteins modifies the proteins and thus generates foreign targets, which elicit an immune response. ACD is caused by synthetic molecules, heavy metals ions and the natural compound urushiol from poison ivy. For the *in vivo* mouse model haptens such as 1-chloro-2,3,4-trinitro-benzene (TNCB), 1-fluoro-2,4-dinitro-benzene (DNFB) or fluorescein isothiocyanate (FITC) are used to induce CHS [68].

During the sensitization phase haptens indirectly or directly activate keratinocytes, mast cells and DCs especially Langerhans cells in the epidermis. These activated innate immune cells start to secrete soluble compounds like TNF-α, IL-1β, and IL-18, which promote maturation of Langerhans cells and further dermal DCs (dDCs). Upon antigen uptake and maturation, Langerhans cells and dDCs migrate to the skin draining lymph nodes (dLN), where they present antigens to naive T cells. Antigen-specific T cells get activated, differentiate into effector T cells and clonally expand [69, 70].

Upon reexposure of the skin with the same hapten, the elicitation phase of the CHS reaction starts (Figure 6). Skin cells like keratinocytes, mast cells and endothelial cells get activated and secrete cytokines and chemokines. The secretion leads to the infiltration of inflammatory cells including neutrophils, mast cells and antigen-specific memory T cells to the site of reexposure [71]. Recruited antigen-specific T cells secrete proinflammatory cytokines like IFN-γ, TNF-α, several interleukins and GM-CSF. These compounds finally exert effector functions like cytotoxic effects on epidermal cells, activation of T cells and recruitment of neutrophils leading to dermal edema and spongiosis [72-74].

For decades, $CD4^+$ T cells especially TH1 cells were supposed to be the main players during the elicitation phase of CHS, due to high concentrations of IFN-γ at the site of reexposure. In line with this, activated Langerhans cells secrete high levels of IL-12 [75]. Moreover, TH2 and TH17 cells seem to promote CHS development and progression [66]. Interestingly, the TH1/TH2 balance is mainly influenced by the type of antigen. It has been demonstrated in Balb/c mice that the CHS reaction to DNFB is

TH1 dominated, whereas the FITC-induced CHS reaction is mainly TH2 based [67]. However, more recently it has been shown that $CD4^+$ as well as $CD8^+$ T cells participate in the elicitation phase of CHS [76, 77]

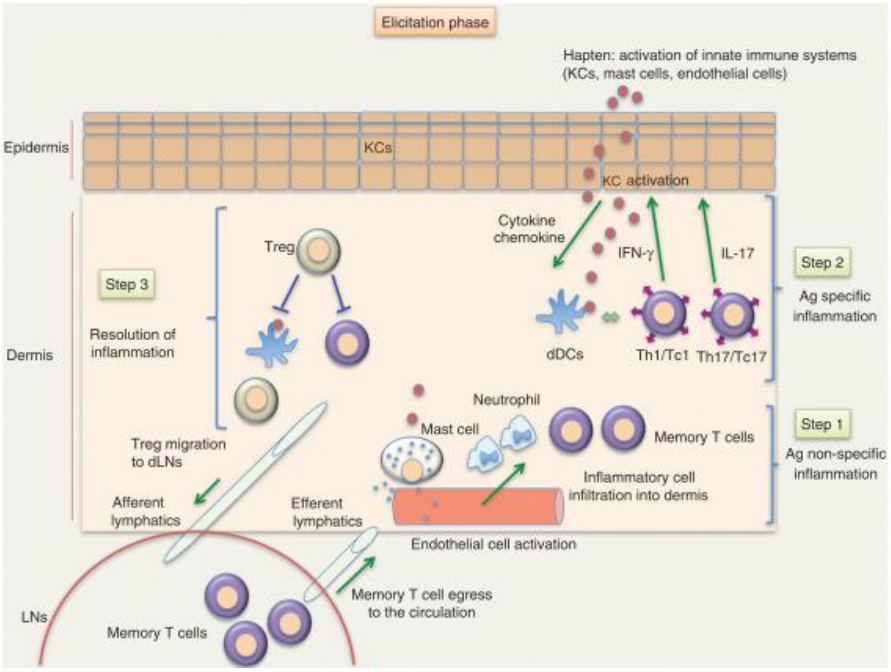

Figure 6: Overview of the immune response during the elicitation phase of a CHS reaction. Upon reexposure of the skin with the same hapten, cells of the skin like keratinocytes, mast cells and endothelial cells get activated and secrete cytokines and chemokines. The secretion leads to the infiltration of inflammatory cells including neutrophils, mast cells and antigen-specific memory T cells locally to the site of reexposure (step 1). Recruited antigen-specific T cells secrete proinflammatory cytokines like IFN-γ and several interleukins (step 2). These compounds finally exert effector functions like cytotoxic effects on epidermal cells, further activation of T cells and recruitment of neutrophils leading to dermal edema and spongiosis. During ongoing CHS reaction the number of Treg cells permanently increases and the elicitation phase of CHS is self-limiting in the end (step 3) [66].

In addition to effector T cells, Treg cells migrate to the site of inflammation to dampen the immune response by various mechanisms, including the secretion of IL-10. IL-10 impairs DCs maturation and decreases T cell activation. During an ongoing CHS reaction the number of Treg cells permanently increases and the elicitation phase of CHS is self-limiting in the end [78].

Taken together, the CHS model provides a suitable model to study the T cell response during cell-dependent hypersensitivity reactions.

1.3.2 Inflammatory diseases

Inflammatory bowel diseases (IBD) including Crohn`s disease (CD) and ulcerative colitis (UC) are defined as chronic inflammatory disorders of the small intestine and/or colon. About 0,1 % of the western population suffers from IBD with symptoms like rectal bleeding, severe diarrhea, abdominal pain, fever and weight loss [79]. The

precise mechanism of disease induction and progression is still elusive. However, several recurring characteristic of IBD were identified. It is widely accepted that environmental factors and the genetic background can negatively influence the epithelial barrier function and the innate or adaptive immune response causing an imbalance of the gut homeostasis. Hence, an exaggerated activation of the mucosal immune system occurs, which leads to a dysregulated immune response against antigens from commensal bacteria in the gut. In consequence, a chronic inflammation of the gut is evolving, which is mainly T cell-mediated and accompanied by a massive increase of proinflammatory cytokines [80, 81].

To further understand the immunological mechanism of the disease, animal models that mimic the human disease have been developed. Among the huge number of different mouse models of intestinal inflammation, three major categories can be distinguished: the chemically-induced, the cell transfer-induced and the genetically-engineered mouse models of experimental colitis. The *in vivo* models differ concerning the method of induction and the nature of inflammation [82-84].

A widely used model to study the impact of T cells concerning IBD initiation and progression is the T cell transfer model of chronic colitis first described by Powrie and colleagues [85]. In this model the adoptive transfer of $CD45RB^{high}CD4^{+}$ naive T cells from wildtype (WT) donor mice into syngeneic, immunodeficient recipient mice causes inflammation of the colon (colitis) 5-8 weeks post T cell transfer. As recipient mice any T cell deficient mice can be used such as recombinase activating gene-1/2 deficient (Rag1/2KO), severe combined immunodeficiency (SCID) or $CD3^{-/-}$ mice. The T cell transfer induces the so called wasting syndrome with symptoms like diarrhea/loose stool, mild to severe weight loss, reduced physical activity and piloerection. Histopathological features comprise transmural inflammatory cell infiltrate (lymphocytes, monocytes), mucosal injury, goblet cell loss, epithelial cell hyperplasia, erosions and crypt abscesses. Overall, the inflamed colon shrinks, but gains weight. The increasing colon weight-to-length ratio correlates with the histopathology, so that it can be used as an index for inflammation [86-89].

In this mouse model, inflammation is induced by activation of the transferred, naive T cells by the antigens of commensal bacteria in the gut. The activated T cells polarize mainly into TH1 and TH17 effector cells, expand and exert their effector functions by secretion of cytokines (Figure 7). Due to massively elevated levels of IFN-γ and TNF-α in the lamina propria (LP), predominantly TH1 cells have been described to cause the inflammation in this mouse model of colitis [85, 90]. With the discovery of TH17

cells, IL-17 (IL-17A/IL-17F) and other TH17-related cytokines have been identified to contribute to the inflammation as well. In accordance with this, an involvement of TH17 cells to the pathology has been observed in humans with IBD [91-93]. Currently, the T cell transfer model of colitis is supposed to be a model with a mixed TH1/TH17 immune response responsible for disease onset and progression [94]. However, since the number of IFN-γ-secreting cells is much higher than the number of IL-17-producing cells in the LP, TH1 cells still seem to be the main players.

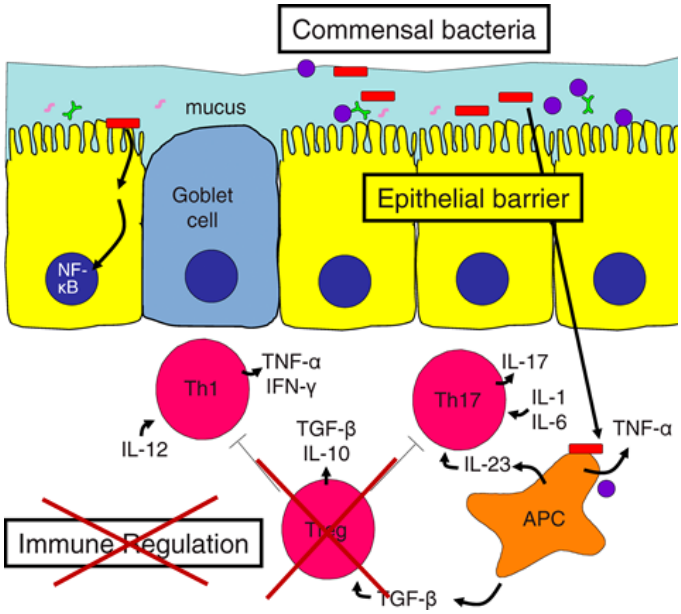

Figure 7: Overview of the immune response in the T cell transfer model of chronic colitis.
In the T cell transfer model of chronic colitis the adoptive transfer of CD45RB^{high}CD4^{+} naive T cells from WT donor mice into syngeneic immunodeficient recipient mice causes inflammation of the colon 5-8 weeks post T cell transfer. Transferred, naive T cells elicit an immune response against the antigens of the commensal bacteria in the gut of recipient mice. The activated T cells polarize mainly into TH1 and TH17 effector cells, expand and exert their effector functions via the secretion of cytokines like IFN-γ and IL-17. The transfer of Treg-depleted, naive T cells implicates an uncontrolled T cell response due to missing immune regulation, finally causing chronic inflammation (adapted from [95]).

In addition to the effector cytokines TNF-α, IFN-γ and IL-17, mainly secreted by differentiated TH1 and TH17 cells, multiple other cytokines have been identified to promote intestinal inflammation [96]. Among those cytokines especially IL-12 and IL-23, produced by APCs, have been associated with a pathogenic role in IBD [97, 98]. Both cytokines belong to the IL-12 family and share their p40 subunit. As mentioned before, IL-12 is the master cytokine for TH1 cell differentiation (see chapter 1.2.4). It is a heterodimeric cytokine composed of two subunits p40 and p35 [99, 100], whereas IL-23 is a heterodimer of the p40 and a unique p19 subunit [101]. In CD patients elevated levels of IL-12 in the colon correlated with disease severity [102-104]. The pathogenic role of IL-12 is underlined by different mouse models of intestinal inflammation. Increased secretion of IL-12 has been detected in the

inflamed colon of mice using different colitis models. Furthermore, the treatment with an IL-12 antibody ameliorated inflammation in different experimental models of colitis [105-108]. Noteworthy, in the T cell transfer model of chronic colitis it has been demonstrated that IL-12 promotes proinflammatory cytokine secretion and dampens Treg cell function [109]. In line with this, it was shown in different colitis models that IL-23 acts as a proinflammatory cytokine, which promotes immunopathology by enhancing T cell proliferation and suppressing Treg cell differentiation [91, 110, 111].
Interestingly, the transfer of naive, Treg-depleted T cells induces colitis, whereas the transfer of mature CD45RB^{low} T cells, which contain a small subset of Treg cells, does not induce colitis. Furthermore, the cotransfer of naive T cells with WT Treg cells neither leads to colitis development [112-114]. Therefore, the T cell transfer model of chronic colitis provides a suitable model to investigate the role of Treg cells regarding colitis induction and progression. It is well known, that Treg cells antagonize the pathological effector T cell response in the gut [52]. However, the question why Treg cells accumulate in the colon of IBD patients without being able to effectively suppress the inflammation is still elusive.
Taken together, the T cell transfer model of chronic colitis provides a suitable model to study the T cell response during intestinal inflammation.

1.4 CD83

CD83 is a 45 kDa, highly glycosylated, type 1 transmembrane glycoprotein and a member of the Ig superfamily first described by Zhou and colleagues in 1992 [115]. Genetic and structural analysis revealed that the mammalian CD83 protein is well conserved. The murine *cd83 gene* is located on chromosome 13 band A5, whereas the human *cd83 gene* locus is positioned within a homologous region of human chromosome 6p23 [116]. Both of them are composed of five exons. The murine CD83 protein consists of 196 amino acids (aa) [117] and shares 63 % sequence identity with the human CD83 protein consisting of 186 aa [115, 118]. Additionally, the recently identified porcine CD83 shows 81 % and 71 % homology to the human and the murine CD83 protein, respectively [119].
CD83 gene expression is mainly regulated by NF-κB and IRFs (Figure 8) [117, 120]. Upon translation transmembrane CD83 exhibits an extracellular Ig-like, an intracellular and a cytoplasmic domain [115, 118]. The CD83 extracellular domain resembles a V-type Ig domain, which harbors three N-glycosylation sites and five

cysteines. By using recombinant CD83 it has been shown, that the fifth cysteine is able to form a homodimer via a covalent intermolecular disulfide bound. The four residual cysteines seem to be involved in the formation of intramolecular disulfide bonds [119, 121]. Crystal structure analysis of the extracellular CD83 domain confirmed the formation of dimers and additionally, showed the formation of trimers. Furthermore, crystal structure analysis of CD83 revealed strong structural homologies to CD80, CD86 and PD-L2, all members of the B7 family and CD48, a member of the signaling lymphocyte activation (SLAM) family [122]. These structural homologies possibly indicate a similar function for CD83. However, the cytoplasmic CD83 domain encompasses 39 aa (human) and 44 aa (murine) without any tyrosine, which excludes signal transduction via immunoreceptor tyrosine-based inhibitory or activatory motifs (ITIM/ITAM) [115, 118]. Nevertheless, signaling via any other conserved or unknown motif is possible.

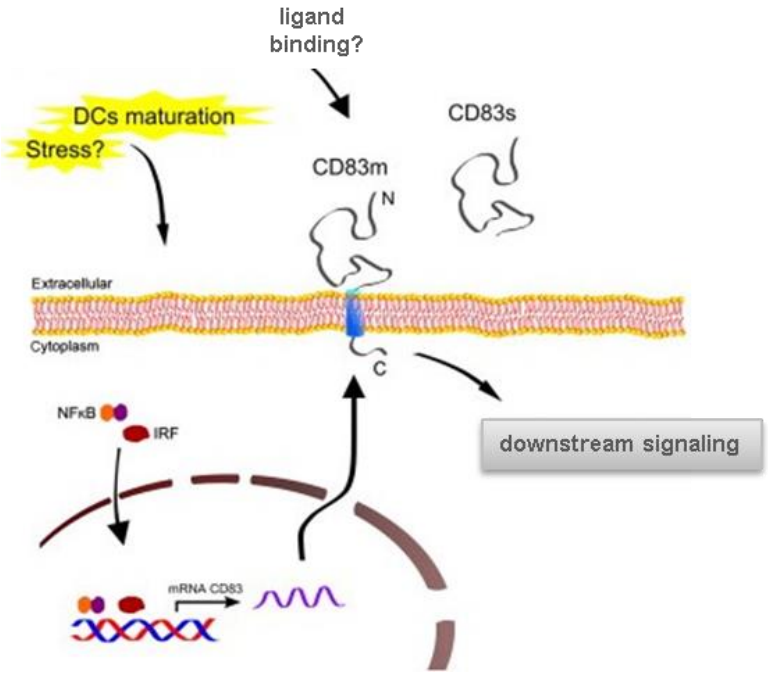

Figure 8: Overview of the induction of CD83 expression.
CD83 is a 45 kDa type 1 transmembrane glycoprotein and a member of the Ig superfamily. CD83 is strongly upregulated on DCs upon maturation. However, CD83 is also expressed on the surface of many other cell types upon activation. CD83 gene expression is mainly regulated by interaction of NF-κB and IRFs. Upon translation transmembrane CD83 exhibits an extracellular Ig-like, an intracellular and a cytoplasmic domain. In addition to transmembrane CD83 protein, another naturally occurring isoform of CD83 exists, the so called sCD83, which consist of the extracellular domain of the transmembrane protein (adapted from [123]).

Due to the fact that CD83 is strongly upregulated on DCs upon maturation, CD83 was designated as a marker for activated, mature DCs [117, 124, 125]. However, CD83 is also expressed on the surface of many other cell types including activated T and B cells [115, 126-129], activated macrophages and neutrophils [130-133], NK

cells [134, 135] and thymic epithelial cells [136]. In addition to the full-length transmembrane CD83 protein, another naturally occurring isoform of CD83 has been identified in the sera of healthy individuals, the so called soluble CD83 (sCD83), which consist of the above described extracellular domain of the transmembrane protein [137, 138]. The precise generation of sCD83 is still elusive, but latest data indicated the generation of sCD83 by alternative splice variants and/or shedding [137, 139]. Four different splice variants have been identified. The largest splice variant encodes for the full-length transmembrane CD83 protein, whereas the other three encode for shorter protein isoforms, which likely include sCD83 [139]. Strikingly, application of sCD83 has been described as a promising therapeutic compound to dampen immune responses in several disease models. However, the pharmacological potential of sCD83 and the broad expression of transmembrane CD83 on several immune cells underline the importance of further research on CD83.

1.4.1 Functionality of transmembrane CD83

With regard to the function of CD83, first striking results were obtained by Fujimoto and colleagues. The authors have described a specific defect in the development of single positive $CD4^+$ T cell and thus significantly reduced numbers of peripheral $CD4^+$ T cells in general CD83 knockout mice ($CD83^{-/-}$) . By the use of bone-marrow chimeras, they identified CD83 expression on thymic epithelial cells to be essential for adequate $CD4^+$ T cell development and selection in the thymus [136]. These results were later confirmed by Garcia-Martinez and colleagues by analysis of CD83 mutant mice, which resemble the above described phenotype of $CD83^{-/-}$ mice [140].

The fact that human and murine DCs upregulate CD83 upon activation suggests a costimulatory role for DC-expressed CD83 [117, 141-143]. Indeed, several studies have reported that CD83 deletion decreases MHCII expression on DCs, B cells and other antigen presenting cells in the thymus and the periphery, thereby misbalancing the immune response [136, 144]. Recently, the transmembrane domain of CD83 was demonstrated to provide an increase in MHCII expression by antagonizing the interaction of MHCII with the ubiquitin ligase MARCH1, which normally induces the ubiquitination and degradation of interacting molecules [145]. Additionally, several viruses including varicella-zooster virus and human cytomegalovirus were shown to

dampen the cell surface expression of CD83 on mature DCs upon infection. The downregulation of CD83 was accompanied by a reduced DC-mediated T cell activation [146, 147]. Furthermore, downregulation of CD83 surface expression on DCs mediated via interference with nuclear export of CD83 mRNA also reduced T cell stimulatory capacity [148]. Further studies underlined that knockdown of CD83 by the use of small interfering RNA (siRNA) decreases the ability of mature DCs to stimulate T cells [149, 150]. Taken together, these studies suggest a costimulatory role of CD83 expression on mature DCs.

On the contrary, other studies reported a normal development of CD83-deficient DCs and no significant effect of CD83 deletion on T cell stimulatory capacity could be observed [140, 151]. Even an enhanced T cell stimulatory capacity of CD83-deficient DCs was demonstrated [144]. In addition, in the *in vivo* mouse model of dextran sodium sulfate (DSS)-induced colitis DC-specific CD83 conditional knockout mice exhibited aggravated colitis symptoms compared to WT mice. In line with this, mucosal overexpression of CD83 protected mice from DSS-induced colitis, thereby a protective function of CD83, regulating DC activation and inhibiting the proinflammatory response, was assumed [152]. In summary, these studies suggest a regulatory role of DC-expressed CD83 during immune responses. Even if the results of DC-expressed CD83 are still controversial, all results indicate a more pivotal role of CD83 than being just a marker for mature DCs.

In addition to DCs, CD83 is also expressed on activated T cells. Several studies demonstrated an elevated surface expression of CD83 on human and murine $CD4^+$ T cells upon activation *in vitro*. CD83 expression on T cells is upregulated in response to stimulation with antigenic peptides, TCR-specific monoclonal antibodies and phytohaemagglutinin within hours and declines within two to three days post activation [126, 127, 129, 153-155]. Separated analysis of sorted $CD4^+CD25^+$ Treg cells and $CD4^+CD25^-$ Tconv cells revealed that both subsets upregulate CD83 expression upon stimulation. Nevertheless, CD83 expression on Treg cells was faster and higher in comparison to the expression on Tconv cells [127].

As already mentioned, the analysis of $CD83^{-/-}$ and CD83 mutant mice revealed a pivotal role for CD83 expression on thymic epithelial cells for adequate $CD4^+$ T cell development. Noteworthy, a remaining small population of $CD4^+$ T cells in the periphery of $CD83^{-/-}$ and CD83 mutant mice displayed an altered phenotype with a diminished response to allogenic stimulation [136, 140]. Furthermore, by adoptive

transfer experiments with the CD83-deficient $CD4^+$ T cells it was demonstrated that CD83 expression on $CD4^+$ T cells is necessary for T cell longevity [126]. Taken together, these results gave some first hints of an altered peripheral T cell activation, function and survival in the absence of intrinsic CD83 expression on T cells. However, the defect in T cell development in $CD83^{-/-}$ mice requires a conditional deletion or specific overexpression of CD83 to investigate the role of T cell-expressed CD83.

Until now, a few studies specifically addressed the effect of T cell-expressed CD83 on intrinsic T cell activation and function. Additionally, the extrinsic effect of T cell-expressed CD83 on the immune response in general was investigated. Interesting results were obtained by overexpression of CD83 using retroviral gene transfer. It was shown that transduction of $CD4^+CD25^-$ T cells with a CD83-encoding retrovirus confers immunosuppressive functions to these cells. Noteworthy, the inhibitory phenotype was accompanied by the induction of Foxp3 in 25 % of transduced cells. In addition, the adoptive transfer of the retrovirally-transduced $CD83^+$ T cells diminished the symptoms and immune response during the elicitation phase of a CHS reaction comparable to the transfer of freshly isolated Treg cells. Furthermore, the adoptive transfer of these cells prevented the onset of paralysis and dampened the inflammation in an experimental autoimmune encephalomyelitis (EAE) model [127]. In line with this, stimulation of $CD4^+$ T cells with TGF-β maintained CD83 expression on $CD4^+$ T cells, which correlated with the expression of Foxp3 and CD25 in these cells [153]. Moreover, a detailed analysis of sorted $CD83^+$ and $CD83^-CD4^+$ T cells from CD83eGFP reporter mice revealed that $CD83^+CD4^+$ T cells exhibit a Treg-like phenotype, indicated by a significantly increased expression of Treg-associated molecules including CTLA-4, CD25, Nrp-1 and Foxp3 compared to $CD83^-CD4^+$ T cells. In addition, $CD83^+CD4^+$ T cells mediated suppressive function *in vitro*. *In vivo* the cotransfer of $CD83^+CD4^+$ and $CD45RB^{high}CD4^+$ T cells into Rag1KO mice dampened the development of colitis compared to the cotransfer of $CD83^-CD4^+$ T cells [156]. Taken together, these studies clearly indicate a positive correlation of CD83 expression on T cells with a suppressive phenotype. Recently, Doebbeler and colleagues addressed the question, whether CD83 expression on $CD4^+$ T cells is essential for Treg cell differentiation and function. The group generated and analysed a Treg-specific CD83 conditional knockout mouse (CD83cKO). Interestingly, mice with a Treg-specific deletion of CD83 displayed significantly reduced frequencies of Treg cells in the periphery compared to WT littermates. These results were in line

with an impaired differentiation of naive T cells from CD83cKO mice into iTreg cells *in vitro*. However, the remaining T cells from Treg-specific CD83cKO mice showed a proinflammatory phenotype with decreased expression levels of Treg-specific markers and increased activation marker expression. Nevertheless, remaining CD83-deficient Treg cells were able to exert suppressive function similar to WT Treg cells *in vitro*. *In vivo* Treg-specific CD83cKO mice displayed an exacerbated disease onset and progression of EAE, most likely due to the reduced Treg cell frequencies [157].
In summary, CD83 expression on T cells correlates with a suppressive phenotype and Treg-specific deletion of CD83 leads to reduced Treg cell frequencies in the periphery. These results suggest a positive influence of CD83 expression on Treg cell differentiation and phenotype.

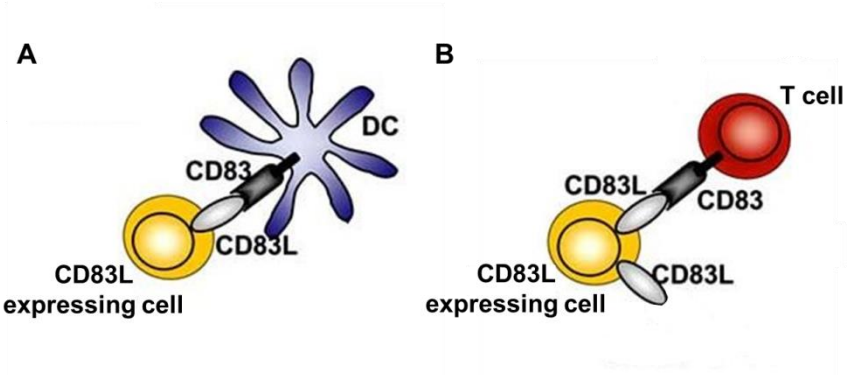

Figure 9: Supposed interaction of DC- and T cell-expressed CD83 with its ligand.
DCs (A) and T cells (B) do upregulate CD83 upon stimulation. CD83 possibly exerts its function by binding to a ligand (CD83L) on any CD83L-expressing cell. Signal transduction via receptor-ligand interaction might induce costimulatory or coinhibitory signals (adapted from [158]).

Independent of a costimulatory or coinhibitory function of transmembrane CD83, it is still elusive how CD83 exerts its function. One possibility is the binding of CD83 to a ligand. In Figure 9 binding of DC- and T cell-expressed CD83 to its ligand (CD83L) is schematically summarized. Indeed, several studies indicate the existence of a ligand for CD83, albeit the results concerning the CD83-ligand expressing cell population are controversial. Until now CD83 binding to human DCs [138], monocytes [159], human $CD8^+$ T cells [159, 160] and murine B2 cells [154] has been reported. Due to the fact that all of these cell populations express CD83 upon stimulation a homotypic interaction is also possible. For DC-expressed CD83 a homotypic interaction has been described recently [152]. However, the authors did not directly proof a biophysical interaction. Moreover, CD83 and its putative ligand could also be expressed on the same cell. In addition to a possible CD83-CD83L interaction, CD83 could also exert its function via the release of sCD83, which might be generated by enzymatic cleavage of transmembrane CD83 [137]

1.4.2 Functionality of sCD83

As described before, CD83 expression on T cells might be associated with an immunoregulatory function, whereas the function of DC-expressed CD83 is discussed controversially. However, results regarding the function of sCD83, the extracellular domain of the transmembrane CD83 protein, during immune responses are quiet concordant. Several studies have reported an anti-inflammatory and immunosuppressive effect *in vitro* and *in vivo*.

sCD83 has been discovered in the sera of healthy individuals and in the supernatant of activated DCs and B cells by Hock and colleagues [137]. Recently, sorted $CD83^+CD4^+$ T cells from CD83 reporter mice, which exert immunosuppressive function *in vitro*, were additionally shown to release sCD83 into the supernatant [156]. Furthermore, elevated levels of sCD83 have been detected in the sera of patients with hematological malignancies [161] and a direct negative correlation of sCD83 concentration in the sera of chronic lymphocytic leukemia patients and treatment-free survival has been observed [162]. Noteworthy, it was demonstrated that sCD83, released by tumor cells, inhibits $CD4^+$ and $CD8^+$ T cell proliferation [163]. Moreover, increased concentration of sCD83 has been detected upon infection of DCs with human cytomegalovirus accompanied by decreased T cell activation [164]. In summary, these data suggest an immunoregulatory function of sCD83 expression under normal conditions mediated via inhibition of unspecific effector cell activation. Additionally, sCD83 expression seems to be used as immune escape mechanism of malignant diseases and viruses.

Strikingly, in several *in vivo* models the treatment with sCD83 showed an immunosuppressive effect and prevented or ameliorated disease onset, symptoms and progression. Treatment with sCD83 has been reported to inhibit the onset and progression of murine EAE [165], systemic lupus erythematosus [166] and experimental autoimmune uveitis [167]. The application of sCD83 diminished transplant rejection in allogenic skin, heart, cornea and kidney transplantation models as well [168-171]. Moreover, it was reported that sCD83 treatment reduced mortality and disease symptoms in a murine chemically-induced colitis model accompanied by a significantly reduced secretion of proinflammatory cytokines [172]. Taken together, these data indicate a promising therapeutic potential of sCD83 for the modulation of immune responses *in vivo*.

The mechanism by which sCD83 exerts its immunosuppressive function has not been identified yet. However, application of sCD83 and recombinant CD83 fusion proteins has been shown to dampen DC maturation and DC-mediated T cell stimulation [121, 138, 139, 154, 165, 170]. Moreover, it was reported in different *in vivo* models that IDO expression is induced by sCD83, which seems to be relevant for the anti-inflammatory effect of sCD83 [168, 170, 172]. Recently, the myeloid differentiation factor-2 (MD-2), which serves as coreceptor within the TLR4/MD-2 receptor complex, has been identified as a high affinity-binding partner for sCD83. The authors demonstrated that sCD83 decreases T cell activation by binding to the TLR4/MD-2 complex on $CD14^+$ monocytes. Binding of sCD83 prevented the interaction with bacterial LPS, which would initiate a proinflammatory signaling cascade [173]. Noteworthy, alterations in the signaling cascade finally led to the expression of anti-inflammatory molecules including IDO. Nevertheless, it is important to mention that sCD83 might additionally interact with other molecules/receptors on other cells.

In summary, the supposed immunomodulatory role of DC- and T cell-expressed CD83 and the pharmacological potential of sCD83 as an anti-inflammatory agent underline the importance of further research on transmembrane and soluble CD83.

2 Aim

CD83 has been described as a marker for mature DCs for years, because it is strongly upregulated on DCs upon maturation. However, CD83 is also expressed on the surface of many other cell types including activated B and T cells.

Increasing evidence indicates that CD83 expression on T cells contributes to regulation of T cell activation and immune responses in general. However, the precise function of T cell-expressed CD83 is still elusive. Therefore, the aim of the present study was to carefully characterize the role of CD83 expression in Treg as well as Tconv cells.

First of all, the molecular and functional phenotype of naive, T cell-specific CD83 conditional knockout mice (CD4KO) was examined. *In vitro* analysis of $CD4^+$ T cells from CD4KO mice included T cell activation, proliferation and differentiation. In addition, CD4KO mice were analysed in an ongoing immune response to determine the impact of CD83 expression in T cells on the initiation and course of disease. For that purpose the CHS mouse model was chosen, because the T cell response is important for the disease outcome in this model. To carefully dissect the impact of CD83 expression in Tconv cells the T cell transfer model of chronic colitis was performed as well.

The broad expression of CD83 on several immune cells raised the question, whether CD83 has a conserved function or might act in distinct ways on different immune cells. Even though CD83 is a well-established marker for mature DCs, the function of DC-expressed CD83 is still discussed controversially. Using a DC-specific CD83 conditional knockout mouse (CD11cKO) the role of CD83 expression in DCs was analysed in more detail and compared to the function of CD83 expression in T cells.

3 Materials & Methods

3.1 Materials

3.1.1 Mice

CD83$^{flox/flox}$ mice

CD83$^{flox/flox}$ mice were generated on Balb/c background in cooperation with Ari Waisman, Mainz. The transgenic mice carry a CD83 gene, in which exon 3 is flanked with two loxp sites.

CD83$^{flox/flox}$ x CD4cre mice

CD83$^{flox/flox}$ x CD4cre mice were generated by breeding CD83$^{flox/flox}$ mice with CD4cre mice [174]. The transgenic mice have a T cell-specific deletion of the CD83 gene expression.

CD83$^{flox/flox}$ x CD4cre x HA mice

CD83$^{flox/flox}$ x CD4cre x HA mice were generated by backcrossing CD83$^{flox/flox}$ x CD4cre with TCR-HA mice. TCR-HA transgenic mice express a TCR specific for the peptide 110-120 from influenza virus hemagglutinin ($HA_{110-120}$) presented via MHCII (I-Ed) [175]. Hence, these mice do have CD83-deficient T cells specific for the $HA_{110-120}$ peptide.

CD83$^{flox/flox}$ x CD11ccre mice

CD83$^{flox/flox}$ mice were bred with CD11ccre mice [176] to generate CD83$^{flox/flox}$ x CD11ccre mice, which do have a CD11c^{+} dendritic cell-specific deletion of the CD83 gene expression.

CD83eGFP

CD83eGFP reporter mice express the enhanced green fluorescent protein (eGFP) under the control of the CD83 gene locus. An IRES2-eGFP reporter cassette was inserted right after the stop codon in exon 5 of the CD83 gene using homologous DNA recombination [177].

Rag2KO

Rag2KO mice are deficient for the recombination activating gene 2 (Rag2) [178]. Due to missing Rag2 expression these mice do not contain T and B cells and are immunodeficient.

Thy1.1

The T cells of these mice express the allele 1 of the Thy1/CD90 protein (Thy1.1/CD90.1).

TCR-HA x Thy1.1

T cells from TCR-HA x Thy1.1 mice express the allele 1 of the Thy1/CD90 protein (Thy1.1/CD90.1) and a TCR specific for the peptide 110–120 from influenza virus hemagglutinin (HA) presented via MHCII (I-Ed).

3.1.2 Nucleic acids, Enzymes, Peptides

Table 1: Nucleic acids, Enzymes, Peptides

Nucleic acid, Enzyme, Peptide	Manufacturer
Collagenase Type IV	Merck, Darmstadt, Germany
Deoxynucleotide set	Merck, Darmstadt, Germany
GeneRuler 100bp Plus DNA ladder	Thermo Fisher Scientific, Braunschweig, Germany
GoTaq Hot Start Polymerase	Promega, Mannheim, Germany
$HA_{110-120}$ peptide (I-Ed) from Influenza virus	Proimmune, Oxford, United Kingdom
M-MLV RT (H-) Point Mutant	Promega, Mannheim, Germany
Oligo(dT) 12-18 Primer	Thermo Fisher Scientific, Braunschweig, Germany
Proteinase K, recombinant, PCR Grade	Merck, Darmstadt, Germany
Primer	MWG-Biotech, Ebersberg, Germany Eurofins, Hamburg, Germany
Random Primers	Thermo Fisher Scientific, Braunschweig, Germany

3.1.3 Antibodies and Dyes for flow cytometry

Table 2: Fluorochrome-conjugated antibodies

Epitope	Fluorochrome	Clone	Manufacturer
CD3	AF647	145-2C11	BD Bioscience, Heidelberg, Germany
	PE	145-2C11	BD Bioscience, Heidelberg, Germany
CD4	APC	RM4-5	BD Bioscience, Heidelberg, Germany
	FITC	GK1.5	BD Bioscience, Heidelberg, Germany
	PB	RM4-5	BD Bioscience, Heidelberg, Germany
	PE	H129.19	BD Bioscience, Heidelberg, Germany
CD8	APC	53-6.7	BD Bioscience, Heidelberg, Germany
	BV510	53-6.7	BioLegend, San Diego, USA
	PB	53-6.7	BD Bioscience, Heidelberg, Germany
CD11b	PE	M1/70	BD Bioscience, Heidelberg, Germany
CD11c	V450	HL3	BD Bioscience, Heidelberg, Germany
CD25	FITC	PC61	BD Bioscience, Heidelberg, Germany
	PE	7D4	BD Bioscience, Heidelberg, Germany
	PE-Cy7	PC61	BD Bioscience, Heidelberg, Germany
CD40	FITC	3/23	BD Bioscience, Heidelberg, Germany
CD44	APC	IM7	BD Bioscience, Heidelberg, Germany
CD45RB	APC	16A	BioLegend, San Diego, USA
CD62L	PE	MEL-14	Thermo Fischer Scientific, Braunschweig, Germany
	PE-Cy5	MEL-14	Thermo Fischer Scientific, Braunschweig, Germany
CD69	FITC	H1.2F3	BD Bioscience, Heidelberg, Germany
	PE-Cy7	H1.2F3	BD Bioscience, Heidelberg, Germany
CD80	PE-Cy7	16-10A1	BD Bioscience, Heidelberg, Germany
CD83	APC	Michel 19	BD Bioscience, Heidelberg, Germany
CD86	PE-Cy7	GL1	BD Bioscience, Heidelberg, Germany
CD90.1	FITC	OX-7	BD Bioscience, Heidelberg, Germany
CD90.2	PE	53-2.1	BD Bioscience, Heidelberg, Germany
CD152	PE	UC10-4F10-11	BD Bioscience, Heidelberg, Germany
CD297	PE	J43	BD Bioscience, Heidelberg, Germany
IL-17A	BV421	TC11-18H10.1	Biolegend, San Diego, USA

IFN-γ	PE	XMG1.2	BD Bioscience, Heidelberg, Germany
Foxp3	APC	FJK-16s	Thermo Fischer Scientific, Braunschweig, Germany
	FITC	FJK-16s	Thermo Fischer Scientific, Braunschweig, Germany
	PE	FJK-16s	Thermo Fischer Scientific, Braunschweig, Germany
	PerCP-Cy5.5	FJK-16s	Thermo Fischer Scientific, Braunschweig, Germany
I-A/I-E (MHCII)	BV510	M5/114.15.2	BioLegend, San Diego, USA
Ki-67	PE-Cy7	SolA15	Thermo Fischer Scientific, Braunschweig, Germany
p-Zap-70 (Tyr319)	AF488	/	Cell Signaling, Frankfurt am Main, Germany

Table 3: Dyes

Dye	Manufacturer
eBioscience™ Cell Proliferation Dye efluor670	Thermo Fischer Scientific, Braunschweig, Germany
Invitrogen™ CellTrace™ CFSE Proliferation Kit	Thermo Fischer Scientific, Braunschweig, Germany

3.1.4 Antibodies and Cytokines for stimulation/neutralization

Table 4: Unconjugated antibodies

Epitope	Clone	Manufacturer
anti-IL-2	JES6-1A12	Thermo Fischer Scientific, Braunschweig, Germany
anti-IL-4	11B11	Thermo Fischer Scientific, Braunschweig, Germany
anti-IFN-γ	XMG12	R&D systems, Minneapolis, USA
anti-CD28	37.51	BD Biosciences, Heidelberg, Germany

Table 5: Cytokines

Cytokine	Manufacturer
rmIL-2	Thermo Fischer Scientific, Braunschweig, Germany
rmIL-12	R&D systems, Minneapolis, USA
rmIL-1β	R&D systems, Minneapolis, USA
rmIL-6	R&D systems, Minneapolis, USA
rmIL-21	R&D systems, Minneapolis, USA
rmIL-23	R&D systems, Minneapolis, USA
rh-TGFβ	R&D systems, Minneapolis, USA

3.1.5 Chemicals

Table 6: Chemicals

Chemicals	Manufacturer
1-fluoro-2,4-dinitrobenzene	Merck, Darmstadt, Germany
β-Mercapthoethanol	Merck, Darmstadt, Germany
LE Agarose	Biozym, Oldendorf, Germany
Acetone, AR, ≥99.5%	Merck, Darmstadt, Germany
AutoMACS Pro Washing Solution	Miltenyi Biotec, Bergisch Gladbach, Germany
AutoMACS Running Buffer	Miltenyi Biotec, Bergisch Gladbach, Germany
Brefeldin A (BFA)	Merck, Darmstadt, Germany
DEPC-water	Carl Roth, Karlsruhe, Germany
Ethidium bromide 1 %	Carl Roth, Karlsruhe, Germany
Ethylenediaminetetraacetic acid (EDTA)	Carl Roth, Karlsruhe, Germany
Ethylene glycol tetraacetic acid (EGTA)	Carl Roth, Karlsruhe, Germany
Ethanol, absolute and denatured	Carl Roth, Karlsruhe, German
Fetal calf serum (FCS)	Biochrom GmbH, Berlin, Germany
FACS Flow Sheath Fluid	BD Biosciences, Heidelberg, Germany
FACS Clean Solution	BD Biosciences, Heidelberg, Germany
FACS Rinse Solution	BD Biosciences, Heidelberg, Germany
IGEPAL® CA-630	Merck, Darmstadt, Germany
Ionomycin Calcium Salt	Merck, Darmstadt, Germany
Lipopolysaccharide (LPS)	InvivoGen, San Diego, USA

Magnesium chloride ($MgCl_2$)	Carl Roth, Karlsruhe, Germany
Paraformaldehyde (PFA)	Carl Roth, Karlsruhe, Germany
Phorbol-12-myristate-13-acetate (PMA)	Merck, Darmstadt, Germany
Penicillin	Merck, Darmstadt, Germany
Streptomycin	Merck, Darmstadt, Germany
Trypan blue	Merck, Darmstadt, Germany

3.1.6 Consumables

Table 7: Consumables

Consumables	Manufacturer
Culture plates (6-, 12-, 24-, 96- well)	Greiner BioOne, Frickenhausen, Germany
Cell strainer (40, 70, 100 µm)	Falcon, Durham, USA
Disposable syringe (2, 10 ml)	BD Biosciences, Heidelberg, Germany
Micro tubes (1,5; 2 ml)	Sarstedt, Nümbrecht, Germany
Needle	BD Biosciences, Heidelberg, Germany
Petri dish, 92 x 16 mm	Sarstedt, Nümbrecht, Germany
Pipettes 5, 10, 25 ml	Greiner BioOne, Frickenhausen, Germany
Pipette tips 10, 200, 1000 µl	Biozym, Thermo Fisher Scientific, Sarstedt
Tubes (15, 50ml)	Greiner BioOne, Frickenhausen, Germany
Round bottom plates (96-well)	Greiner BioOne, Frickenhausen, Germany
Syringe 1ml	BD Biosciences, Heidelberg, Germany

3.1.7 Kits

Table 8: Kits

Kit	Manufacturer
$CD4^+$ T cell Isolation Kit, mouse	Miltenyi Biotec, Bergisch Gladbach, Germany
$CD8^+$ T cell Isolation Kit, mouse	Miltenyi Biotec, Bergisch Gladbach, Germany
$CD11c^+$ MicroBeads UltraPure, mouse	Miltenyi Biotec, Bergisch Gladbach, Germany
ELISA Kit for Cluster Of Differentiation 83 (CD83)	Cloud-Clone Corp., Katy, USA
Foxp3/Transcription factor Staining Buffer Set	Thermo Fisher Scientific, Braunschweig, Germany

Mouse Luminex Assay LXSAMS	R&D systems, Minneapolis, USA
RNeasy Mini Kit	Qiagen, Hilden, Germany

3.1.8 Media and Buffer

Table 9: Media and Buffer

Media/Buffer	Compound
ACK-Buffer	Aqua dest. + 8,29 g/l Ammonium chloride + 1 g/l Monopotassium phosphate + 0,1 mM EDTA
IMDM complete media	IMDM with GlutaMax™ I and 25mM HEPES + 10 % (v/v) FCS + 100 µg/ml Streptomycin + 100 U/ml Penicillin + 25 µM ß-Mercaptoethanol
FACS-Buffer	PBS-Buffer + 2 % (v/v) FCS + 2 mM EDTA
PBS-Buffer	Aqua dest. + 8 g/l Sodium chloride + 2 g/l Potassium chloride + 1.44 g/l Disodium phosphate + 0.2 g/l Potassium phosphate
PBS/EDTA-Buffer	PBS-Buffer + 3 mM EDTA
PBS/FCS-Buffer	PBS-Buffer + 2 % (v/v) FCS
RPMI 1640 complete media	RPMI with GlutaMax™ I and 25 mM HEPES

	+ 10 % (v/v) FCS + 100 µg/ml Streptomycin + 100 U/ml Penicillin + 2 mM L-Glutamine
RPMI/FCS media	RPMI with GlutaMax™ I and 25 mM HEPES + 20 % (v/v) FCS
RPMI/FCS/EGTA/$MgCl_2$ media	RPMI with GlutaMax™ I and 25 mM HEPES + 1 % (v/v) FCS + 1 mM EGTA + 1,5 mM $MgCl_2$
Tail-Buffer	Aqua dest. + 100 mM Tris HCL + 200 mM NaCl + 5 mM EDTA + 0,2 % SDS

3.1.9 Equipment

Table 10: Equipment

Equipment	Manufacturer
autoMACS® Pro Separator	Miltenyi Biotec, Bergisch Gladbach, Germany
Binokular Axiovert Z1	Carl Zeiss Microscopy GmbH, Jena, Germany
BD FACS Aria II cell sorter	BD Biosciences, Heidelberg, Germany
Centrifuge MULTIFUGE 3SR+	Thermo Fisher Scientific, Waltham, USA
Centrifuge 5417R	Eppendorf AG, Hamburg, Germany
Flow cytometer BD LSRII	BD Biosciences, Heidelberg, Germany
GelDoc station	INTAS®, Göttingen, Germany
Electrophoresis Apparatus Horizon 11.14	Analytik Jena, Jena, Germany
Heracell 150i CO2-Incubator	Thermo Scientific, Darmstadt, Germany
Luminex AtheNA Multy-Lyte System	Progen, Heidelberg, Germany

Sunrise^{TM} absorbance microplate reader	Tecan, Männedorf, Switzerland
Magnetic stirrer Variomag	Thermo Scientific, Darmstadt, Germany
NanoDrop 1000 Spectrophotometer	Peqlab, Erlangen, Germany
Neubauer counting chamber (0.0025mm^2)	Superior, Marienfeld, Germany
Thermocycler T3000	Biometra, Goettingen, Germany
Thermomixer comfort	Eppendorf, Hamburg, Germany
Work Bench Msc Advantage	Thermo Scientific, Darmstadt, Germany
Vortexer D-6013	Neo Lab, Heidelberg, Germany
Water bath	GFL, Burgwedel, Germany

3.1.10 Software

Table 11: Software

Software	Manufacturer
BD FACSDIVA^{TM} Software	BD Biosciences,Heidelberg, Germany
Graph Pad Prism 7.03	GraphPad Software, La Jolla, USA
Magellan^{TM} Tracker V7.2	Tecan, Männedorf, Switzerland

3.2 Methods

3.2.1 Animal procedures

All animals used in this project were bred and housed in the animal facility of the University Hospital Essen under specific pathogen-free conditions. The performed animal experiments were in accordance with the guidelines of the German Animal Protection Law and approved by State Agency for Nature, Environment and Consumer Protection (LANUV), North Rhine-Westphalia, Germany. Female as well as male mice from the age of six weeks were used for experiments. Mice were housed in a 12:12-hour light and dark cycle and received water and food *ad libitum*.

3.2.1.1 Contact hypersensitivity model

To investigate the role of T cell-expressed CD83 *in vivo,* the CHS model was used. In this model the treatment and challenge of the skin with a hapten induces an immune

response against hapten-complexed proteins leading to a local contact hypersensitivity reaction at the site of challenge.

CD83 conditional knockout (CD4KO) mice and wildtype (CD4WT) littermates were treated with 100 µl of a 0,5 % DNFB-solution in acetone and olive oil (3:1) on the shaved back to initiate the immune response (sensitization phase). Five days after sensitization, mice were challenged with 20 µl of a 0,2 % solution of DNFB on the right ear (elicitation phase). The inflammation parameter indicated by the ear swelling was calculated by measuring the ear thickness before and two days post treatment. Furthermore, T cells of the spleen and the dLNs next to the treated ear were analysed by flow cytometry for the expression of several cell surface markers, intracellular cytokines and transcription factors as described in chapter 3.2.3.2.

3.2.1.2 T cell transfer model of chronic colitis

In the T cell transfer model of chronic colitis, the transfer of naive, Treg-depleted T cells from donor mice into congenic immunodeficient recipient Rag2KO mice leads to the induction of chronic colitis due to an induced TH1/TH17 immune response against the antigens from commensal bacteria in the gut.

In order to induce chronic colitis, spleens from CD4WT and CD4KO donor mice were isolated. Single cell suspensions were prepared (see chapter 3.2.3.1) and $CD4^+$ T cells were enriched using magnetic activated cell sorting (MACS) (see chapter 3.2.3.3.1). From the enriched $CD4^+$ T cells naive $CD45RB^{high}CD4^+$ T cells were isolated via fluorescence activated cell sorting (FACS) (see chapter 3.2.3.3.2). Subsequently, 5×10^5 FACS-sorted, naive $CD4^+CD45RB^{high}$ T cells were diluted in 100 µl PBS-Buffer and injected intraperitoneally (i.p.) into immunodeficient Rag2KO mice. Body weight was assessed over time. About five weeks post induction, when colitis symptoms like diarrhea and bloody stool were detected, mice were sacrificed by carbon monoxide asphyxiation. Blood samples were obtained by puncturing the heart with a syringe. Subsequently, blood was centrifuged at 10000 x g for 15 min and analysed for different cytokines and soluble CD83 (sCD83) (see chapter 3.2.3.5). In addition, spleen, mesenteric lymph nodes (mLNs) and colon were excised and prepared (see chapter 3.2.3.1) for flow cytometric analysis. To examine immunopathology, spleen weight and colon weight to length ratio were determined. Furthermore, histopathologic analysis of colons was performed by Robert Klopfleisch (Freie Universität, Berlin). In short, colons were flushed with PBS-Buffer, embedded in a tissue cassette and placed in 4 % PFA-solution for fixation. Afterwards, colons

were embedded in paraffin, trimmed to 4 µm sections using a microtome and stained with hematoxylin and eosin (H&E). Oral, middle and rectal part of the colon were microscopically analysed and scored for different histopathological parameters (infiltration of inflammatory cells in the lamina propria and tela submucosa, mucous defective, neutrophil infiltration, hyperplasia, crypt abscess). The scoring scale ranged from zero for no pathology up to three for severe pathology.

3.2.2 Molecular biological methods

3.2.2.1 Genotyping of transgenic mice

In order to determine, whether mice carry a WT and/or transgenic allele of the gene of interest, tail biopsies were taken four weeks after birth. Biopsies were digested overnight in 90 µl Tail-Buffer containing 10 µl Proteinase K (10 mg/ml) at 56 °C followed by heat inactivation for 10 min at 95 °C in a Thermomixer comfort (Eppendorf). Subsequently, samples were centrifuged at 10000 x g for 10 min and the supernatant was diluted 1:50 in Aqua dest. Depending on the mouse strain polymerase chain reactions (PCR) with primer pairs specific for the target genes (Table 12) were performed to determine the genotype.

Table 12: Primer pair sequences for genotyping

Transgene	Sequence 5`-3`	Annealing temperature T_a (°C)
CD83eGFP	CAGTGACCCTTCCTAAGACAGAAAC	61
	CTTCAAGAAGCTTCCAGAGGAACTG	
CD83^{floxflox}	AATGTGGCCCAACACAAAG	55
	AGAGAAACACTGGCTGGGAGAC	
CD4cre	ACGACCAAGTGACAGCAATG	60
	CTCGACCAGTTTAGTTACCC	
CD11ccre	ACTTGGCAGCTGTCTCCAAG	63
	GCGAACATCTTCAGGTTCTG	
FICwt/ FICtg	TGTGTGATAGTGCCCGTGGTTC	61
	TTCGCAAGAAGAGGAGCCAACG	
	CTGCTTCCTTCACGACATTCAAC	61
	AAGTGCTTTGTGCGAGTGGAGAGC	
Rag2KO/neo	GCTATTCGGCTATGACTGGG	57

	GAAGGCGATAGAAGGCGATG ATGTCCCTGCAGATGGTAACA GCCTTTGTATGAGCAAGTAGC	57
TCR-HA	CCTGAACTGGGGATTCTACTCTTCC AGTCAGCTTATTATTGCCTCCACTC	58

The PCR was performed by adding 1 µl of diluted genomic DNA from the biopsies to 19 µl Mastermix (Table 13). Afterwards samples were run on Thermocycler T300 (Biometra). The PCR program (Table 14) for the different amplification products only differed concerning the annealing temperature.

Table 13: Mastermix for genotyping PCR

Mastermix for PCR
1x Green GoTaq Flexi Buffer (5x)
1,5 mM MgCl2
1 mM dNTPs
5 µM forward primer
5 µM reverse primer
0,5 U GoTaq Hot Start Polymerase
ad 20 µl Aqua dest.

Table 14: PCR program for genotyping

	10 cycles			**27 cycles**		
temperature (°C)	95	T_a	72	95	T_a	72
time (mm:ss)	00:30	1:30	1:30	00:15	00:45	00:45

3.2.2.2 RNA isolation

RNA of stimulated primary spleen cells was isolated using the RNeasy Mini Kit (Qiagen) according to manufacturer`s instructions. After isolation RNA concentration was determined using NanoDrop spectrophotometer (Peqlab). Until further usage RNA was stored at -80 °C.

3.2.2.3 Synthesis of complementary DNA

For synthesis of complementary DNA (cDNA), 0,5 µl Oligo(dT) and 0,5 µl Random Primer (Thermo Fisher Scientific) were added to 1-2 µg RNA diluted in 13 µl DEPC

water (Table 15) and incubated in the Thermocycler T3000 (Biometra) for 10 min at 70 °C. Subsequently, samples were cooled down, the residual compounds of the Mastermix were added on ice and synthesis of cDNA was performed at 42 °C for 1 h. In the end, samples were heated to 95 °C to inactivate the enzyme (Table 16).

Table 15: Mastermix for cDNA synthesis

Mastermix for cDNA synthesis
0,5 µl Oligo(dT)
0,5 µl Random Primer
1 µl dNTPs (10 mM)
4 µl M-MLV RT 5x Reaction Buffer
1 µl M-MLV RT (H-) Point Mutant

Table 16: Program for cDNA synthesis

Step	1	2	3
temperature (°C)	70	42	95
time (mm:ss)	10:00	60:00	05:00

3.2.2.4 Semi-quantitative PCR

To validate the deletion of the CD83 gene expression in CD83 conditional knockout mice, semi-quantitative PCR was performed. Therefore, PCR with primer pairs specific for the CD83 gene was carried out. RPS9 housekeeping gene PCR served as control (Table 17).

Table 17: Primer pair sequences for semi-quantitative PCR

Transgene	Sequence 5'-3'	Annealing temperature T_a (°C)
RPS9	CTGGACGAGGGCAAGATGAAGC	55
	TGACGTTGGCGGATGAGCACA	
CD83	TCGAGGCCCCCAGGAGAA	55
	TTGCAGGTGAAAATGATGAGTGTC	

After RNA isolation of stimulated primary spleen cells (see chapter 3.2.3.1.2) and subsequent cDNA synthesis (see chapter 3.2.2.3), PCR was performed by adding

1 µl cDNA to 19 µl Mastermix (Table 18). Afterwards, samples were run on Thermocycler T300 (Biometra) using the specific PCR-program (Table 19).

Table 18: Mastermix for semi-quantitative PCR

Mastermix for Semi-quantitative PCR
1x Green GoTaq Flexi Buffer (5x)
1,5 mM $MgCl_2$
1 mM dNTPs
5 µM forward primer CD83/ RPS9
5 µM reverse primer CD83/RPS9
0,5 U GoTaq Hot Start Polymerase
ad 20µl Aqua dest.

Table 19: Program for semi-quantative PCR

	10 cycles			**27 cycles**		
temperature (°C)	95	T_a	72	95	T_a	72
time (mm:ss)	00:30	1:30	1:30	00:15	00:45	00:45

3.2.2.5 Agarose gel electrophoresis

Amplification products of the PCR were analysed by electrophoresis on a 1 % agarose gel containing ethidium bromide to visualize the DNA under ultraviolet light afterwards. Visualization was performed using a GelDoc Station (Intas).

3.2.3 Cell biological methods

3.2.3.1 Preparation on single cell suspensions

To obtain single cell suspensions from different organs several protocols were used. Before removing spleen, lymph nodes or colon, mice were sacrificed by carbon monoxide asphyxiation.

3.2.3.1.1 Spleen

To exclude erythrocytes from single cell suspensions, spleens were rinsed with ACK-Buffer while simultaneously squeezing with tweezers. The whole cell suspension was filtered through a 70 µm cell strainer, which was rinsed with FACS-Buffer afterwards. Cell suspension was centrifuged for 10 min at 300 x g. The cell pellet was

resuspended in adequate volume of IMDM complete media or FACS-Buffer and kept on ice until further use.

3.2.3.1.2 Lymph nodes

Lymph nodes were homogenized with the stamp of a syringe in a 70 µm cell strainer located in a petri dish with 10 ml FACS-Buffer. The cell strainer was washed with 5 ml FACS-Buffer, cell suspension was centrifuged at 300 x g for 10 min and the cell pellet was resuspended in IMDM complete media. Until further use, the cell suspension was kept on ice.

3.2.3.1.3 Lamina propria lymphocytes

To isolate lymphocytes from the colon lamina propria (LP) colons were removed, flushed with PBS-Buffer and cut into small pieces of about 1 cm length. The colon pieces were washed twice in 40 ml PBS/EDTA-Buffer at 37 °C for 15 min under permanent stirring. Afterwards buffer was removed and colon pieces were stirred again in RPMI/FCS/EGTA/$MgCl_2$ media for 15 min at 37 °C repeated twice. Colon pieces were transferred into a 50 ml tube containing 10 ml RPMI/FCS/EGTA/$MgCl_2$ media and mixed for 15 s on a Vortexer (Neo Lab). Tissue was washed in a 70 µm cell strainer with PBS-Buffer and homogenized with a scissor in a beaker glass subsequently. For digestion tissue was stirred for 1 h at 37 °C in 30 ml RPMI/FCS media supplemented with collagenase IV (100 U/ml). After 30 min incubation cell suspension was raised up and down in a syringe ten times to further homogenize tissue. The step was repeated after another 30 min stirring. Afterwards the cell suspension was filtered through a 40 µm cell strainer and centrifuged at 300 x g for 10 min. The cells were resuspended in IMDM complete media and filtered again through a 30 µm cell strainer and stored on ice until further use.

3.2.3.2 Flow cytometry

Flow cytometric analysis was performed using the Flow cytometer BD LSRII (BD Bioscience). Data was visualized and analysed with FACS DIVA software (Version 8.0.1).

3.2.3.2.1 Surface staining

The expression of different surface proteins was analysed by flow cytometry. Therefore, single cell suspensions were seeded in a 96-well round bottom plate, centrifuged at 300 x g for 5 min and resuspended in FACS-Buffer supplemented with the fluorochrome-conjugated antibodies of interest. After 10 min incubation at 4 °C in the dark, cells were washed by adding FACS-Buffer followed by centrifugation at 300 x g for 5 min. To perform FACS analysis, cells were resuspended in an adequate volume of FACS-Buffer.

3.2.3.2.2 Intracellular staining

For intracellular cytokine staining cells were restimulated for 4 h in IMDM complete media supplemented with 1 µg/ml ionomycin, 10 ng/ml phorbol-12-myristate-13 acetate (PMA) and 5 µg/ml Brefeldin (BFA). After surface staining (see chapter 3.2.3.2.1), cells were washed with PBS-Buffer, resuspended in 2 % paraformaldehyde and incubated 15 min at 4°C in the dark for fixation. Cells were washed again with PBS-Buffer and incubated 4 min at room temperature (RT) in 0,1 % IGEPAL-630 to permeabilize the cells. Intracellular staining with fluorochrome-conjugated antibodies diluted in FACS-Buffer was performed for 30 min at 4°C in the dark followed by another washing step. Finally, cells were resuspended in an adequate volume of FACS buffer and analysed.

3.2.3.2.3 Intranuclear staining

Intranuclear staining for the transcription factor Foxp3 was performed with Foxp3/Transcription Factor Staining Buffer Set (Thermo Fisher Scientific) according to manufacturer`s instructions. The kit was also used to stain for nuclear protein Ki67 and Granzyme B, which is located in granules inside the cell.

3.2.3.3 Cell separation

3.2.3.3.1 Magnetic activated cell sorting

For separation and enrichment of different cell populations from mouse spleen, single cell suspensions were prepared (see chapter 3.2.3.1.1) and cells of interest isolated using $CD4^+$/$CD8^+$ T cell isolation kit, mouse or CD11c MicroBeads UltraPure, mouse and autoMACS® Pro Separator (Miltenyi Biotec) following manufacturer`s

instructions. MACS-sorted $CD4^+$/$CD8^+$ T cells and $CD11c^+$ DCs were resuspended in FACS-Buffer until further processing.

3.2.3.3.2 Fluorescence activated cell sorting

Prior to fluorescence activated cell sorting cells of interest were enriched using MACS technology (see chapter 3.2.3.3.1). MACS-sorted $CD4^+$ T cells were stained with fluorochrome-conjugated antibodies against surface proteins CD4 and CD25 for separation of $CD4^+CD25^-$ and $CD4^+CD25^+$ T cells using BD FACS Aria II cell sorter (BD Bioscience). FACS-sorted cells were resuspended in IMDM complete media and used for different assays (see chapter 3.2.3.4).

3.2.3.4 *In vitro* functional assays

3.2.3.4.1 Proliferation Assay

$CD4^+CD25^-$ T cells were isolated using BD FACS Aria II cell sorter (BD Bioscience) and stained with either Cell Proliferation Dye efluor670 or CellTrace™ CFSE (Thermo Fischer Scientific) according to manufacturer`s instructions. 1×10^5 stained $CD4^+CD25^-$ T cells were cocultured with 4×10^5 irradiated splenocytes and stimulated with 1 µg/ml anti-CD3 or left untreated. After three days of culture proliferation was determined by measuring loss of efluor670 or CFSE signal via flow cytometry.

3.2.3.4.2 Inhibition Assay

To analyse the inhibitory function of Treg cells, FACS-sorted $CD4^+CD25^+$ cells were cocultured with MACS-sorted, efluor670 labeled $CD4^+CD25^-$ responder T cells in the presence of 4×10^5 irradiated splenocytes and 1 µg/ml anti-CD3. Treg and responder T cells were cocultured in different ratios. Unstimulated and anti-CD3 stimulated responder T cells cultured without Treg cells served as control. Inhibition of Treg cells was determined as proliferation of corresponding responder T cells.

3.2.3.4.3 Differentiation on TH1, TH17 and induced Treg cells *in vitro*

To determine the differentiation capacity of $CD4^+$ T cells into TH1, TH17 and iTreg cells *in vitro,* 1×10^6 FACS-sorted $CD4^+CD25^-$ T cells were cultivated in IMDM complete media for three (iTregs) or six days (TH1, TH17) in a 24-well plate, which was precoated with 5 µg/ml anti-CD3 for 1,5 h at 37 °C. IMDM complete media was

supplemented with different cytokines and antibodies promoting appropriate T cell differentiation (Table 20). TH0 differentiation always served as control. To assess the amount of *in vitro* induced Treg cells, cells were harvested after three days of cultivation, stained for Foxp3 (see chapter 3.2.3.2.3) and analysed by flow cytometry. For TH1 differentiation cells were splitted 1:1 at day three and fresh media was added. At day five half the media was replaced by fresh media. After six days of culture, TH1 differentiation was examined by measuring IFN-γ production after 4 h restimulation (see chapter 3.2.3.2.2). To differentiate TH17 cells, half the media was replaced by fresh media after three days. On day five cells were splitted 1:1 and fresh media was added. TH17 cells were analysed one day later by flow cytometry for intracellular IL-17 production post 4 h restimulation (see chapter 3.2.3.2.2).

Table 20: Differentiation media

T cell subtype	Supplement	Concentration
TH0	anti-CD28	1 µg/ml
TH1	rmIL-12	20 ng/ml
	anti-IL-4	200 ng/ml
	anti-CD28	1 µg/ml
TH17	rmIL-1β	20 ng/ml
	rmIL-6	50 ng/ml
	rmIL-21	100 ng/ml
	rmIL-23	20 ng/ml
	rh-TGFβ	2 ng/ml
	anti-IL-4	200 ng/ml
	anti-IFN-γ	200ng/ml
	anti-IL-2	200 ng/ml
	anti-CD28	1 µg/ml
iTreg	rmIL-2	100 U/ml
	rh-TGFβ	5 ng/ml
	anti-CD28	1 µg/ml

3.2.3.4.4 Coculture

To assess, whether T cell-specific deletion of CD83 affects phenotype of any other cell populations cocultures were prepared. Hence, 5×10^4 MACS-sorted $CD11c^+$ spleen cells from Thy1.1 mice were cocultured with $2{,}5 \times 10^5$ FACS-sorted $CD4^+CD25^-$ T cells from TCR-HA specific CD4KO and CD4WT mice for 16 h without stimulation or in the presence of 1 µg/ml $HA_{110\text{-}120}$ peptide. Activation of $CD11c^+$ DCs was analysed by flow cytometry afterwards.

3.2.3.5 Detection of cytokines and sCD83

Concentrations of different cytokines in mouse serum or supernatants, taken from *in vitro* culture of primary mouse spleen cells, were determined using Mouse Luminex Assay LXSAMS-09 (R&D Systems) according to manufacturer`s instructions. The assay was measured and quantified on Luminex AtheNA Multi-Lyte System (Progen) using Luminex IS software.

The concentration of sCD83 in mouse serum was quantified using the ELISA Kit for Cluster Of Differentiation 83 (CD83) (Cloud-Clone Corp.) according to manufacturer`s instructions. The ELISA plate was measured on Sunrise™ absorbance microplate reader (Tecan) with Magellan™ Tracker V7.2.

4 Results

4.1 T cell-specific CD83 expression

CD83 is a well-established marker for mature DCs. However, CD83 expression was also detected on many other cell types such as thymic epithelial cells, NK cells, macrophages, activated B and T cells [115, 118, 126, 130, 134, 136, 179]. In contrast to CD83 expression on DCs, information about T cell-expressed CD83 is relatively limited and results obtained so far are quite controversial. To gain further insight, we studied the impact of CD83 expression on different T cell subsets in more detail.

4.1.1 $CD83^-$ and $CD83^+$ T cells have a different expression profile

To investigate the role of CD83 expression on T cells, first $CD83^-$ and $CD83^+$ T cells were analysed for their phenotype.

Therefore, $CD83^-$ and $CD83^+$ $CD4^+$ as well as $CD8^+$ T cells from CD83eGFP reporter mice were separated by FACS. The gating strategy used for separation is depicted in Figure 10A. Among all $CD4^+$ or $CD8^+$ T cells, enriched by MACS before, lymphocytes were identified concerning their size and granularity. In a next step, single cells were discriminated, followed by identification of $CD83^-$ and $CD83^+$ T cells by the eGFP signal and surface staining of the CD4 or CD8 coreceptor (Figure 10A). $CD83^-$ as well as $CD83^+$ T cells were analysed for the expression of several surface molecules by flow cytometry before and after stimulation *in vitro*. Naive $CD83^+$ $CD4^+$ T cells showed increased expression of CD69 and PD-1 compared to $CD83^-$ $CD4^+$ T cells (Figure 10B). Upon stimulation $CD83^-$ as well as $CD83^+$ $CD4^+$ T cells significantly upregulated CD69 and PD-1 expression compared to their naive counterparts. Interestingly, $CD83^+$ $CD4^+$ T cells showed significantly less CD69 expression upon stimulation compared to $CD83^-$ $CD4^+$ T cells. In contrast, PD-1 expression was significantly increased on stimulated $CD83^+$ compared to $CD83^-$ $CD4^+$ T cells. In addition, $CD8^+$ T cells were analysed (Figure 10C). $CD83^-$ and $CD83^+$ $CD8^+$ T cells exhibited the same expression pattern like $CD4^+$ T cells, indicating a conserved function of CD83 on $CD4^+$ and $CD8^+$ T cells. Noteworthy, $CD83^-$ $CD4^+$ as well as $CD8^+$ T cells did upregulate CD83 expression upon stimulation, even to a smaller extent compared to $CD83^+$ T cells (Figure 10B-C).

Taken together, these results give a first hint that intrinsic CD83 expression might balance T cell activation.

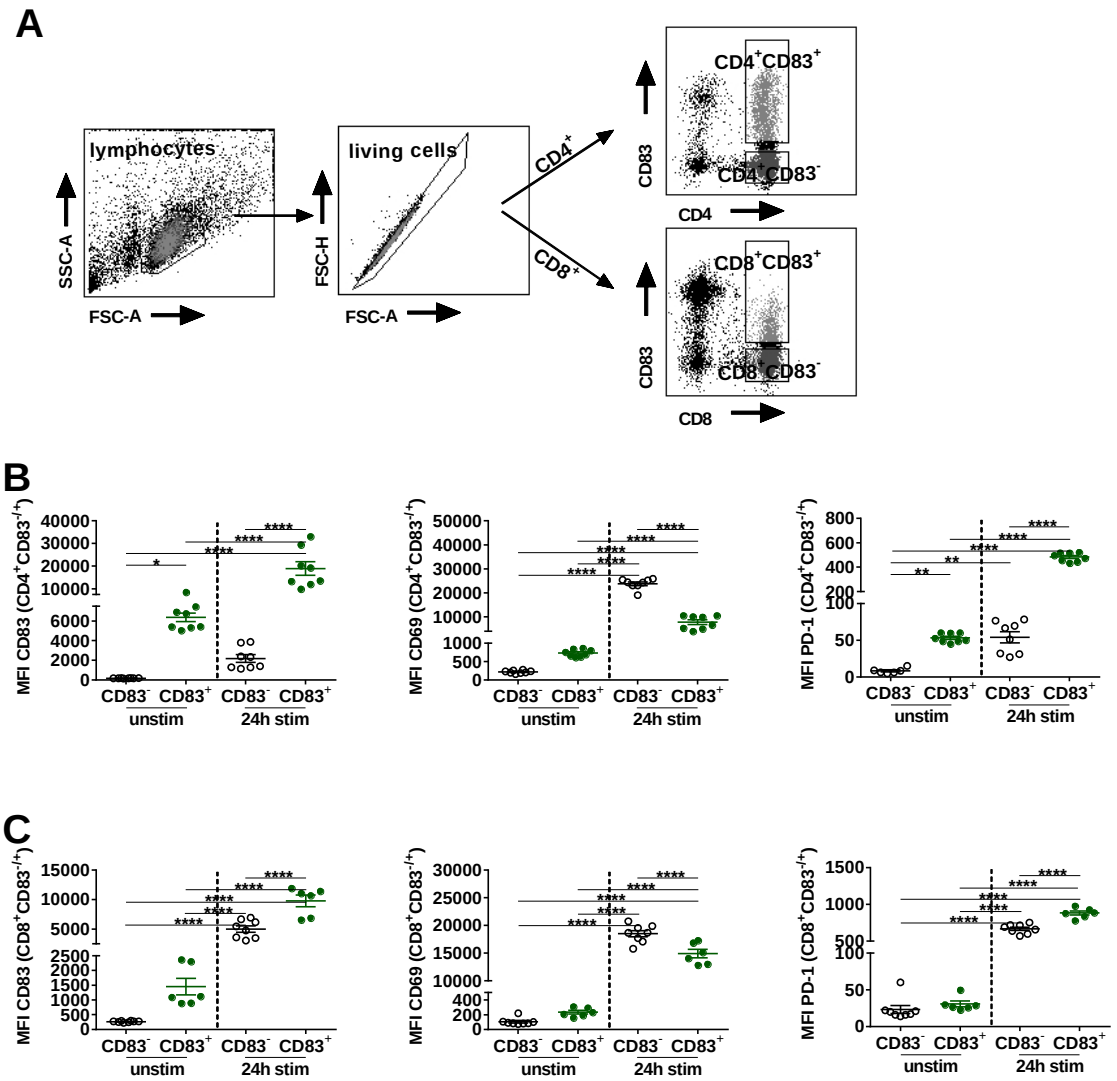

Figure 10: CD83⁻ and CD83⁺ T cells show a different expression profile.
(A-C) CD83⁻ and CD83⁺ CD4⁺ as well as CD8⁺ T cells from CD83eGFP reporter mice were separated by FACS. Sorted CD83⁻ and CD83⁺ T cells were either left unstimulated or stimulated for 24 h with anti-CD3 followed by analysis of the expression of different surface markers by flow cytometry. **(A)** Gating strategy for separation of CD83⁻ and CD83⁺ CD4⁺ as well as CD8⁺ T cells by FACS is shown. **(B-C)** MFI of CD83, CD69 and PD-1 expression of sorted CD83⁻ (white circles) as well as CD83⁺ (green circles) CD4⁺ and CD8⁺ T cells is depicted. Pooled data from two independent experiments (n = 6-8 mice) is presented as mean ± SEM. Statistics were performed using Two-way ANOVA and Tukey's multiple comparisons test (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

4.1.2 Characterization of T cell-specific CD83 conditional knockout mice

4.1.2.1 CD83 expression on CD4⁺ T cells from CD4WT and CD4KO mice

To get deeper insight into the function of CD83 expression on T cells, a T cell-specific CD83 conditional knockout mouse was generated by breeding $CD83^{flox/flox}$ mice with transgenic CD4cre mice ($CD83^{flox/flox}$ x CD4cre). In $CD83^{flox/flox}$ mice exon 3 is flanked with two loxp sites. Crossing these mice with CD4cre mice results in

excision of exon 3 and hence, ablation of CD83 expression specifically in T cells (Figure 11A).

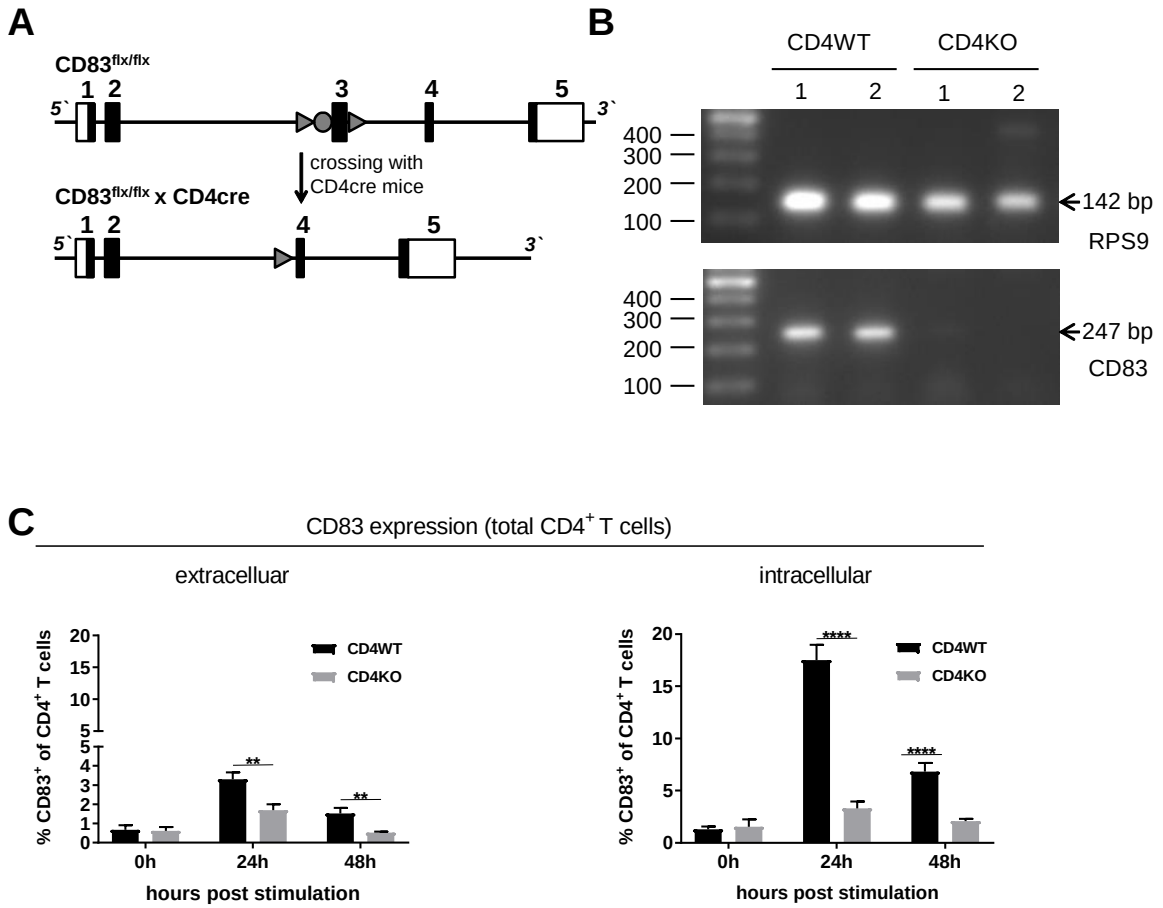

Figure 11: T cell-specific CD83 conditional knockout mice.
(A) Schematic drawing of the strategy to generate T cell-specific CD83 conditional knockout mice ($CD83^{flx/flx}$ x CD4cre mice). **(B)** $CD4^+$ T cells from CD4WT and CD4KO mice were enriched by MACS and stimulated with anti-CD3 for 24 h. RNA was isolated, cDNA generated and CD83 as well as RPS9 (control) RNA expression determined via PCR. Depicted are the PCR results from one representative out of two independent experiments. **(C)** Spleen cells from CD4WT (black bars) and CD4KO (grey bars) mice were isolated and stimulated for depicted time points with anti-CD3. Cells were analysed for extracellular (left) as well as intracellular (right) CD83 expression by flow cytometry. Results from two to three independent experiments (n = 8-14 mice) are presented as mean ± SEM. Statistics were performed using Mann-Whitney test (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

CD83 deletion in T cells was confirmed on RNA level by CD83-specific PCR of MACS-sorted, stimulated $CD4^+$ T cells from CD83 wildtype (CD4WT) and T cell-specific CD83 conditional knockout (CD4KO) mice (Figure 11B). Furthermore, CD83 expression on $CD4^+$ T cells was investigated by flow cytometry (Figure 11C). CD83 expression was upregulated upon *in vitro* stimulation with anti-CD3 on $CD4^+$ T cells of CD4WT mice. Extracellular as well as intracellular CD83 expression peaked 24 h post stimulation and declined 48 h post stimulation. Interestingly, intracellular CD83 expression in activated T cells was about five times higher compared to extracellular

CD83 expression. Additionally, the T cell-specific CD83 conditional knockout could be confirmed on protein level. Significantly reduced extracellular and intracellular CD83 expression of $CD4^+$ T cells from CD4KO mice was detected at all time points post stimulation compared to CD4WT mice (Figure 11C).

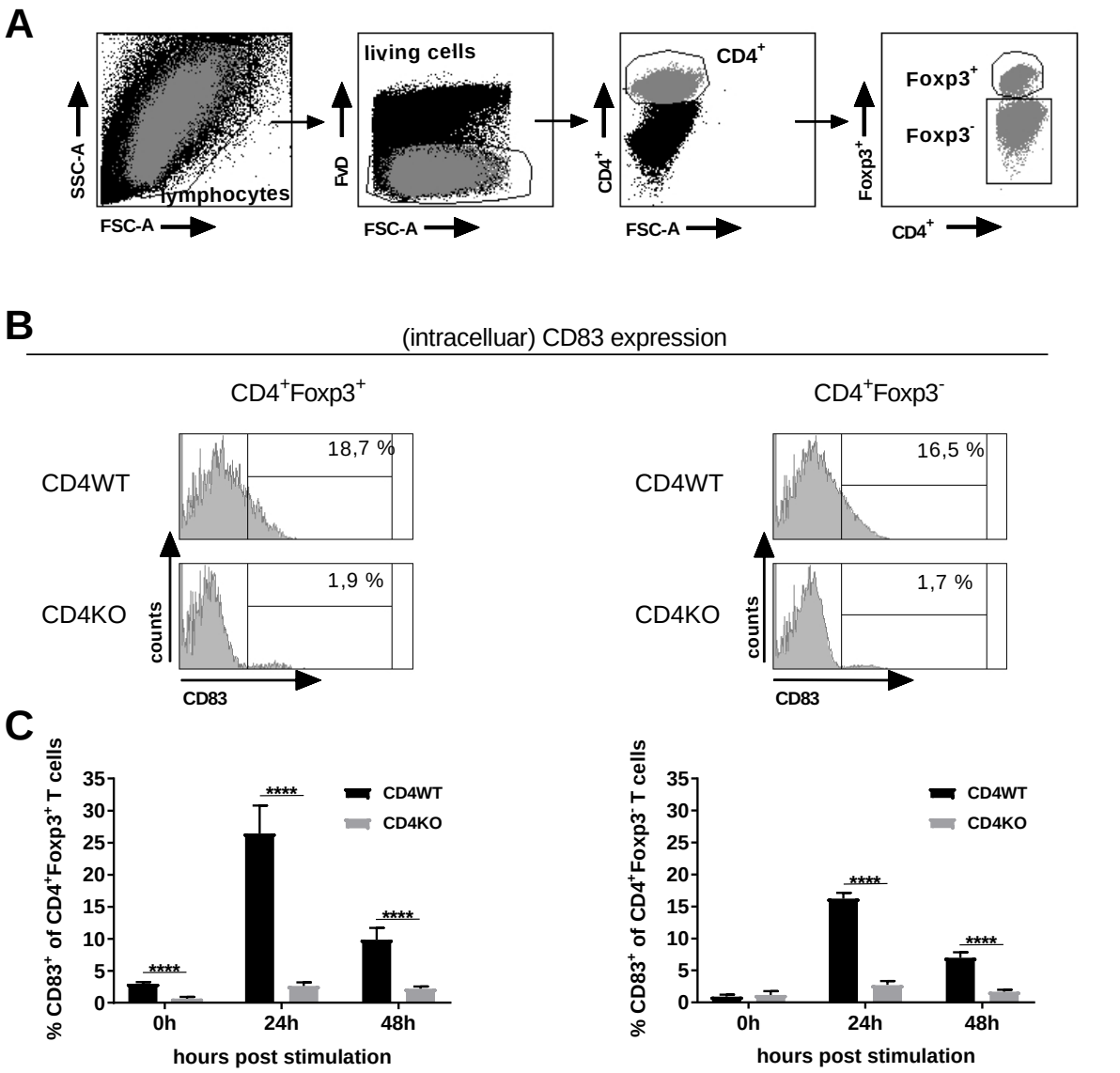

Figure 12: CD83 expression on $CD4^+Foxp3^+$ Treg and $CD4^+Foxp3^-$ Tconv cells isolated from CD4WT and CD4KO mice.
(A-C) Spleen cells from CD4WT and CD4KO mice were isolated and left unstimulated or stimulated for depicted time points with anti-CD3. **(A)** Gating strategy to determine Treg ($Foxp3^+$) and Tconv ($Foxp3^-$) cells by flow cytometry. **(B)** Representative histograms of $CD83^+$ Treg (left) and Tconv (right) cells from CD4WT and CD4KO mice 24 h post stimulation. **(C)** Percentages of splenic $CD83^+$ of Treg and Tconv cells isolated from CD4WT (black bars) and CD4KO mice (grey bars) are shown. Results from two independent experiments (n = 8-9 mice) are presented as mean ± SEM. Statistics were performed using Mann-Whitney t-test (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

CD83 expression on T cells was described to be associated with regulatory T cells [127, 156, 157]. Therefore, CD83 expression was quantified on Foxp3$^+$ regulatory T cells (Treg cells) and Foxp3$^-$ conventional T cells (Tconv cells) (Figure 12).

Figure 12A shows the gating strategy to identify Treg and Tconv cells. Initially, the lymphocyte population was determined by granularity and size and dead cells were excluded by staining with fixable viability dye (FvD). Upon gating on all CD4$^+$ T cells, Treg cells were defined as Foxp3$^+$ and Tconv cells as Foxp3$^-$. In accordance with the results from the analysis of total CD4$^+$ T cells, intracellular CD83 expression of Foxp3$^+$ as well as Foxp3$^-$ T cells peaked 24 h post stimulation (Figure 12C). Although the percentages of CD83$^+$ Treg cells were higher (Figure 12C left) compared to the percentages of CD83$^+$ Tconv cells (Figure 12C right), both T cell subsets upregulated CD83 expression upon *in vitro* stimulation. As expected Treg as well as Tconv cells from CD4KO mice showed significantly reduced CD83 expression compared to T cells from CD4WT mice.

Thus, T cell-specific CD83 conditional knockout mice provide a suitable model to study the impact of CD83 expression on T cell function.

4.1.2.2 T cell numbers in different lymphoid organs of CD4WT and CD4KO mice

It has been described that mice with a ubiquitous deletion of CD83 have a defective development of CD4$^+$ T cells in the thymus and in consequence decreased numbers of CD4$^+$ T cells in the periphery [136, 140]. To study, whether T cell-specific CD83 deletion has an impact on T cell development, T cell numbers in spleen from CD4KO mice were quantified (Figure 13).

Overall, CD4WT and CD4KO mice had similar spleen weight and same numbers of total spleen cells (Figure 13A). In addition, percentages of CD4$^+$ and CD8$^+$ T cells of viable cells as well as absolute CD4$^+$ and CD8$^+$ T cell numbers did not differ between CD4WT and CD4KO mice (Figure 13B left). Similar frequencies of CD4$^+$ and CD8$^+$ T cells were also detected in the mLNs and thymus of CD4WT and CD4KO mice (Figure 13B right).

To address the question, whether T cell-specific CD83 deletion affects Treg or Tconv cell differentiation, Foxp3 expression was analysed by flow cytometry (Figure 14).

Interestingly, CD4KO mice showed significantly increased numbers and percentages of splenic Foxp3$^+$ Treg cells and vice versa decreased numbers and percentages of Foxp3$^-$ Tconv cells compared to CD4WT mice (Figure 14A). Moreover, Foxp3 expression of total CD4$^+$ T cells isolated from CD4KO mice was significantly

increased compared to the Foxp3 expression on total CD4$^+$ T cells from CD4WT mice as measured via the mean fluorescence intensity (MFI) (Figure 14B top). However, Foxp3 expression was similar on Foxp3$^+$ Treg cells from CD4KO and CD4WT mice (Figure 14B bottom), indicating elevated abundance of Foxp3$^+$ Treg cells in CD4KO mice, but no increase in Foxp3 expression in Treg cells itself.

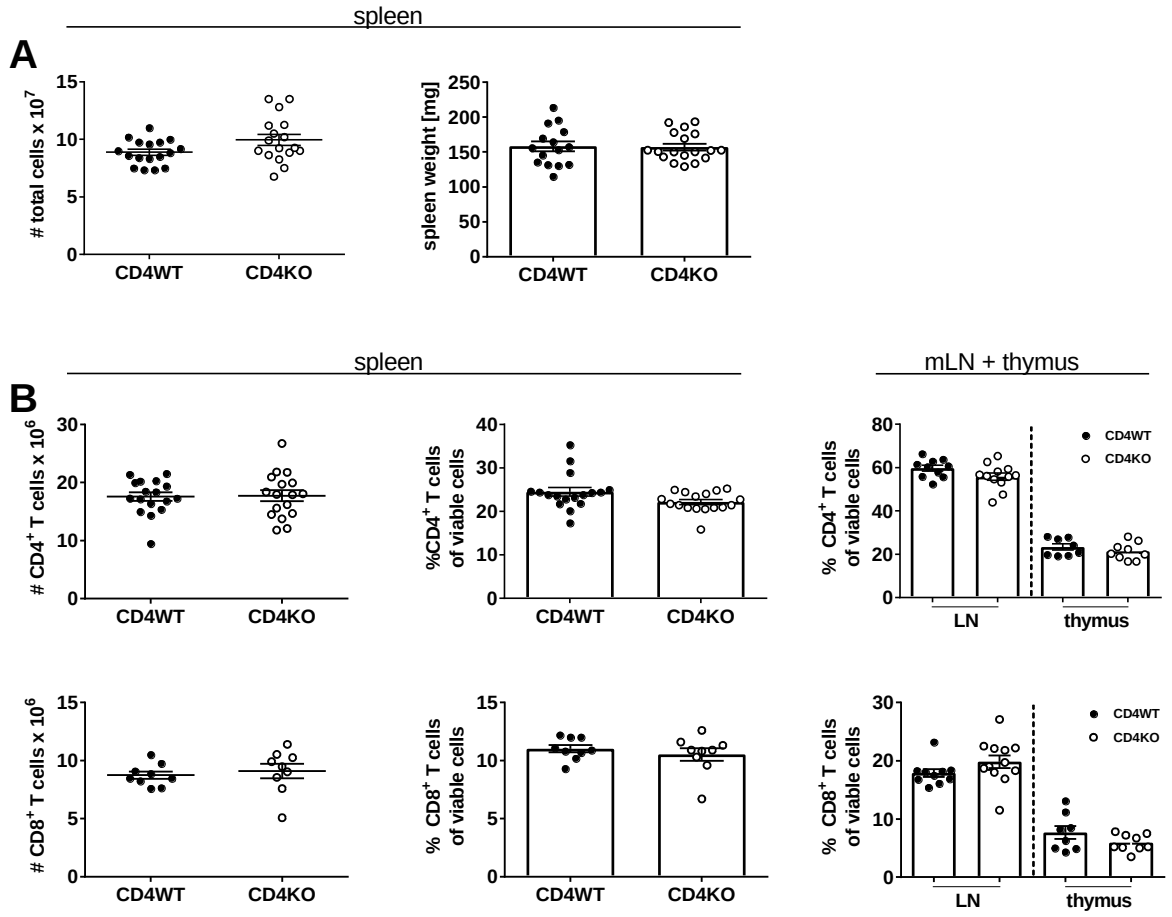

Figure 13: CD4WT and CD4KO mice display the same CD4$^+$ and CD8$^+$ T cell numbers.
(A-B) Spleen, mLNs and thymus from CD4WT and CD4KO mice were excised and total cell numbers were determined. **(A)** Spleen weight, total cell numbers as well as **(B)** cell numbers and percentages of CD4$^+$ (upper panel) and CD8$^+$ (lower panel) T cells in different organs are shown. Results from three to five independent experiments (n = 8-17 mice) are summarized as mean ± SEM. Student`s t-test and Mann-Whitney test were used for statistical analysis (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

In summary, CD4WT and CD4KO mice display equal CD4$^+$ and CD8$^+$ T cell numbers in different lymphoid organs. Noteworthy, CD4KO mice show significantly increased numbers and percentages of Foxp3$^+$ Treg cells in the spleen compared to CD4WT mice.

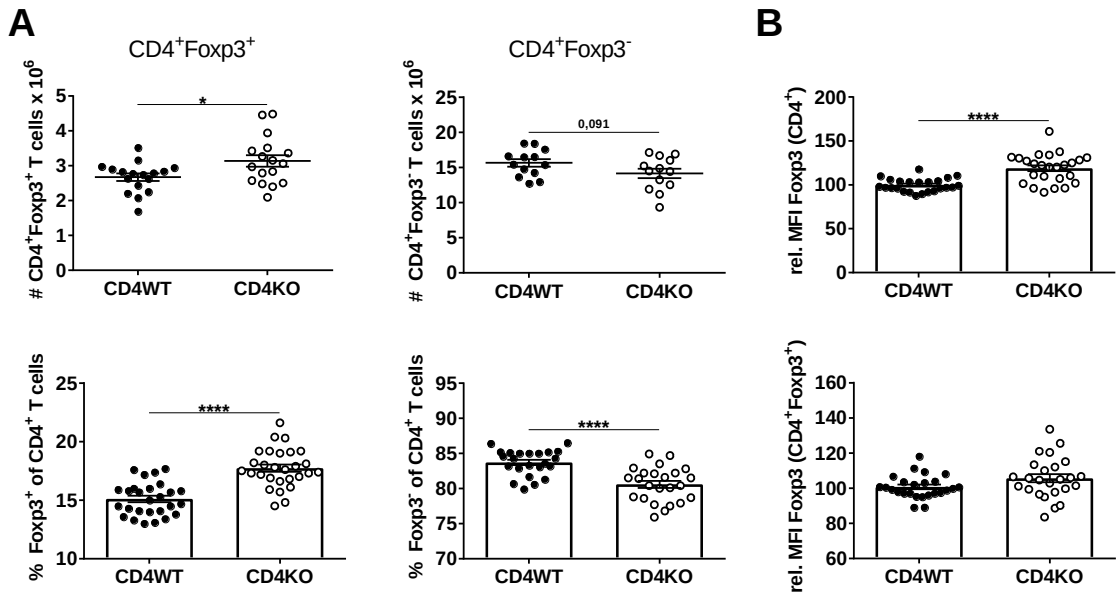

Figure 14: Numbers of splenic Treg as well as Tconv cells differ between CD4WT and CD4KO mice.
(A-B) Spleens from CD4WT and CD4KO mice were excised and total cell numbers from single cell suspensions were determined. **(A)** Absolute cell numbers and percentages of Foxp3$^+$ Treg (left) as well as Foxp3$^-$ Tconv (right) cells are shown. **(B)** Relative MFIs of Foxp3 expression on total CD4$^+$ (top) and Foxp3$^+$ (bottom) T cells are depicted. Results from four to eight independent experiments (n = 13-24 mice) are summarized as mean ± SEM. Student`s t-test and Mann-Whitney test were used for statistical analysis (*, p < 0,05; **, p < 0,01; ***, p < 0,001; ****, p < 0,0001).

4.1.2.3 CD83 expression is not essential for induction and suppressive function of Treg cells *in vitro*

As we observed elevated frequencies of Foxp3$^+$ Treg cells in CD4KO mice, we asked, whether T cell-specific CD83 deletion modulates Treg cell differentiation and/or suppressive function (Figure 15).

Hence, *in vitro* induction of Treg cells was analysed. Therefore, splenic CD4$^+$CD25$^-$ T cells from CD4WT and CD4KO mice were isolated by FACS and stimulated with Treg cell polarizing media *in vitro*. No differences were observed between the *in vitro* Treg induction of FACS-sorted CD4$^+$CD25$^-$ T cells from CD4WT and CD4KO mice (Figure 15A). To further address the question, whether the suppressive function of Treg cells is impaired by the loss of CD83 expression, coculture experiments were performed. Therefore, CD4$^+$CD25$^+$ T cells, which are about 90 % Foxp3 positive (data not shown) and hence termed Treg cells in the following, were isolated via FACS. FACS-sorted CD4$^+$CD25$^+$ Treg cells isolated from CD4WT and CD4KO mice and MACS-sorted CD4$^+$CD25$^-$ responder T cells from Thy1.1 mice were cocultured in different ratios. After three days of culture, proliferation of responder T cells was determined as parameter for Treg cell suppressive activity. Noteworthy, CD4$^+$CD25$^+$ Treg cells

from CD4WT and CD4KO mice exhibited similar inhibitory activity *in vitro* (Figure 15B).

These results show that, even though overall percentages of $CD4^+Foxp3^+$ Treg cells are increased in CD4KO mice, T cell-specific CD83 deletion neither modulates the capacity of $CD4^+CD25^-$ T cells to differentiate into Treg cells nor alters the suppressive function of $CD4^+CD25^+$ Treg cells *in vitro*.

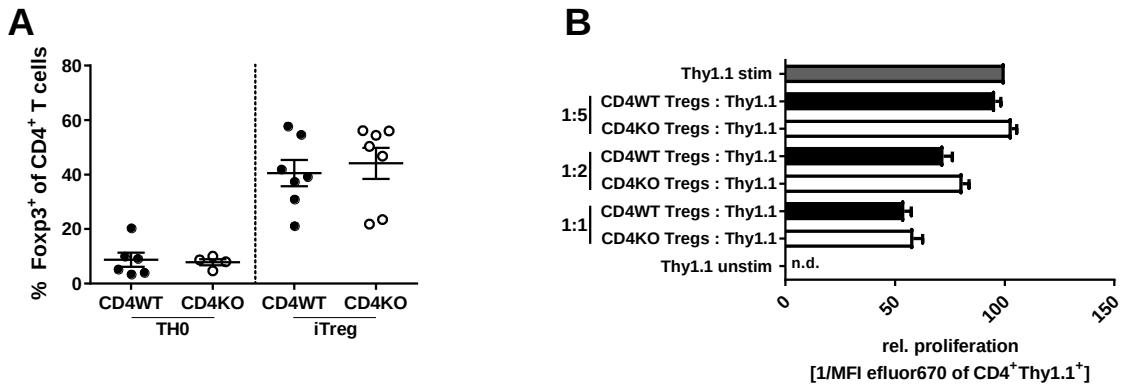

Figure 15: *In vitro* Treg induction and inhibitory capacity of Treg cells is not altered upon T cell-specific CD83 deletion.
(A) Splenic $CD4^+CD25^-$ T cells from CD4WT (black circles) and CD4KO (white circles) mice were isolated by FACS and stimulated with anti-CD3, anti-CD28, IL-2 and TGF-β. After three days of culture Foxp3 expression was measured by flow cytometry as parameter for *in vitro* Treg induction. Percentages of $Foxp3^+$ of $CD4^+$ T cells are shown. **(B)** Splenic $CD4^+CD25^+$ Treg cells of CD4WT (black bars) and CD4KO (white bars) mice were isolated by FACS and cocultivated with $CD4^+CD25^-$ T responder cells from Thy1.1 mice. Responder T cells were isolated by MACS and stained with the proliferation dye efluor670 before. Irradiated splenocytes and anti-CD3 were added to the culture. Stimulated $CD4^+CD25^-$ T cells from Thy1.1 mice cultivated alone (grey bar) served as control. Proliferation of responder T cells, depicted as relative MFI of the efluor670 signal, is presented as parameter for *in vitro* Treg inhibitory capacity. Results from two to three independent experiments (n = 4-7 mice) are presented as mean ± SEM. Statistics were performed using Student`s t-test and Mann-Whitney test.

4.1.2.4 Tconv cells from CD4KO mice do have an activated phenotype

In addition to the Treg cells (4.1.2.3), Tconv cells from CD4KO mice were further analysed. Hence, phenotype and function of Tconv cells was investigated in more detail.

To exclude an impact of CD83 deletion on other cell types and to avoid an effect of the increased Treg cell frequencies in CD4KO mice, Tconv cells were isolated before analysis. Thus, $CD4^+CD25^-$ T cells, which are about 90 % negative for Foxp3 (data not shown) and hence termed Tconv cells in the following, were separated via FACS.

First of all, activation of Tconv cells was assessed. Interestingly, naive Tconv cells isolated from the spleen of CD4KO mice exhibited a more activated phenotype, determined by significantly increased CD69, CD25, CD44 and decreased CD62L

expression (Figure 16). However, upon stimulation with irradiated splenocytes from Thy1.1 WT mice and anti-CD3, Tconv cells from CD4KO and CD4WT mice were both able to upregulate all of the analysed activation markers.

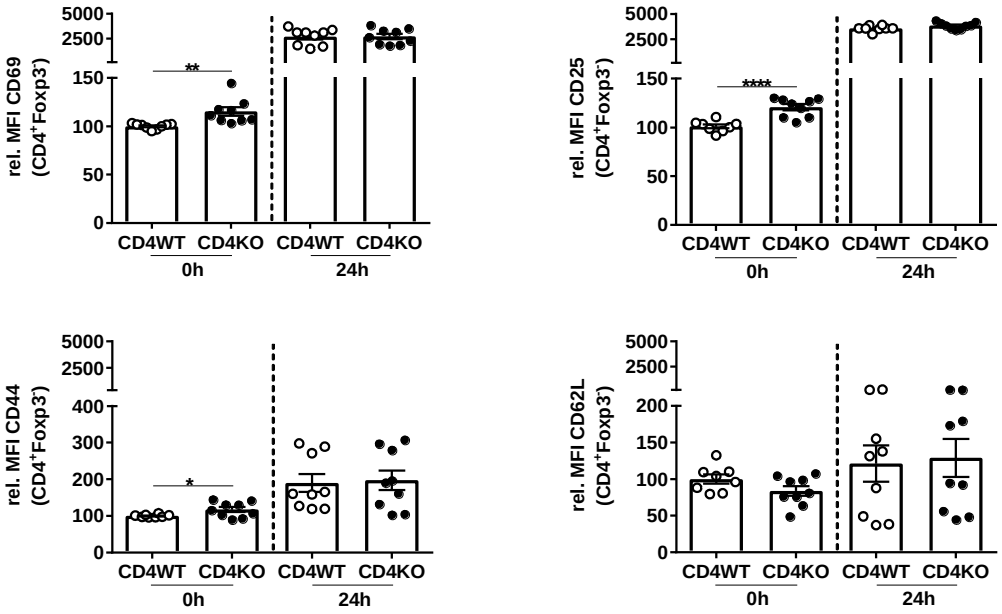

Figure 16: CD83-deficient Tconv cells are more activated compared to WT Tconv cells.
(A) Splenic $CD4^+CD25^-$ Tconv cells from CD4WT and CD4KO mice were isolated by FACS left unstimulated or stimulated for 24 h with irradiated splenocytes and anti-CD3. T cells were analysed for the expression of different surface markers by flow cytometry. Relative MFI of CD69, CD25, CD44 and CD62L expression of $Foxp3^-$ T conv cells is presented. Results from three independent experiments (n = 8-9 mice) are presented as mean ± SEM. Statistics were performed using Student`s t-test and Mann-Whitney test (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

To gain further insight into the role of CD83 expression on Tconv cell function, the proliferative capacity was investigated. For this purpose FACS-sorted, splenic $CD4^+CD25^-$ Tconv cells isolated from CD4WT and CD4KO mice were stained with the proliferation dye efluor670 and stimulated with anti-CD3 in the presence of irradiated splenocytes from Thy1.1 WT mice or left unstimulated as control. As shown in Figure 17A, CD83-deficient Tconv cells showed significantly increased proliferative activity compared to Tconv cells from CD4WT mice. Furthermore, cytokine secretion in the supernatant of stimulated cells was analysed by Luminex technology. After three days of culture, a significantly higher concentration of the proinflammatory cytokine IFN-γ and a slightly increased IL-2 concentration were detected in the supernatant of stimulated Tconv cells from CD4KO mice (Figure 17B). To further investigate, whether ablation of CD83 expression in T cells might affect TH cell differentiation, the capacity of Tconv cells from CD4WT and CD4KO mice to differentiate into TH1 cells was investigated. FACS-sorted $CD4^+CD25^-$ Tconv

cells were cultured under TH1 polarizing conditions and analysed for IFN-γ expression via flow cytometry afterwards. Interestingly, Tconv cells isolated from CD4KO mice showed significantly enhanced differentiation into TH1 cells compared to Tconv cells isolated from CD4WT mice (Figure 17C), whereas the capacity to differentiate into TH17 cells was not altered (data not shown). Hence, CD83 deletion seems to influence TH1, but not TH17 differentiation at least *in vitro*.

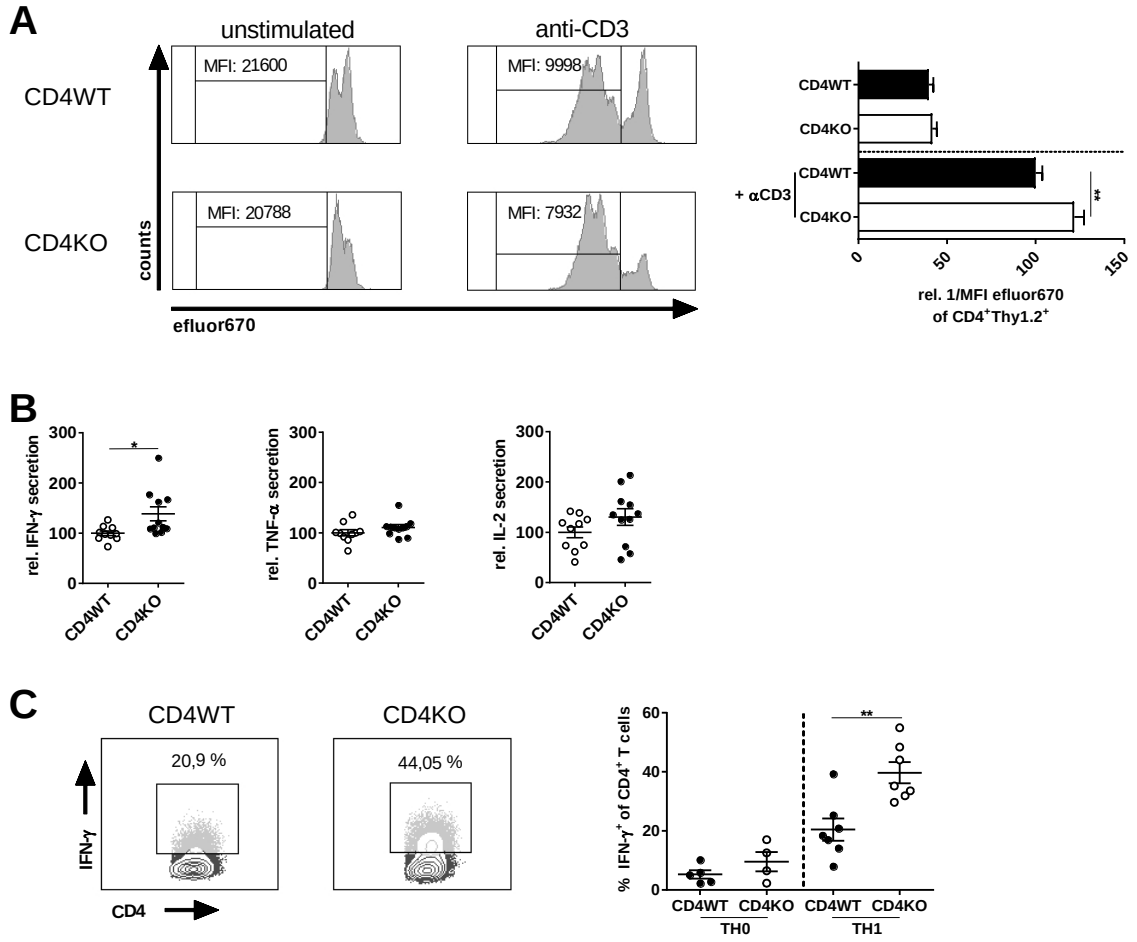

Figure 17: CD83-deficient Tconv cells show stronger proliferation as well as increased TH1 differentiation compared to WT Tconv cells *in vitro.*
(A) Splenic $CD4^+CD25^-$ Tconv cells isolated from CD4WT and CD4KO mice were isolated by FACS, stained with the proliferation dye efluor670 and stimulated with anti-CD3 in the presence of irradiated splenocytes for three days. Unstimulated $CD4^+CD25^-$ Tconv cells served as control. Representative histograms of the efluor670 signal of unstimulated and stimulated $CD4^+Thy1.2^+$ T cells from CD4WT and CD4KO are shown (left). Proliferation of Tconv cells is summarized as relative MFI of the efluor670 signal (right). **(B)** Concentrations of different cytokines in the supernatant of stimulated cells from (A) were determined by Luminex technology. Relative IFN-γ, TNF-α and IL-2 concentrations are shown. **(C)** Splenic $CD4^+CD25^-$ Tconv cells of CD4WT and CD4KO mice were isolated by FACS and stimulated with anti-CD3, anti-CD28, IL-12 and anti-IL-4 for TH1 differentiation. After six days of culture intracellular IFN-γ expression was measured by flow cytometry. Representative dot plots of $IFN\text{-}\gamma^+CD4^+$ T cells from CD4WT and CD4KO mice are shown (left). Percentages of $IFN\text{-}\gamma^+$ of $CD4^+$ T cells are summarized (right). Results from three to five independent experiments (n = 4-15 mice) are presented as mean ± SEM. Statistics were performed using Student`s t-test and Mann-Whitney test (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

Taken together, Tconv cells from CD4KO mice exhibit a more activated phenotype determined by significantly increased expression of activation marker molecules, enhanced proliferation as well as elevated TH1 differentiation compared to Tconv cells from CD4WT mice *in vitro*.

4.1.2.5 CD83-deficient CD4$^+$ T cells exhibit increased TCR-signaling

To investigate the mechanism by which CD83 deletion alters CD4$^+$ T cell activation, downstream signaling of the TCR was assessed by flow cytometry. As one of the first signaling events downstream of the TCR, phosphorylation of the zeta-chain-associated protein kinase 70 (ZAP-70) takes place. ZAP-70 binds to the intracellular part of the two ζ-chains from the TCR. Interestingly, CD83-deficient Tconv cells showed significantly enhanced phosphorylation of ZAP-70 upon *in vitro* stimulation compared to CD4WT Tconv cells (Figure 18 left). The same tendency could be observed for Treg cells (Figure 18 right).

Due to the fact that phosphorylated ZAP-70 induces downstream inflammatory signaling pathways, these results further indicate a more proinflammatory phenotype of CD83-deficient Tconv cells.

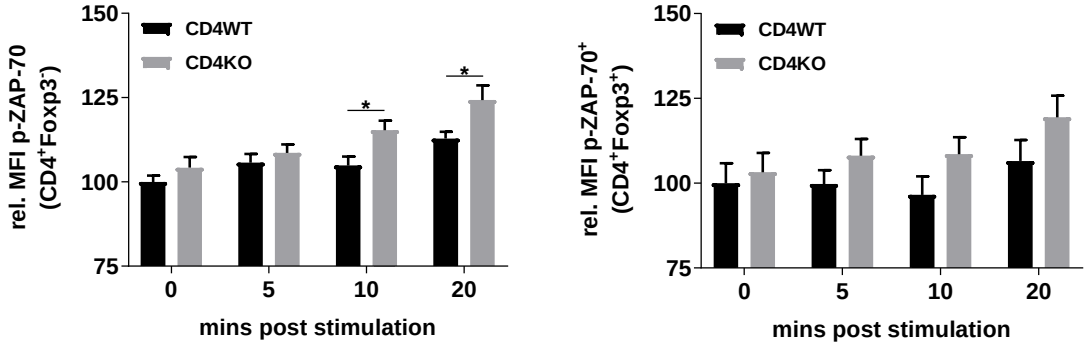

Figure 18: CD83-deficient CD4$^+$ T cells show increased phosphorylation of ZAP-70 compared to WT CD4$^+$ T cells.
Spleen cells from CD4WT and CD4KO mice were isolated, left unstimulated or stimulated for 5, 10 and 20 min with anti-CD3 and anti-CD28. Phosphorylation of ZAP-70 was determined by flow cytometry. Presented is the relative MFI of p-ZAP-70 of CD4$^+$Foxp3$^-$ (left) and CD4$^+$Foxp3$^+$ (right) T cells from CD4WT and CD4KO mice. Results from three independent experiments (n = 10-13 mice) are summarized as mean ± SEM. Student`s t-test was used for statistical analysis (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

In summary, T cell-specific CD83 deletion does not modulate Treg induction nor suppressive function *in vitro*, whereas Tconv cells exhibit a more activated phenotype upon CD83 deletion *in vitro*. Mechanistically these alterations might be a

consequence of enhanced TCR signaling indicated by increased ZAP-70 phosphorylation.

4.1.3 Effect of T cell-specific CD83 deletion during immune responses *in vivo*

The *in vitro* experiments indicate that CD83 plays an important role in balancing proinflammatory immune responses. To address the question, whether CD83 expression on $CD4^+$ T cells is also relevant in the more complex *in vivo* situation, the immune response of CD4KO mice was analysed in a mouse model of allergic contact reactions (contact hypersensitivity model) and inflammatory bowel diseases (T cell transfer model of chronic colitis). In both diseases $CD4^+$ T cells are known to play an important role for disease initiation and progression [66, 88].

4.1.3.1 T cell-specific CD83 deletion increases inflammation during a contact hypersensitivity reaction

CHS reactions are a group of T cell-derived skin inflammations, in which the TH1 cell immune response is critical for pathogenesis. Therefore, the CHS model is a suitable model to study the role of T cell-expressed CD83 during dysregulated immune responses. The treatment with a hapten induces an immune response against modified self-proteins complexed with the hapten, leading to a local CHS reaction of the skin at the site of reexposure.

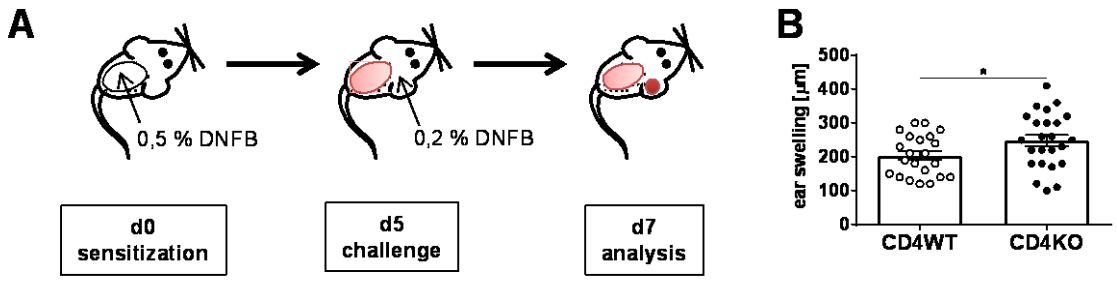

Figure 19: CD4KO mice show increased inflammation in an *in vivo* CHS model compared to CD4WT mice
(A) Experimental setup of the CHS model. Mice were sensitized with DNFB on the shaved back at day zero. Five days post sensitization mice were challenged with DNFB on the right ear. Two days post challenge mice were sacrificed and spleen and dLNs excised. **(B)** Ear swelling, calculated by measuring the ear thickness before and two days post challenge, is depicted. Results from five independent experiments (n = 23-24 mice) are summarized as mean ± SEM. Student`s t-test was used for statistical analysis (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

CD4WT and CD4KO mice were sensitized with DNFB on the shaved back at day zero (Figure 19A). Five days later mice were challenged with DNFB on the right ear. Two days post reexposure, mice were sacrificed and analysed. Strikingly, CD4KO

mice developed a significantly enhanced CHS reaction, determined by increased ear swelling after challenge with DNFB compared to CD4WT mice (Figure 19B).

It is well described that the $CD4^+$ T cell response is critical for pathogenesis of CHS reactions. Therefore, $CD4^+$ T cells in spleen and draining lymph nodes (dLNs) close to the inflamed ear were analysed by flow cytometry. As described for naive CD4KO mice (see chapter 4.1.2.4), the percentages of $Foxp3^+$ Treg cells were also increased in spleen and dLNs of CD4KO mice compared to WT littermates during CHS reaction (Figure 20B left). In line with this, the percentages of $Foxp3^-$ Tconv cells were significantly decreased in spleen and dLNs of CD4KO mice (Figure 20B right).

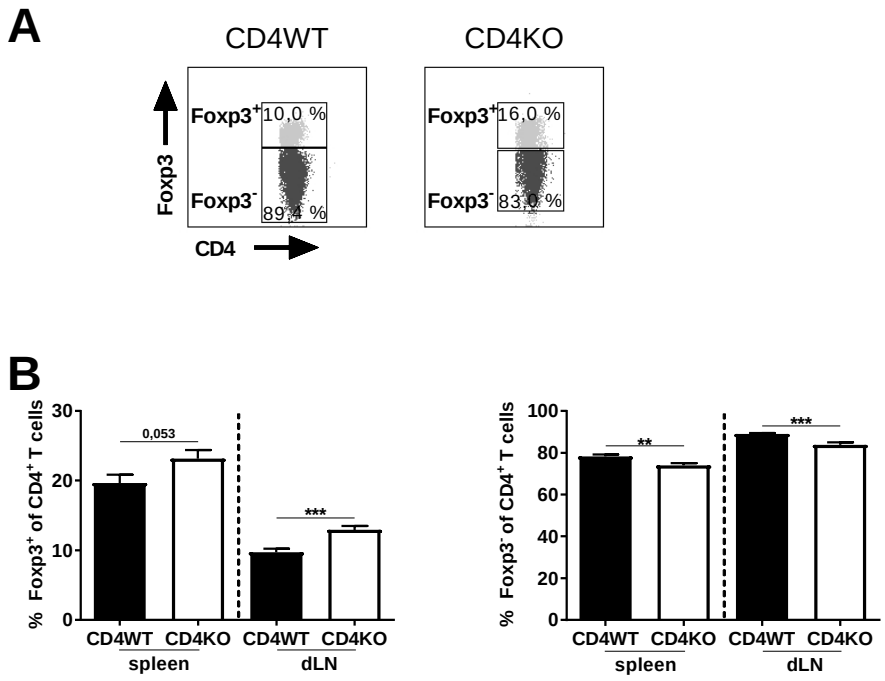

Figure 20: CD4KO mice exhibit increased percentages of Treg cells in spleen and dLNs during CHS reaction compared to CD4WT mice.
(A-B) Mice were sensitized with DNFB on the shaved back at day zero. Five days post sensitization mice were challenged with DNFB on the right ear. Two days post challenge mice were sacrificed, spleen and dLNs excised and Foxp3 expression determined by flow cytometry. **(A)** Representative dot plots of $Foxp3^+$ Treg and $Foxp3^-$ Tconv cells from CD4WT (left) and CD4KO (right) mice. **(B)** Percentages of $Foxp3^+$ (left) as well as $Foxp3^-$ T cells (right) of $CD4^+$ T cells in spleen and dLNs of CD4WT and CD4KO mice are shown. Results from three independent experiments (n = 16-18 mice) are summarized as mean ± SEM. Student`s t-test was used for statistical analysis (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

However, $CD4^+$ T cells isolated from spleen and dLNs of CD4KO mice expressed significantly elevated levels of CD69 and CD44 and decreased levels of CD62L compared to $CD4^+$ T cells from WT littermates (Figure 21A). Moreover, significantly increased percentages of IFN-γ^+ and IL-17^+ $CD4^+$ T cells were detected in the dLNs of CD4KO mice compared to CD4WT mice (Figure 21B).

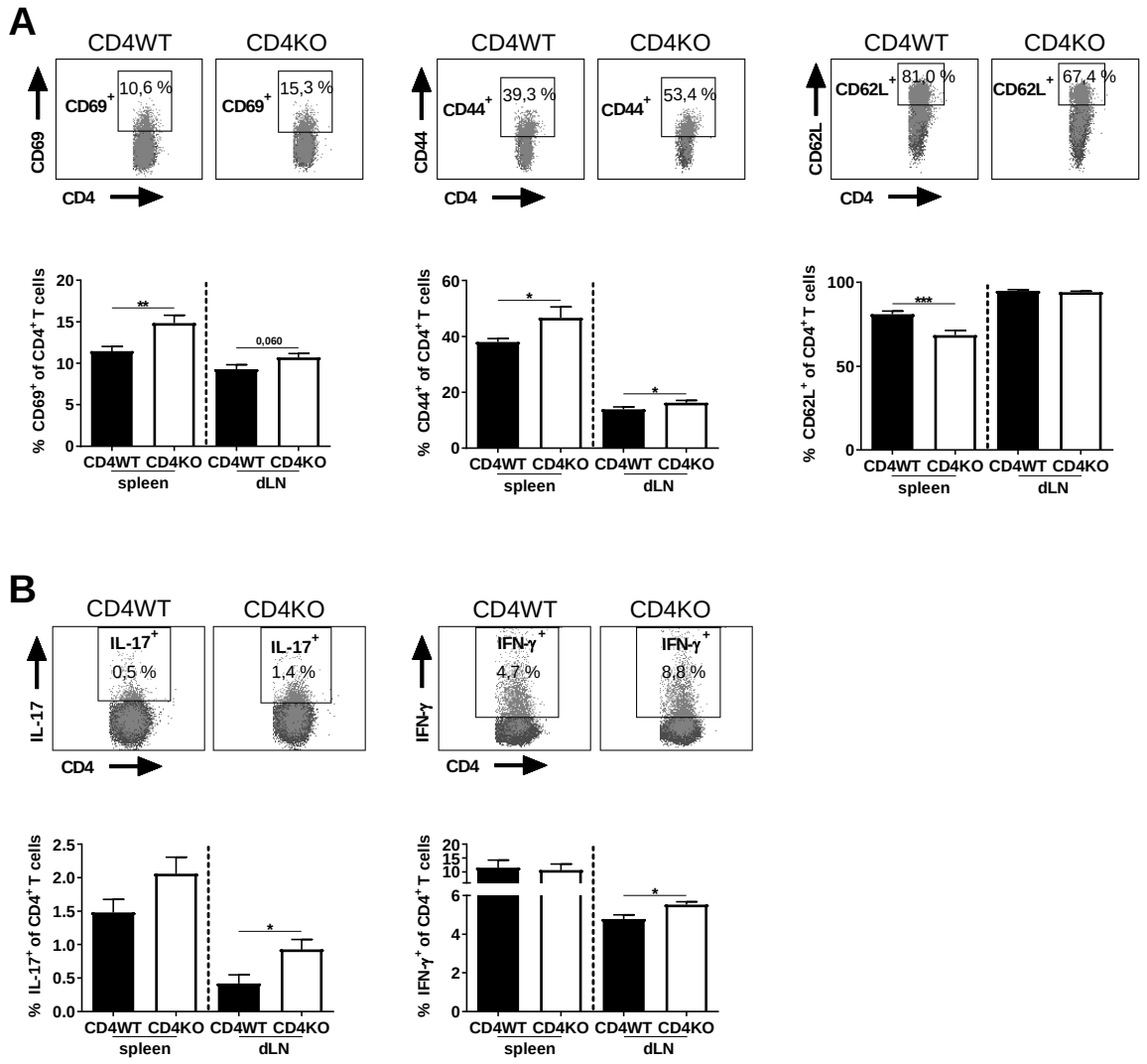

Figure 21: CD4⁺ T cells from CD4KO mice exhibit a more activated phenotype and an increased secretion of inflammatory cytokines during CHS reaction compared to CD4WT mice.
(A-B) Mice were sensitized with DNFB on the shaved back at day zero. Five days post sensitization mice were challenged with DNFB on the right ear. Two days post challenge mice were sacrificed, spleen and dLNs excised and surface marker and cytokine expression determined by flow cytometry. **(A)** Representative dot plots (top) of CD69⁺ (left), CD44⁺ (middle) and CD62L⁺ (right) splenic CD4⁺ T cells from CD4WT and CD4KO mice. Summarized percentages of CD69⁺, CD44⁺ and CD62L⁺ of CD4⁺ T cells in spleen and dLNs of CD4WT and CD4KO mice are shown (bottom). **(B)** Representative dot plots (top) of IL-17⁺ (left) and IFN-γ⁺ (right) CD4⁺ T cells from the dLNs of CD4WT and CD4KO mice. Percentages of IL-17⁺ and IFN-γ⁺ of CD4⁺ T cells in spleen and dLNs of CD4WT and CD4KO mice are shown (bottom). Results from two to four independent experiments (n = 10-20 mice) are summarized as mean ± SEM. Student`s t-test and Mann-Whitney test were used for statistical analysis (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

Taken together, CD4KO mice exhibit a significantly increased CHS reaction compared to CD4WT mice, which is accompanied by an increased CD4⁺ T cell activation. These data suggest that CD83 expression on CD4⁺ T cells regulates T cell activation *in vivo*, thereby reducing immunopathological effects.

4.1.3.2 T cell-specific CD83 deletion exacerbates T cell transfer induced colitis in Rag2KO mice

Data obtained from *in vitro* and *in vivo* experiments indicate that T cell-specific deletion of CD83 mainly affects Tconv cells. However, an impact of T cell-specific CD83 deletion on Treg cells could not be excluded. To further analyse this, the T cell transfer model of chronic colitis was used.

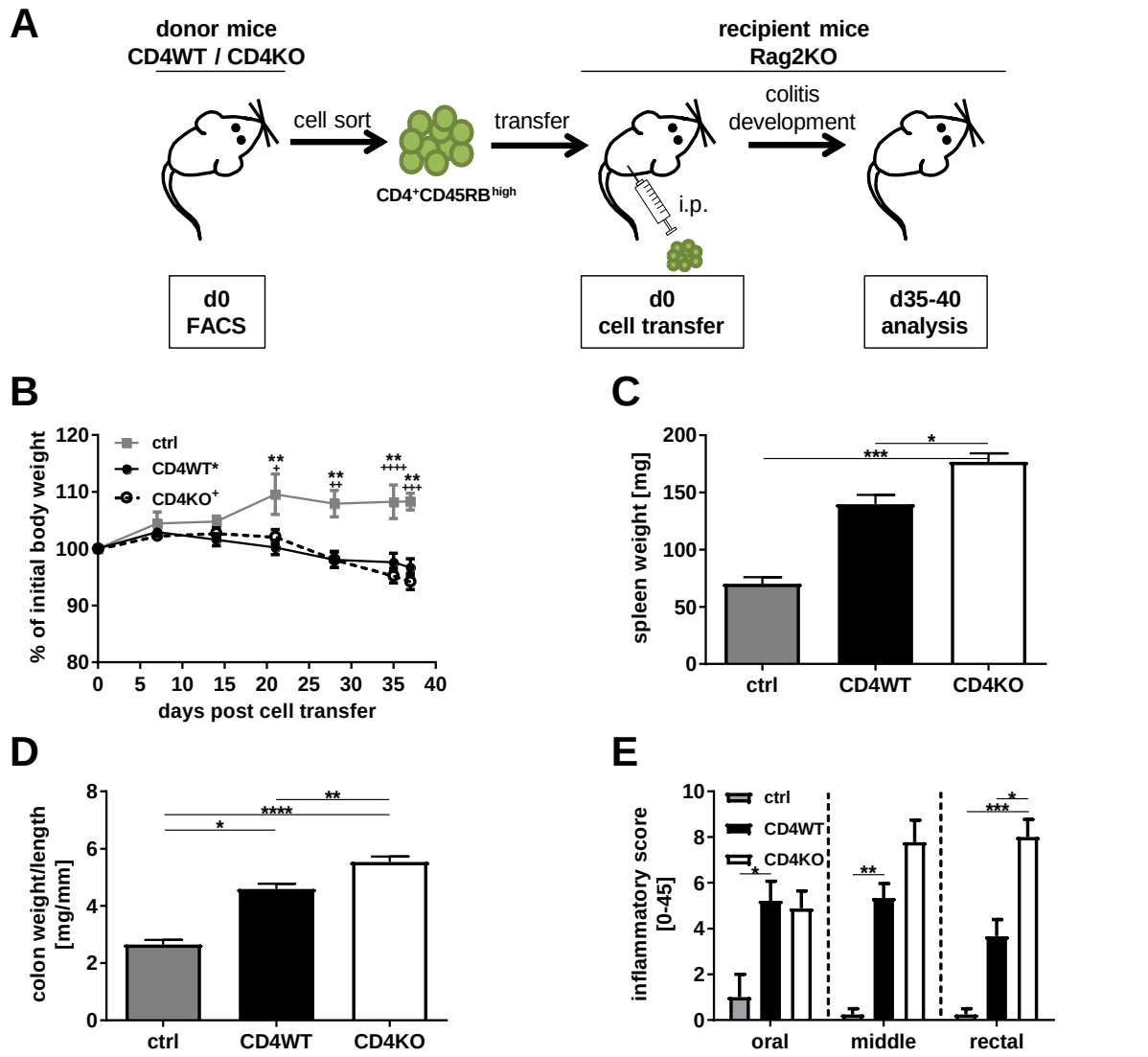

Figure 22: CD83 deletion in CD4⁺ T cells exacerbates T cell transfer induced colitis in Rag2KO mice.
(A) Experimental setup of the T cell transfer induced colitis model in Rag2KO mice. Sorted CD45RB^{high}CD4^{+} T cells isolated from the spleen of CD4KO and CD4WT mice were transferred i.p. into immunodeficient Rag2KO mice. **(B)** Body weight at depicted time points, **(C)** spleen weight, **(D)** colon weight to length ratio and **(E)** inflammatory score of the colon at day 37 after cell transfer are presented. Pooled data from two to seven independent experiments (n = 4-33 mice) is depicted as mean ± SEM. Statistics were performed using Kruskal-Wallis test with Dunn's multiple comparisons test (B: CD4cre WT vs. ctrl: *, p < 0,05; **, p < 0,01; ***, p < 0,001; ****, p < 0,0001; CD4cre TG vs. ctrl: $^{+}$, p < 0,05; $^{++}$, p < 0,01; $^{+++}$, p < 0,001; $^{++++}$, p < 0,0001), (C-E: *, p < 0,05; **, p < 0,01; ***, p < 0,001; ****, p < 0,0001).

In this model of chronic colitis the transfer of naive, Treg-depleted T cells from donor mice into syngeneic immunodeficient recipient Rag2KO mice leads to the induction of chronic colitis due to a TH1/TH17 immune response against the antigens from commensal bacteria in the gut. By transferring naive, Treg-depleted $CD4^+$ T cells from T cell-specific CD4KO and CD4WT mice Treg-specific effects can be excluded and the influence of CD83 deletion in Tconv cells can be investigated.

Figure 22A shows the strategy to initiate the T cell transfer induced chronic colitis in Rag2KO mice. In short, $CD45RB^{high}CD4^+$ naive T cells from CD4WT and CD4KO mice were isolated via FACS and injected intraperitoneally (i.p.) into immunodeficient Rag2KO mice (Figure 22A). Rag2KO mice injected with naive T cells from CD4WT and CD4KO mice showed a significant loss of body weight over time compared to control mice injected with PBS (Figure 22B). About five weeks post cell transfer, when colitis symptoms like diarrhea and bloody stool were detected, mice were sacrificed and colitis parameters were quantified. Strikingly, Rag2KO mice adoptively transferred with naive T cells from CD4KO mice exhibited significantly increased spleen weight and colon weight/length ratio compared to Rag2KO mice, which received naive T cells from WT littermates (Figure 22C-D). Furthermore, histopathologic analysis of colons was performed. Especially in the rectal part of the colon the transfer of naive T cells from CD4KO mice induced a stronger pathology compared to the transfer of T cells from CD4WT mice (Figure 22E).

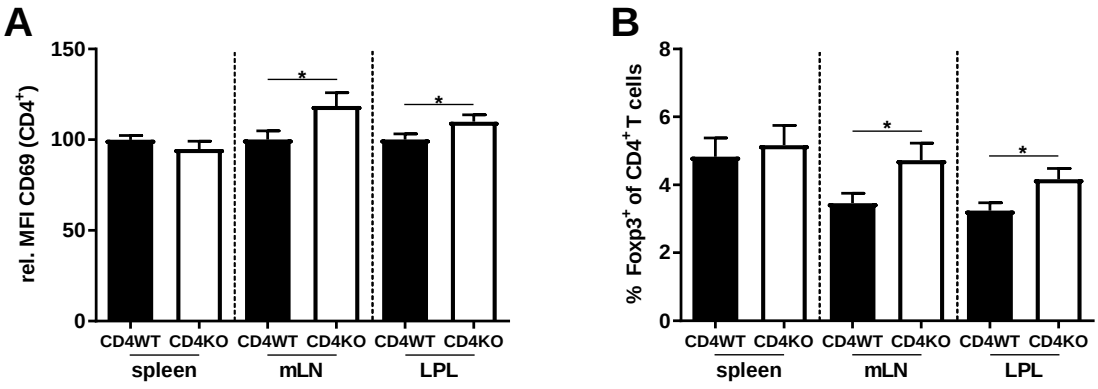

Figure 23: Transferred, CD83-deficient $CD4^+$ T cells are more activated and exhibit higher percentages of Treg cells compared to transferred, WT $CD4^+$ T cells.
(A-B) FACS-sorted $CD45RB^{high}CD4^+$ T cells isolated from spleens of CD4KO and CD4WT mice were transferred i.p. into immunodeficient Rag2KO mice. **(A)** Relative MFI of CD69 expression on transferred $CD4^+$ T cells from CD4WT and CD4KO mice in spleen, mLNs and LP of Rag2KO mice is presented. **(B)** Percentages of $Foxp3^+$ T cells of transferred $CD4^+$ T cells from CD4WT and CD4KO mice in spleen, mLNs and LP of Rag2KO mice are shown. Results from five to six independent experiments (n = 17-29 mice) are summarized as mean ± SEM. Student`s t-test was used for statistical analysis (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

To get deeper insight into the underlying mechanism, immune cells from the spleen, mLNs and LP of the colon were isolated and analysed by flow cytometry. $CD4^+$ T cells isolated from mLNs and LP of Rag2KO mice, which were injected with naive T cells from CD4KO mice, showed significantly increased expression of the activation marker CD69 compared to transferred $CD4^+$ T cells from CD4WT mice (Figure 23A). Interestingly, the percentages of $Foxp3^+$ Treg cells were significantly elevated in mLNs and LP of Rag2KO mice injected with $CD4^+$ T cells from CD4KO mice (Figure 23B). Noteworthy, the increased Treg cell frequencies seem to rather result from increased expansion and/or infiltration due to increased inflammation than from *de novo* induction, because *in vitro* Treg differentiation is not altered in CD83-deficient $CD4^+$ T cells (see chapter 4.1.2.3).

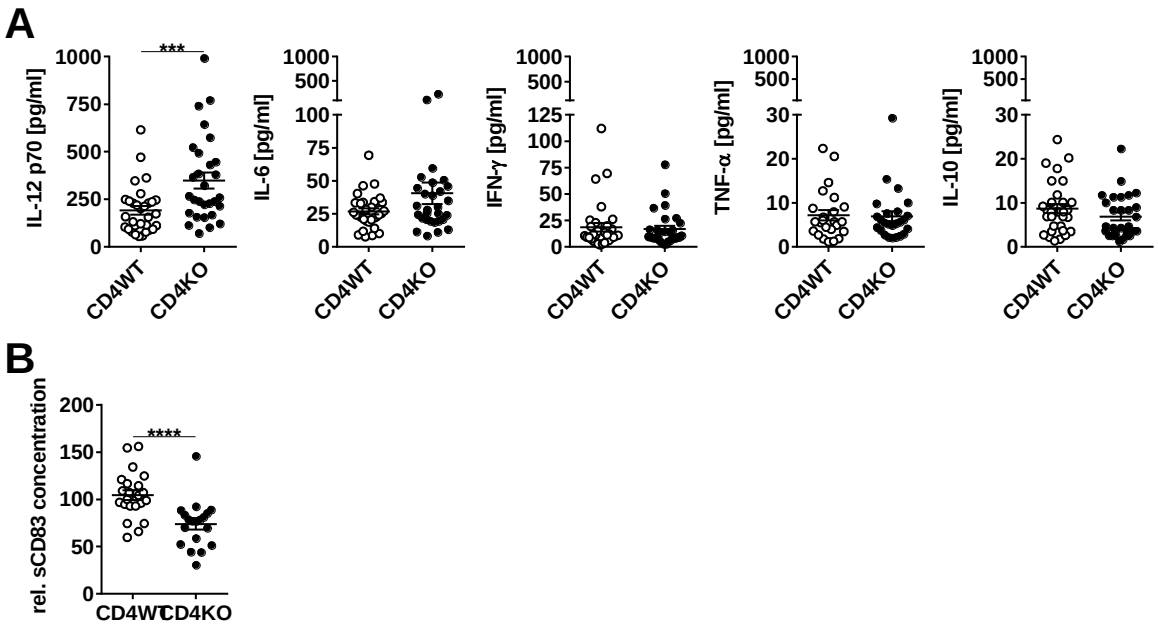

Figure 24: Rag2KO mice that received $CD4^+$ T cells from CD4KO mice display increased IL-12p70 and decreased sCD83 concentration in the serum compared to Rag2KO mice transferred with $CD4^+$ T cells from CD4WT mice.
(A-B) Sorted $CD45RB^{high}CD4^+$ T cells isolated from the spleen of CD4KO and CD4WT mice were transferred i.p. into immunodeficient Rag2KO mice **(A)** Concentrations of different cytokines in the serum of Rag2KO mice injected with $CD4^+$ T cells from CD4WT and CD4KO mice determined by Luminex technology are depicted. **(B)** Concentration of sCD83 in the serum of Rag2KO mice injected with $CD4^+$ T cells from CD4WT and CD4KO mice analysed with a sCD83 ELISA kit is presented. Results from two to six independent experiments (n = 11-22 mice) are summarized as mean ± SEM. Student`s t-test was used for statistical analysis (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

The cytokine secretion strongly correlates with the inflammatory response. Hence, the cytokine profile in the serum of Rag2KO mice was analysed using Luminex technology. The concentration of the DC-related cytokine IL-12p70 was significantly increased in the serum of Rag2KO mice, which received naive T cells from CD4KO

mice, compared to Rag2KO mice, transferred with CD4WT T cells (Figure 24A). IL-6, predominantly secreted by DCs, was also slightly increased in Rag2KO injected with CD83-deficient $CD4^+$ T cells. However, the concentrations of the cytokines IFN-γ, TNF-α and IL-10 were not altered. In addition, the concentration of sCD83 was analysed. Interestingly, Rag2KO mice injected with CD83-deficient $CD4^+$ T cells had significantly lower concentration of sCD83 in the serum (Figure 24B).

Overall the increased concentrations of DC-related cytokines and decreased concentration of sCD83 in the serum underlines an elevated proinflammatory immune response in Rag2KO mice that received CD83-deficient, naive $CD4^+$ T cells.

Due to the increased concentration of DC-related cytokines the activation of DCs was also investigated. Interestingly, the frequency of $CD11c^+MHII^{high}$ DCs was significantly increased in the spleen of Rag2KO mice transferred with CD83-deficient, naive $CD4^+$ T cells compared to the transfer of naive T cells from CD4WT mice (Figure 25A). In addition, these DCs exhibited a more activated phenotype determined by increased expression of CD40 (Figure 25B).

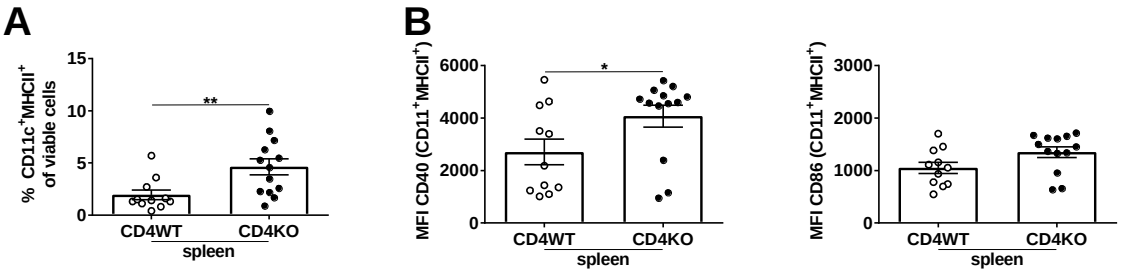

Figure 25: Rag2KO mice that received $CD4^+$ T cells from CD4KO mice show elevated frequencies of DCs in the spleen compared the mice transferred with $CD4^+$ T cells from CD4WT mice.

(A) Sorted $CD45RB^{high}CD4^+$ T cells isolated from the spleen of CD4KO and CD4WT mice were transferred i.p. into immunodeficient Rag2KO mice. Percentages of $CD11c^+MHCII^{high}$ DCs and MFI of CD40 and CD86 on $CD11c^+MHCII^+$ DCs are shown. Results from two independent experiments (n = 10-11 mice) are summarized as mean ± SEM. Student`s t-test was used for statistical analysis (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

In summary, the transfer of CD83-deficient, naive $CD4^+$ T cells into Rag2KO mice aggravated colitis symptoms compared to the transfer of CD4WT T cells. Enhanced inflammation is indicated by increased pathology of the colon, elevated activation of T cells, enhanced DC frequencies and higher concentrations of proinflammatory cytokines in the serum. Due to the transfer of naive, Treg-depleted $CD4^+$ T cells the effect can be traced back to CD83-deficiency in Tconv cells. These results support

the results from the *in vitro* analysis of CD4KO mice, which show a stronger activation of CD83-deficient CD4$^+$ Tconv cells compared WT Tconv cells.

4.1.4 Influence of T cell-specific CD83 expression on DCs

The results obtained from the transfer of CD83-deficient, naive T cells into Rag2KO mice indicate an impact of T cell-expressed CD83 on DCs. To analyse, whether the impact on DCs is a consequence of elevated inflammation or a direct effect of T cell-expressed CD83 on DCs, a coculture experiment was performed.

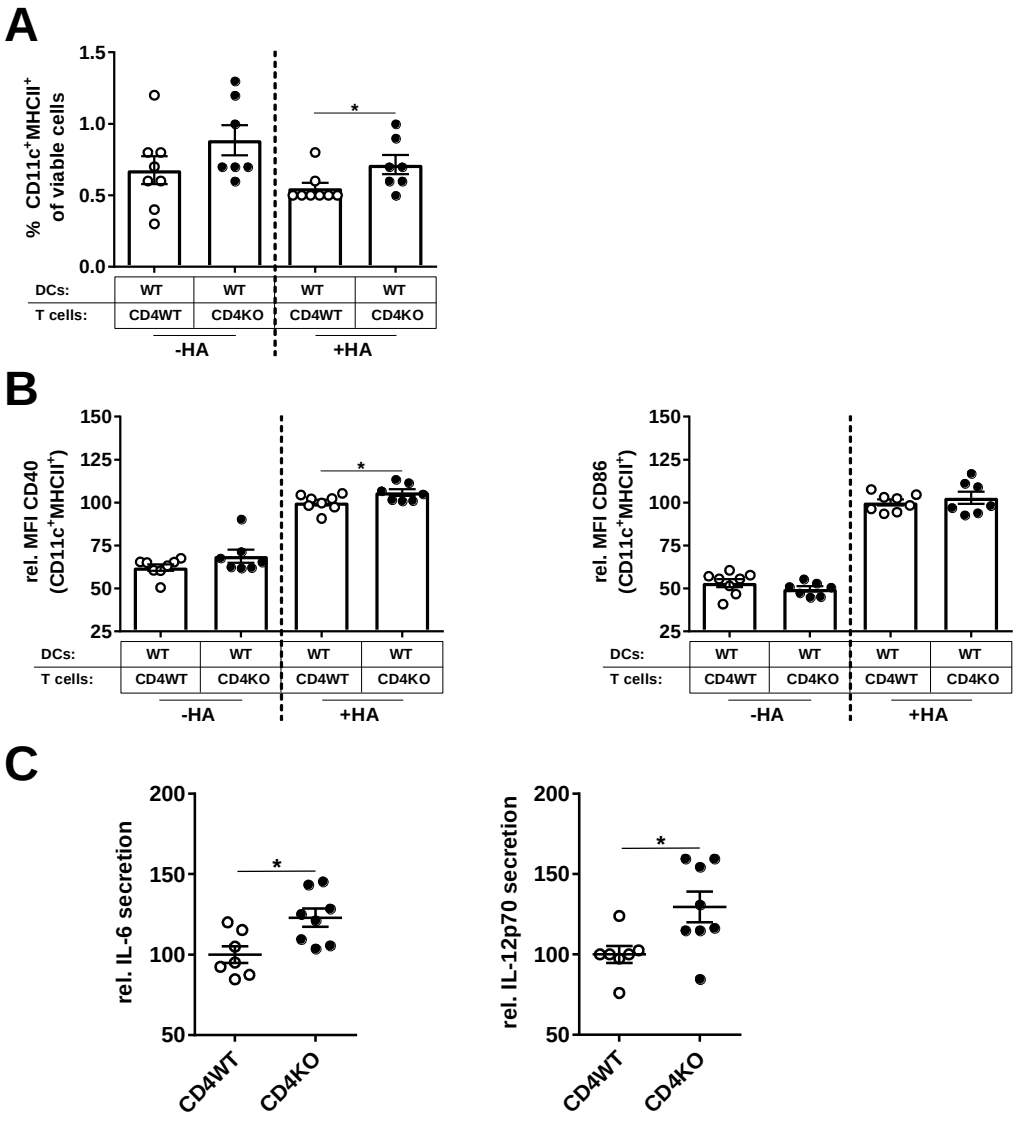

Figure 26: T cell-specific deletion of CD83 leads to increased DC activation *in vitro*.
(A-C) CD4$^+$CD25$^-$ T cells from HA-specific CD4WT and CD4KO mice enriched by FACS were cocultured with MACS-sorted CD11c$^+$ DCs from WT Balb/c mice in the presence or absence of $HA_{110-120}$ antigen. After 16 h of coculture activation of DCs was determined flow cytometry. **(A)** Percentages of CD11c$^+$MHCII^{high} DCs of viable cells and **(B)** relative MFI of CD40 and CD86 on CD11c$^+$MHCII^{high} DCs are presented. **(C)** Cytokine concentrations in the supernatant of stimulated cocultures from (A) were determined by Luminex technology. Results from two to three independent experiments (n = 7-8 mice) are summarized as mean ± SEM. Student`s t-test was used for statistical analysis (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

For this purpose, $CD4^+CD25^-$ Tconv cells from HA-specific CD4WT and CD4KO mice ($CD83^{flox/flox}$ x CD4cre x HA) enriched by FACS were cocultured with MACS-sorted $CD11c^+$ DCs from WT Balb/c mice in the presence or absence of $HA_{110-120}$ antigen. After 16 h of coculture, activation of DCs was determined flow cytometry. Strikingly, the frequency of $CD11c^+MHCII^{high}$ DCs cocultured with CD83-deficient $CD4^+$ T cells was significantly elevated compared to DCs cocultured with $CD4^+$ T cells from WT mice (Figure 26A). Furthermore, DCs cocultured with CD83-deficient T cells showed significantly higher expression of the activation marker CD40 (Figure 26B), whereas the expression of CD86 was not affected. In addition, significantly elevated concentrations of DC-related cytokines IL-12p70 and IL-6 were detected in the supernatant of DCs cocultured with stimulated CD83-deficient T cells compared to the coculture of DCs and CD4WT T cells (Figure 26C).

These results indicate that CD83 deletion in $CD4^+$ T cells not just intrinsically affects T cells, but also alters DC activation. Whether this effect is mediated via direct receptor-ligand interaction or indirect via e.g. cytokine secretion needs to be analysed in more detail in future.

4.2 DC-specific CD83 expression

Results obtained using the T cell-specific CD83 conditional knockout mice suggest an inhibitory function of T cell-expressed CD83. The broad expression of CD83 on several immune cells raises the question, whether CD83 has a conserved function or might act in distinct ways on different immune cells. CD83 is a well-established marker for mature DCs [124]. However, the function of CD83 in DCs is still discussed controversially [141]. Thus, CD83 expression and function on DCs was analysed in more detail.

4.2.1 Characterization of DC-specific CD83 conditional knockout mice

4.2.1.1 CD83 expression on $CD11c^+$ DCs isolated from CD11cWT and CD11cKO mice

To get further insight in the function of CD83 on DCs, a DC-specific CD83 conditional knockout mouse ($CD83^{flox/flox}$ x CD11ccre) was generated by breeding $CD83^{flox/flox}$ mice with transgenic CD11ccre mice.

CD83 expression was quantified on total CD11c^{+}MHCII^{high} DCs and on specific cDC subsets from DC-specific CD83 conditional knockout mice (CD11cKO) and WT littermates (CD11cWT).

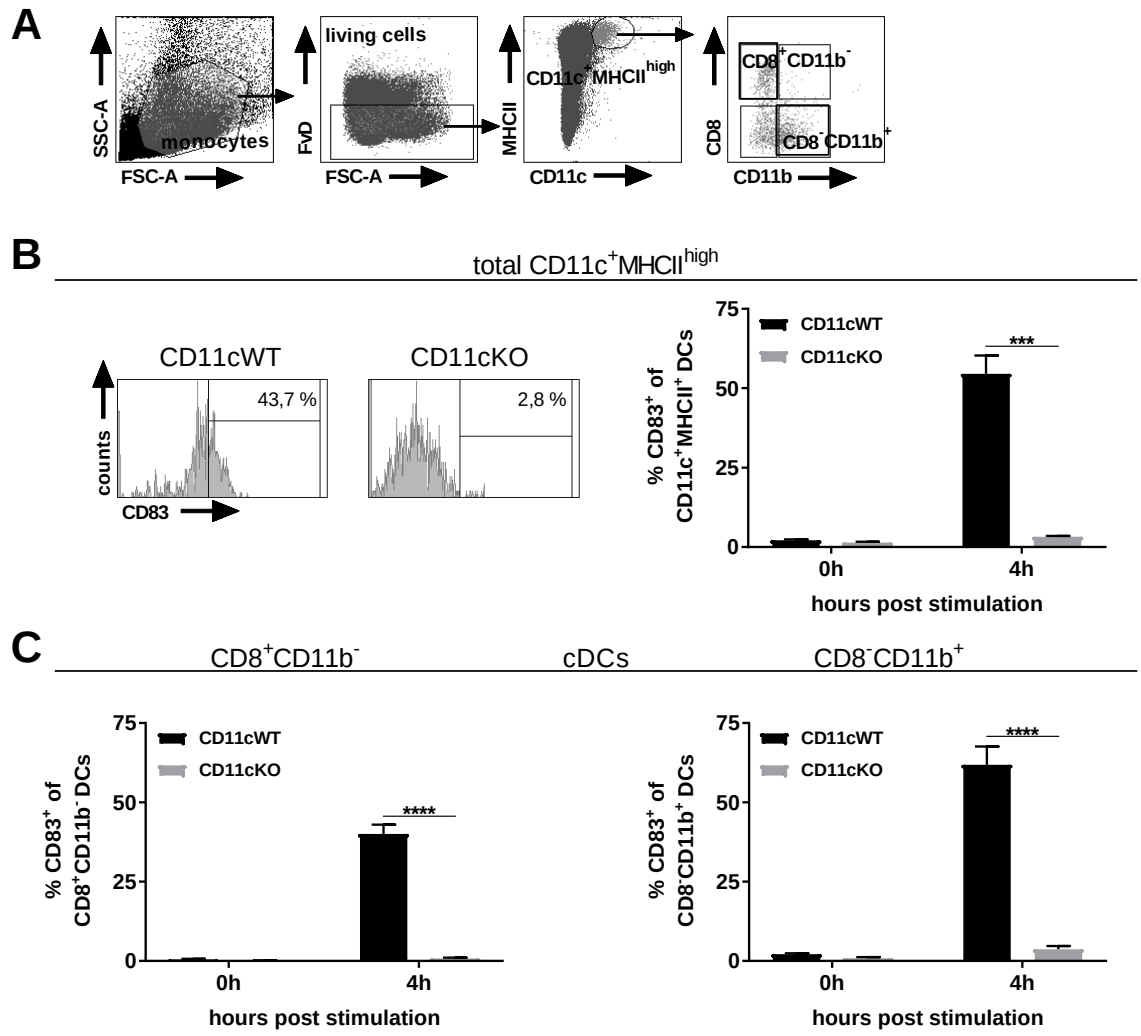

Figure 27: CD83 expression on total CD11c^{+}MHCII^{high} DCs and distinct DC subsets from CD11cWT and CD11cKO mice.
(A-B) Spleen cells from CD11cWT and CD11cKO mice were isolated and left unstimulated or stimulated for 4 h with LPS. **(A)** Gating strategy to determine CD11c^{+}MHCII^{high} DCs and distinct DC subsets (CD8^{+}CD11b^{-} and CD8^{-}CD11b^{+} cDCs) by flow cytometry. **(B)** Representative histograms of CD83 expression on total CD11c^{+}MHCII^{high} DCs from CD11cWT and CD11cKO mice (left) and summarized percentages of CD83^{+} of total CD11c^{+}MHCII^{high} (right) and **(C)** CD8^{+}CD11b^{-}/CD8^{-}CD11b^{+} cDCs from CD11cWT and CD11cKO mice are shown. Results from two independent experiments (n = 7-8 mice) are presented as mean ± SEM. Statistics were performed using Student`s t-test (*, p < 0,05; **, p < 0,01; ***, p < 0,001; ****, p < 0,0001).

Figure 27A shows the gating strategy to identify different DC populations. Initially, the monocyte population was determined by granularity and size and dead cells were excluded by staining with FvD. Upon gating on all CD11c^{+}MHCII^{high} DCs CD8^{+}CD11b^{-} and CD8^{-}CD11b^{+} cDCs were identified. Analysis of CD83 expression on CD11c^{+}MHCII^{high} DCs from CD11cWT mice showed that none of the naive DC

subsets expressed CD83. However, CD83 expression was strongly upregulated on all DC subsets from CD11cWT mice upon activation with LPS (Figure 27B-C). As expected, total CD11c^{+}MHCII^{high} DCs as well as all other DC subsets from CD11cKO mice neither displayed CD83 expression in the naive state nor upon stimulation (Figure 27B-C).

Taken together, these results show that CD83 is expressed on different DC subsets of CD11cWT mice upon stimulation. Furthermore, DC-specific deletion of CD83 could be confirmed on protein level in CD11cKO mice. Thus, CD11cKO mice provide a suitable mouse model to study the impact of cell-intrinsic CD83 expression on the DC phenotype.

4.2.1.2 DC frequencies in spleen of CD11cWT and CD11cKO mice

To investigate, whether ablation of CD83 in CD11c^{+} DCs affects the development of DCs, frequencies of different splenic DCs subsets in CD11cWT and CD11cKO mice were quantified by flow cytometry (Figure 28). CD11cWT and CD11cKO mice exhibited similar percentages of total CD11c^{+}MHCII^{high} DCs (Figure 28A) as well as CD8^{+}CD11b^{-} and CD8^{-}CD11b^{+} cDCs (Figure 29B), indicating that CD11c-specific CD83 expression is not essential for DC development.

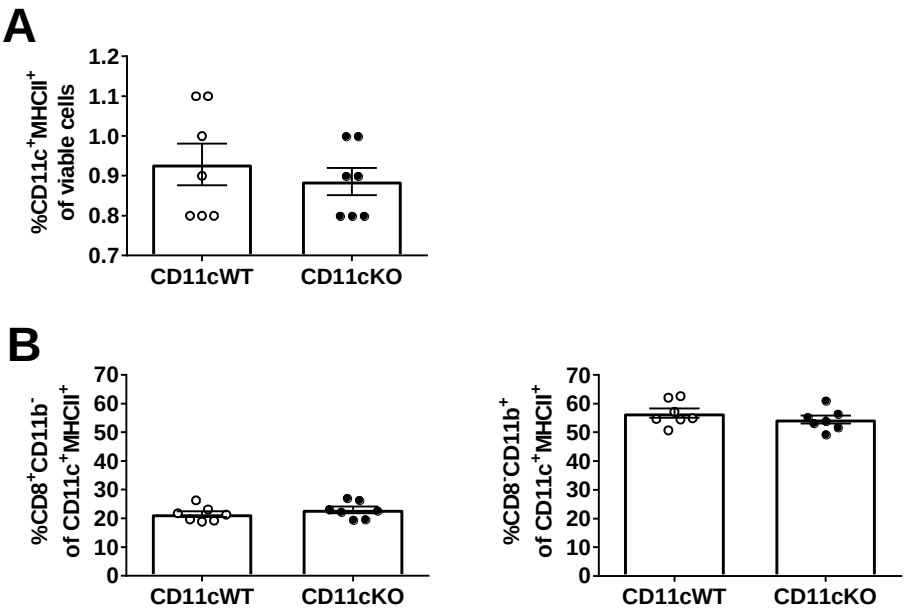

Figure 28: CD11cWT and CD11cKO mice exhibit the same percentages of different DC subsets. **(A-C)** Spleens from CD11cWT and CD11cKO mice were excised and single cell suspensions prepared. Percentage of **(A)** total CD11c^{+}MHCII^{high} DCs and **(B)** CD8^{+}CD11b^{-} as well as CD8^{-}CD11b^{+} cDCs in spleens from CD11cWT and CD11cKO mice are shown. Results from two independent experiments (n = 7 mice) are summarized as mean ± SEM. Mann-Whitney test was used for statistical analysis (*, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$).

4.2.1.3 CD11c^{+}MHCII^{high} DCs from CD11cKO mice have an altered expression of stimulatory molecules

To gain further insight into the role of CD83 in DC biology, the phenotype and function of CD83-deficient DCs was investigated. First, MHCII expression as well as expression of different activation marker on naive and stimulated DCs from CD11cWT and CD11cKO mice was analysed by flow cytometry (Figure 29).

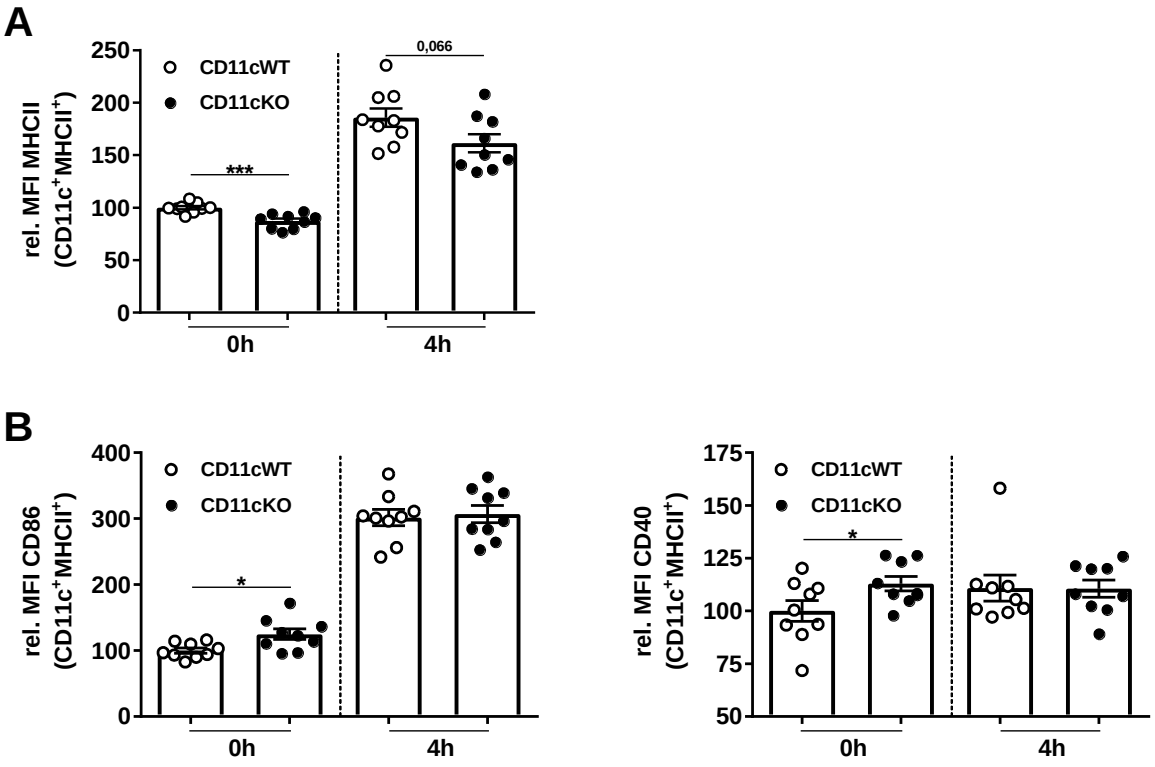

Figure 29: Naive DCs from CD11cKO mice show decreased MHCII expression compared to DCs from CD11cWT mice.
(A) Spleen cells of CD11cWT and CD11cKO mice were isolated, left unstimulated or stimulated for 4 h with LPS. DCs were analysed for the expression of different surface markers by flow cytometry. Relative MFI of MHCII, CD86 and CD40 expression on CD11c^{+}MHCII^{+} DCs is presented. Results from three independent experiments (n = 9 mice) are presented as mean ± SEM. Statistics were performed using Student`s t-test (*, p < 0,05; **, p < 0,01; ***, p < 0,001; ****, p < 0,0001).

MHCII expression was significantly decreased on CD83-deficient CD11c^{+}MHCII^{high} DCs compared to WT DCs (Figure 29A). Upon stimulation, DCs from CD11cWT as well as CD11cKO mice did upregulate MHCII expression, albeit CD83-deficient DCs still exhibited less MHCII expression. On the contrary, unstimulated CD11c^{+}MHCII^{high} DCs isolated from CD11cKO mice showed significantly increased expression of the costimulatory molecules CD86 and CD40 compared to CD11cWT DCs (Figure 29B).

However, expression of both activation markers was similar between DCs from CD11cWT and CD11cKO mice upon stimulation *in vitro*.

Taken together, DC-specific CD83 deletion seems to have an impact on the phenotype of DCs.

4.2.1.4 CD83-deficient CD11c^{+}MHCII^{high} DCs exhibit less T cell stimulatory capacity

To analyse the impact of CD83 expression on the T cell stimulatory capacity of DCs, the proliferation of responder T cells stimulated with CD83-deficient or WT DCs was investigated. Therefore, a coculture assay was performed. MACS-sorted CD11c^{+} DCs from CD11cWT and CD11cKO mice were cocultured with MACS-sorted CD4^{+}CD25^{-} Tconv cells from TCR-HA x Thy1.1 mice in the presence or absence of HA$_{110-120}$ antigen. Proliferation of HA-specific CD4^{+}CD25^{-} responder T cells, stained with the proliferation dye CFSE before cultivation, served as parameter for T cell stimulatory capacity of the DCs.

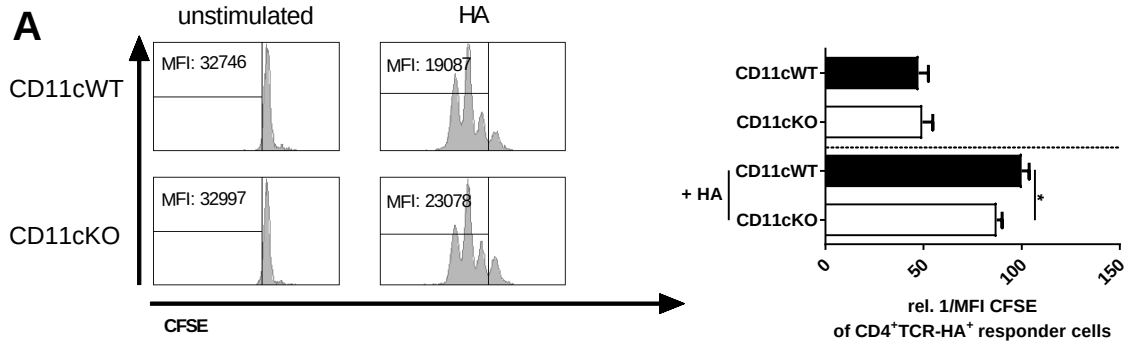

Figure 30: Tconv cells cocultivated with CD83-deficient DCs proliferate less compared to Tconv cells cocultivated with WT DCs.
(A) CD11c^{+} DCs from CD11cWT and CD11cKO mice and TCR-HA-specific CD4^{+}CD25^{-} T cells from TCR-HA x Thy1.1 mice were enriched by MACS and cocultivated in the presence or absence of HA$_{110-120}$ antigen for two days. Proliferation of HA-specific T cells, which were stained with the proliferation dye CFSE before cultivating, was determined as the loss of the CFSE signal by flow cytometry. Representative histograms of the CFSE signal of unstimulated and stimulated HA-specific T cells are shown (left) and proliferation is summarized as relative MFI of the CFSE signal (right).

Interestingly, HA-specific responder T cells stimulated with HA-loaded CD11c^{+}MHCII^{high} DCs from CD11cKO mice proliferated less compared to responder T cells stimulated with antigen-loaded DCs from CD11cWT mice (Figure 30).

Overall, deletion of CD83 expression has no impact on DC development as indicated by the same frequencies of different DCs population in CD11cWT and CD11cKO mice. Noteworthy, CD83-deficient DCs exhibit significantly decreased expression

levels of MHCII, which is in line with a reduced T cell stimulatory capacity. These data suggest that DC-expressed CD83 acts as a costimulatory molecule.

Taken together our data provide evidence that CD83 expressed on T cells and DCs seems to act in different ways. T cell-expressed CD83 delivers cell-intrinsic as well as cell-extrinsic inhibitory signals, indicated by a proinflammatory phenotype of CD83-deficient Tconv cells *in vitro* and *in vivo* as well as an elevated activation of DCs upon coculture with T cells from CD4KO mice *in vitro*. In contrast, CD83 expression on DCs seems to deliver costimulatory signals demonstrated by a decreased T cell stimulatory capacity of CD83-deficient DCs in *vitro*.

5 Discussion

T cell activation is initiated upon TCR-CD3 engagement with a specific peptide-MHC complex presented on the surface of APCs [24]. For appropriate T cell activation interaction of costimulatory molecules, such as the proteins of the CD28/B7 family expressed on the surface of both cells, is necessary [26, 27]. Upon T cell activation, several coinhibitory molecules are upregulated as well. Coinhibitory pathways balance T cell activation under steady state and preserve immune homeostasis. Furthermore, coinhibition regulates T cell activation during acute immune responses to avoid immunopathology and to restore homeostasis. In addition to T cells, these coinhibitory molecules can also be expressed on other hematopoietic cells such as DCs [59, 180]. Interestingly, increasing evidence indicates that CD83, a highly glycosylated, 45 kDa member of the Ig superfamily, contributes to regulation of T cell activation [127, 156, 157]. CD83 is strongly upregulated on DCs upon maturation, hence described as a marker for mature DCs for years [117, 124, 125]. However, CD83 is also expressed on the surface of many other cell types including activated B and T cells [115, 126, 127, 156]. In this study we investigated the role of T cell-expressed CD83 as a potential negative regulator of T cell activation in more detail.

Several studies reported an elevated surface expression of CD83 on human and murine $CD4^+$ T cells upon activation *in vitro* [126, 127, 129, 153, 155, 156]. In accordance with this, we detected an upregulation of CD83 expression on total $CD4^+$ T cells upon *in vitro* stimulation, which peaked 24 h post stimulation. Noteworthy, intracellular CD83 expression was about five times higher compared to extracellular CD83 expression in activated $CD4^+$ T cells (Figure 11C). Because extracellular CD83 expression already declined 48 h post activation, a transport of intracellular CD83 to the cell surface is unlikely. However, a release of intracellularly expressed CD83 is possible, taking into account that the precise mechanism for sCD83 generation is still elusive [137, 139].

Detailed analysis of CD83 expression on $Foxp3^+$ Treg and $Foxp3^-$ Tconv cells revealed an upregulation on both subsets upon activation, albeit the frequency of $CD83^+$ Treg cells was higher compared to $CD83^+$ Tconv cells (Figure 12C). A comparable observation was made by Reinwald and colleagues analysing the CD83 expression on FACS-sorted $CD4^+CD25^+$ Treg and $CD4^+CD25^-$ Tconv cells [127].

Overall, our data demonstrate that Treg as well as Tconv cells upregulate CD83 expression upon stimulation.

To better define the role of naturally expressed CD83 on T cells, we generated a T cell-specific CD83 conditional knockout mouse (CD4KO) (Figure 11A). For general CD83 knockout mice ($CD83^{-/-}$) a specific defect in the development of single positive $CD4^+$ T cells has been described, leading to reduced numbers of peripheral $CD4^+$ T cells [136]. CD4KO mice exhibited the same numbers and frequencies of $CD4^+$ T cells in the thymus and different lymphoid organs, indicating that T cell-specific CD83 deletion has no impact on T cell development (Figure 13B). In fact, this was expected, because Fujimoto and colleagues identified CD83 expression on thymic epithelial cells to be essential for adequate $CD4^+$ T cell development and not CD83 expression on $CD4^+$ T cells itself [136].

Noteworthy, CD4KO mice showed significantly increased numbers and frequencies of $Foxp3^+$ Treg cells in the spleen compared to CD4WT mice (Figure 14A) However, *in vitro* differentiation of naive $CD4^+$ T cells into iTreg cells was unchanged upon T cell-specific CD83 deletion (Figure 15A). In contrast to our results, Doebbeler and colleagues detected reduced Treg cell frequencies in peripheral lymphoid organs of Treg-specific CD83 conditional knockout mice (CD83cKO) and an impaired differentiation of naive T cells isolated from CD83cKO mice into iTreg cells *in vitro*. Hence, they postulated CD83 expression to be essential for Treg cell differentiation and stability [157]. However, Treg-specific CD83cKO mice still exhibited Treg cells, even in reduced amounts [157]. This underlines a rather supportive instead of an essential role regarding CD83 expression in Treg cells. A promoting role for CD83 expression in Treg cell development is also facilitated by data showing an induction of Foxp3 in about 25 % of $CD4^+CD25^-$ T cells transduced with a CD83-encoding retrovirus [127]. As mentioned above, we detected significantly increased frequencies of splenic Treg cells in CD4KO mice compared to CD4WT mice (Figure 14A-B). This might be explained by the observed elevated activation of Tconv cells from CD4KO mice (Figure 16), what might induce Treg cell expansion. Additionally, the results from the T cell transfer model of chronic colitis underline a positive correlation of $CD4^+$ T cell activation and enhanced induction of Treg cells (Figure 23B). In summary, our results clearly demonstrate that CD83 expression is not essential for Treg cell development. Apart from that, our results confirmed that CD83 expression is not essential for the inhibitory function of Treg cells [157], indicated by

an equal suppressive function of CD83-deficient and WT Treg cells *in vitro* (Figure 15B).

In addition to Treg cells, CD83 expression is upregulated on Tconv cells (Figure 12). To clarify the impact of CD83 expression on Tconv cell function, we investigated the phenotype of Tconv cells from CD4WT and CD4KO mice. CD83-deficient, naive Tconv cells exhibited enhanced activation (Figure 16), determined by increased expression of T cell activation marker CD69, CD44 and CD25 [29, 30, 32]. The same phenotype was also observed for naive CD83-deficient Treg cells (data not shown). Our results are in line with the enhanced activation observed for the Treg cells from Treg-specific CD83cKO mice [157]. These data point towards a cell-intrinsic function of T cell-expressed CD83 in the regulation of T cell activation and a contribution to immune homeostasis. A similar function has already been described for coinhibitory receptors like CTLA-4 and PD-1 [59, 180, 181]. In addition, CD83-deficient T cells showed enhanced proliferation, differentiation into TH1 cells and secretion of TH1-related cytokines (Figure 17A-C). Missing regulation of TH cell differentiation can unbalance immune homeostasis causing chronic diseases or can lead to inefficient pathogen clearance [182]. Therefore, differentiation into the distinct TH cell subtypes is a tightly regulated process influenced by many signals such as the costimulatory/coinhibitory molecule expression [183, 184]. As described for the coinhibitory molecules CTLA-4, PD-1 and TIM-3 [185-187], our results indicate that CD83 regulates TH1 cell differentiation as well and might therefore be considered as additional coinhibitory molecule.

Due to the fact that CD83 is upregulated in response to TCR stimulation, regulation of TCR signaling via CD83 is likely. The cytoplasmic CD83 domain does not exhibit a tyrosine, which excludes signal transduction via ITIMs [115, 118]. Nevertheless, signaling via any other conserved or unknown motif or adaptor protein is possible. Indeed, CD83-deficient Tconv cells showed significantly increased phosphorylation of ZAP-70 upon *in vitro* stimulation compared to CD4WT Tconv cells (Figure 18A). These results indicate a negative regulation of signal transduction via ZAP-70 by T cell-expressed CD83. ZAP-70 binds to the intracellular part of the two ζ-chains from the TCR. Thus, phosphorylation of ZAP-70 is one of the first signaling events downstream of the TCR [188]. TCR-mediated activation of ZAP-70 induces the recruitment and phosphorylation of many other signaling molecules such as Akt leading to T cell activation, proliferation and differentiation [188]. Hence, these results correlate with the activated phenotype of CD83-deficient Tconv cells and provide a

possible explanation on how CD83 exerts its cell-intrinsic function. For the coinhibitory receptors CTLA-4 and PD-1 inhibition of Akt signaling has already been demonstrated, albeit CTLA-4 and PD-1 bind different phosphatases and inhibit different signaling molecules [57, 180]. However, how CD83 regulates ZAP-70 phosphorylation has to be further investigated in future.

In summary, our *in vitro* data indicate that CD83 is upregulated upon activation to cell-intrinsically balance $CD4^+$ T cell responses. From these results we hypothesized that T cell-specific deletion of CD83 would have deleterious consequences during inflammatory conditions *in vivo*.

The CHS model is a suitable model to study the T cell response during cell-dependent HRs [66, 67]. In the CHS mouse model the treatment with a hapten induces an immune response against modified self-proteins complexed with the hapten, leading to a local CHS reaction of the skin at the site of reexposure [66]. Misbalanced regulation of the T cell response during the ongoing immune response can lead to enhanced inflammation. Indeed, CD4KO mice exhibited an enhanced CHS reaction compared to CD4WT mice indicated by a stronger ear swelling upon reexposure to the hapten (Figure 19B). The phenotype of CD4KO mice was characterized by significantly elevated activation of $CD4^+$ T cells isolated from the spleen and dLNs of CD4KO mice compared to $CD4^+$ T cells from CD4WT mice (Figure 21A). $CD4^+$ T cells have been described to be responsible for the immunopathology during the elicitation phase of CHS. Due to high concentrations of IFN-γ at the site of reexposure, TH1 cells are supposed to be the main players. Especially the CHS reaction to DNFB has been described to be TH1-dominated [67, 75]. However, TH17 cells seem to promote CHS development and progression as well [66]. Well in line our data showed an increased secretion of IFN-γ and IL-17 by $CD4^+$ T cells isolated from the dLNs of CD4KO mice compared to CD4WT mice (Figure 21B). Hence, results from the CHS mouse model underline a cell-intrinsic function of CD83 for the regulation of T cell responses *in vivo*.

Data obtained from *in vitro* and *in vivo* experiments suggest that T cell-specific deletion of CD83 mainly affects the Tconv cell phenotype and function. To further corroborate these findings, we used the T cell transfer model of chronic colitis, because in this model Treg-depleted, naive $CD4^+$ T cells are transferred. Indeed, the adoptive transfer of naive $CD4^+$ T cells from CD4KO mice resulted in enhanced disease progression compared to the transfer of WT $CD4^+$ T cells, indicated by increased spleen weight, colon weight/length ratio and histopathological score

(Figure 22C-D). In addition, transferred CD83-deficient $CD4^+$ T cells isolated from the mLNs and the LP were more activated compared to WT $CD4^+$ T cells (Figure 23). These data clearly show that the expression of CD83 on Tconv cells is important for the cell-intrinsic regulation of T cell activation.

Moreover, the transfer of CD83-deficient naive T cells resulted in increased levels of IL-12 in the serum of Rag2KO mice compared to mice injected with WT $CD4^+$ T cells (Figure 24A). In the T cell transfer model of chronic colitis the transferred T cells mainly differentiate into TH1 and TH17 cells [85, 88, 94, 113]. In addition to the effector cytokines IFN-γ and IL-17, DC-related cytokines contribute to the pathology in the colon [96]. In Crohn`s disease patients elevated levels of IL-12 in the colon have been reported to correlate with disease severity [102-104]. In addition, increased secretion of IL-12 was detected in the inflamed colon of mice using different colitis models. The deleterious role of IL-12 is further underlined by studies that showed a beneficial effect of treatment with an anti-IL-12 antibody in different colitis models [105-109]. In accordance with this, Rag2KO mice that received CD83-deficient T cells displayed enhanced frequencies of DCs, which were additionally more activated (Figure 25A-B). These results support a cell-extrinsic function of CD83 expression on Tconv cells in terms of downregulation of DCs activation. T cell-expressed CD83 seems to regulate the activation of DCs resulting in a reduced ability of DCs to stimulate T cells, thereby balancing T cell activation in a feedback loop. This phenomenon has already been demonstrated for other coinhibitory molecules such as CTLA-4 and PD-1. Those molecules bind to B7 proteins expressed on DCs, thus dampen DC activation and T cell stimulatory capacity [57-59, 181]. Overall, the results from the T cell transfer model of chronic colitis suggest that CD83 regulates T activation via cell-intrinsic and cell-extrinsic mechanisms in inflamed tissues to dampen immunopathology.

To confirm a cell-extrinsic function of Tconv cell-expressed CD83 on DC activation, we performed a coculture experiment *in vitro*. For this purpose, HA-specific Tconv cells from CD4WT and CD4KO mice were cocultured with $CD11c^+$ DCs from WT Balb/c mice in the presence or absence of $HA_{110\text{-}120}$ antigen. DCs cocultured with CD83-deficient Tconv cells revealed elevated activation upon stimulation (Figure 26C). However, downregulation of costimulatory molecules on DCs, mediated by T cell-expressed CD83, seems to be specific for CD40, because CD86 expression was unchanged. Noteworthy, CD40/CD40L interaction is required for IL-12 and IL-6

secretion by DCs [189-192]. In line with this, significantly enhanced secretion of IL-12 and IL-6 was detected in the supernatant of DCs cocultured with CD83-deficient Tconv cells, further suggesting that CD83 expression in Tconv cells might participate in the regulation of CD40 expression on DCs. This is underlined by the results obtained from the T cell transfer model of chronic colitis. Herein, CD40 expression was upregulated on DCs from Rag2KO mice that received CD83-deficient T cells and IL-12 as well as IL-6 concentrations in the serum were enhanced (Figure 24A, 25B). One might speculate that this is a result of missing CD83 expression on T cells during DC/T cell interaction. A negative impact on the expression of costimulatory molecules on DCs has already been described for the T cell-expressed coinhibitory molecule CTLA-4 [193-195]. Based on our results an impact on costimulatory molecule expression is also likely for T cell-expressed CD83.

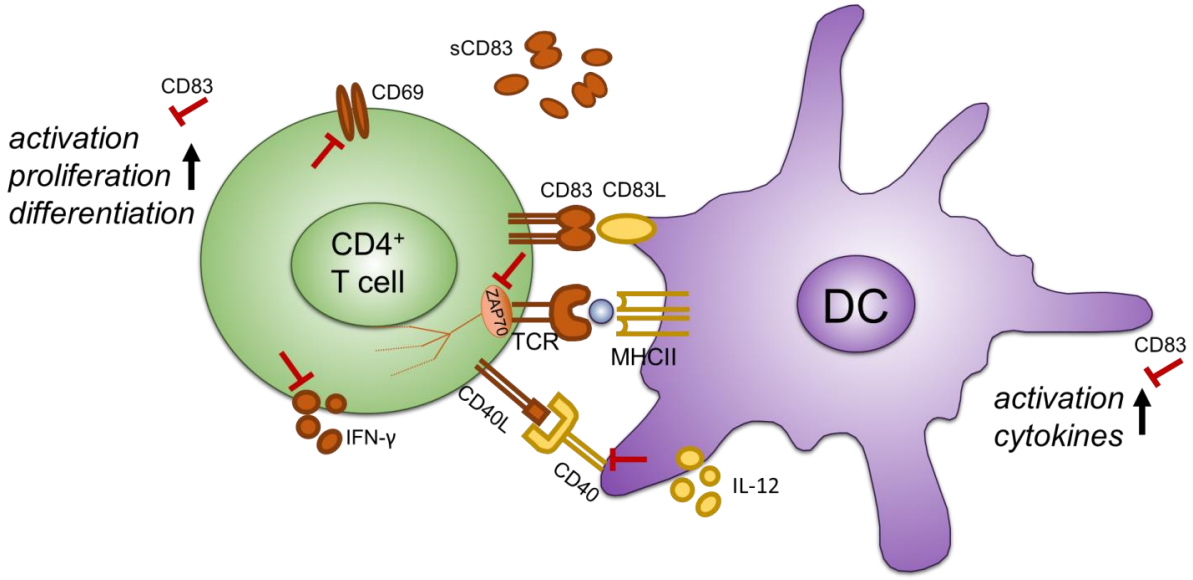

Figure 31: Proposed mechanism of CD83 expression in T cells.
CD83 expression on Tconv cells regulates T cell activation in naive state to keep up homeostasis as well as during an ongoing immune response to dampen immunopathology. On the one hand CD83 might exert its function by cell-intrinsic inhibition of T cell activation, proliferation and differentiation, which is likely controlled by regulation of ZAP-70 signaling. On the other hand interaction of T cell-expressed CD83 with a possible CD83L expressed on DCs might cell-extrinsically regulate DCs activation. In addition sCD83, directly released by the T cell or generated via enzymatic cleavage of transmembrane CD83, could interact with surrounding cells and confer immunosuppressive function.

Cell-extrinsic functions of coinhibitory molecules can be mediated via different mechanism [59, 180, 181]. One possibility of cell-extrinsic function of CD83 is the classical receptor-ligand interaction, inducing negative signaling in the ligand-expressing cell. Indeed, several studies have postulated the existence of a ligand for CD83 (CD83L). Albeit the results regarding the CD83L expressing cell population are controversial [138, 154, 160, 196], ligand expression on DCs has been described

[138]. From our results the expression of a CD83L on DCs is also very likely. However, as long as the ligand for CD83 is unknown, a precise mechanism for CD83 immunoregulatory function is hard to predict.

In addition to a possible CD83-CD83L interaction, CD83 could also exert the cell-extrinsic function via sCD83. Indeed, Rag2KO mice injected with CD83-deficient $CD4^+$ T cells exhibited significantly lower levels of sCD83 in the serum compared to Rag2KO mice transferred with WT $CD4^+$ T cells (Figure 24B). In this experimental setting the origin of sCD83 expression cannot be identified and the release by DCs, T cells or any other cell population is possible. Indeed, sCD83 has already been detected in the supernatant of activated DCs, B and T cells [137, 156]. However, we could not detect significant concentrations of sCD83 in the supernatant of *in vitro* stimulated Tconv cells from CD4WT mice (data not shown). Noteworthy, Kreiser and colleagues were able to detect sCD83 in the supernatant of enriched $CD83^+CD4^+$ T cells upon *in vitro* stimulation [156], whereas the concentration in the supernatant of $CD83^-CD4^+$ T cells was decreased. In consequence, a diminished sCD83 secretion by CD83-deficient $CD4^+$ T cells is likely, so that T cell-specific CD83 deletion could be partially responsible for the decreased sCD83 concentration in Rag2KO mice that received CD83-deficient T cells (Figure 24B). Regardless of the origin, an immunosuppressive function of sCD83 has been described by many different studies, highlighting a suppressive effect on DC maturation and DC-mediated T cell stimulation [121, 138, 139, 154, 169, 197, 198]. IDO expression, which has been described to be induced by sCD83 several times, could be responsible for the anti-inflammatory effect of sCD83 [168, 170, 172]. Hence, sCD83 expression provides another mechanism for $CD83^+CD4^+$ T cells to exert cell-extrinsic function. Whether sCD83 is directly released from the cell or generated by enzymatic cleavage still needs to be clarified.

Taken together, CD83 seems to negatively regulate T cell responses by cell-intrinsic and cell-extrinsic mechanisms as summarized in Figure 31.

Results obtained using the T cell-specific CD83 conditional knockout mice suggest an immunoregulatory function of CD83 expression in T cells. The broad expression of CD83 on several immune cells raises the question, whether CD83 has a conserved function or might act by different mechanisms on different immune cells. CD83 is a well-established marker for mature DCs [128, 141]. In the present study we could show that CD83 is highly expressed on total splenic $CD11c^+MHCII^{high}$ DCs

and on different subsets of cDCs upon *in vitro* stimulation (Figure 27). These results are well in line with reports that have shown CD83 expression on activated murine and human DCs [117, 124, 125, 141, 143, 199].

By using a DC-specific CD83 conditional knockout mouse (CD11cKO), the role of CD83 expression on DCs was analysed in more detail. Noteworthy, DC-specific CD83 deletion did not affect DC development, indicated by similar DCs frequencies and numbers in CD11cWT and CD11cKO mice (Figure 28). However, the function of CD83 in DCs is still discussed controversially [199]. Some studies reported that CD83 deletion decreases MHCII expression on DCs, B cells and other antigen presenting cells [136, 144], whereas other stated a normal MHCII expression on CD83-deficient DCs [140, 151]. The different results might be explained by the use of different mechanism to manipulate CD83 expression. The studies describing an effect on MHCII expression used complete CD83 knockout mice [136, 144], whereas the other ones used CD83 mutant mice, which only exhibit reduced CD83 expression [140, 151]. We detected a significantly decreased MHCII expression on naive and stimulated CD83-deficient $CD11c^{+}MHCII^{high}$ DCs compared to WT DCs (Figure 29A), suggesting that CD83 plays an important role for appropriate MHCII expression on DCs. This is concordant with the observation made by Tze and colleagues. They demonstrated that the transmembrane domain of CD83 provides an increase in MHCII expression by antagonizing the interaction of MHCII with the ubiquitin ligase MARCH1 [145]. In addition, downregulation of CD83 expression on DCs by different viruses, the use of siRNA or interference with the nuclear export has been described to reduce the T cell stimulatory capacity of DCs [146, 147, 149, 150]. In accordance with these studies, HA-specific responder T cells cocultured with HA-loaded DCs from CD11cKO mice proliferated less compared to T cells cocultured with DCs from CD11cWT mice (Figure 30).

Results from our experiments with CD11cKO mice suggest a costimulatory function of DC-expressed CD83. For many members of the B7 family such as CD80 and CD86 a costimulatory role has been described [200-202]. Indeed, structural similarities of CD83 to the B7 family members CD80, CD86 and PD-L2 have been observed recently [122]. Noteworthy, DC-expressed costimulatory molecules can also exert coinhibitory function by binding to a different ligand as described for the CD80/CTLA-4 interaction [57-59]. A diverse function of DC-expressed CD83 mediated by interaction with different ligands is conceivable. This would also explain the immunoregulatory role of CD83 expression on DCs observed by Bates and

colleagues, which is in contrast to the costimulatory role described before. DC-specific CD83 conditional knockout mice developed exacerbated colitis following dextran sodium sulfate challenge, whereas mucosal overexpression of CD83 inhibited the inflammatory immune response and prevented colitis. By the use of different *in vitro* experiments they claimed that the inflammatory response is regulated via cell-cell contact dependent CD83 homotypic interactions [152]. However, our results suggest a more costimulatory function of DC-expressed CD83 at least *in vitro*. Thus, further experiments with DC-specific CD83-deficient mice are necessary to clarify the impact of CD83 expression on DC function *in vivo*.

Taken together our results confirmed the expression of CD83 on activated T cells and DCs. Nevertheless, the broad expression of CD83 is not concurrent with a conserved function on the different immune cell subtypes. Hence, analysis of CD83 expression on different cell types has to be investigated very precisely.
From our results we conclude that CD83 expression on Tconv cells controls T cell activation in naive mice to maintain homeostasis as well as to dampen immunopathology and restore homeostasis during an ongoing immune response. Particularly important is the observation that cell-intrinsic and cell-extrinsic mechanisms of T cell-expressed CD83 contribute to the regulation of T cell responses. Overall, CD83 expression on T cells seems to be upregulated upon activation to balance $CD4^+$ T cell responses. In contrast, our data showed a costimulatory function of CD83 expression on DCs, at least *in vitro*. To elucidate the mechanism of CD83 function, the characterization of the ligand and downstream signaling would be the next important tasks. This would also promote the potential to therapeutically target CD83 in the future.

6 Abstract

The glycoprotein CD83 is a classical marker for mature DCs, but it is also expressed on the surface of many other cell types including activated B and T cells. Increasing evidence indicates that CD83 expression on T cells contributes to the regulation of T cell activation and immune responses in general. However, the precise function of T cell-expressed CD83 is still elusive. Therefore, we carefully investigated the role of CD83 expression in T cells in the present study.

Herein, we identified CD83 to be expressed on regulatory (Treg) as well as conventional (Tconv) T cells upon stimulation. To better define the role of naturally expressed CD83 on T cells, we generated a T cell-specific CD83 conditional knockout mouse (CD4KO). CD4KO mice showed the same numbers and frequencies of $CD4^+$ T cells in the thymus and different secondary lymphoid organs, indicating that T cell-specific CD83 expression has no impact on T cell development. Furthermore, T cell-specific CD83 deletion did neither affect Treg cell induction nor Treg cell inhibitory function *in vitro*. In contrast, Tconv cells from CD4KO mice depicted a significantly altered phenotype. Upon *in vitro* stimulation naive $CD4^+$ Tconv cells from CD4KO mice showed significantly enhanced activation, elevated proliferation as well as increased TH1 cell differentiation compared to Tconv cells from CD4WT mice. In addition, CD83-deficient Tconv cells showed significantly enhanced phosphorylation of ZAP-70 compared to CD4WT Tconv cells upon *in vitro* stimulation, suggesting a regulation of TCR signaling by CD83.

In vivo CD4KO mice developed a more severe CHS reaction compared to CD4WT mice, which was accompanied by significantly increased activation and proinflammatory cytokine secretion of CD83-deficient $CD4^+$ T cells. Moreover, CD83 deletion in $CD4^+$ T cells led to exacerbated T cell transfer induced colitis in Rag2KO mice compared to the transfer of wildtype $CD4^+$ T cells, demonstrating an important role of cell-intrinsic CD83 expression in Tconv cell function.

In addition to the cell-intrinsic effects, T cell-specific CD83 deletion induced elevated DC activation *in vitro* and *in vivo* suggesting a cell-extrinsic function of T cell-expressed CD83 expression in regulation of T cell activation as well.

In summary, we showed for the first time that CD83 expressed on stimulated Tconv cells negatively regulates T cell activation by cell-intrinsic as well as cell-extrinsic mechanisms.

Even though CD83 is used as a marker for mature DCs, the precise function on DCs is still discussed controversially. Our data support a costimulatory function of CD83 expression on DCs indicated by decreased T cell stimulatory capacity of CD83-deficient DCs compared to WT DCs.

Overall, CD83 expression on T cells has an inhibitory function, whereas CD83 expression on DCs seems to exert a more costimulatory function. Therefore, the function of CD83 expression on different immune cells has to be considered differentially.

7 Zusammenfassung

CD83 ist ein klassischer Marker für reife Dendritische Zellen, wird jedoch ebenfalls von vielen anderen Immunzellen, wie aktivierten T- und B-Zellen, exprimiert. In der Literatur wird zunehmend eine immunregulatorische Funktion für die Expression von CD83 auf T-Zellen beschrieben. Die genaue Funktion von T Zell-exprimiertem CD83 ist jedoch weitgehend unklar. Aus diesem Grund wurde in der vorliegenden Arbeit die Funktion von T Zell-exprimiertem CD83 genauer analysiert.

Zunächst konnten wir zeigen, dass CD83 nach Stimulation *in vitro* sowohl von regulatorischen (Treg) als auch von konventionellen (Tkonv) T-Zellen exprimiert wird. Um die Funktion von CD83 auf T-Zellen besser analysieren zu können, wurde eine T Zell-spezifische CD83 konditionelle Knockout-Maus generiert (CD4KO). Die T Zell-spezifische Deletion von CD83 zeigte keinen Einfluss auf die Entwicklung der $CD4^+$ T Zellen. Des Weiteren hatte die Deletion von CD83 keine Auswirkungen auf die Induktion regulatorischer T-Zellen und die inhibitorische Aktivität der Treg-Zellen *in vitro*. Interessanterweise, wiesen die Tkonv-Zellen der CD4KO Mäuse einen veränderten Phänotyp auf. Dieser äußerte sich in einer signifikant erhöhten Expression verschiedener Aktivierungsmarker, einer stärkeren Proliferation und einer gesteigerter TH1-Zell Differenzierung gegenüber den Tkonv-Zellen der CD4WT Mäuse. Außerdem konnte eine erhöhte Phosphorylierung von ZAP-70 in CD83-defizienten Tkonv-Zellen detektiert werden, was auf eine Regulierung der T-Zell-Rezeptor Signaltransduktion durch CD83 schließen lässt.

In vivo entwickelten die CD4KO Mäuse verglichen mit den CD4WT Mäusen eine stärkere CHS Reaktion, die mit einer erhöhten T-Zell-Aktivierung und einer stärkeren Sekretion proinflammatorischer Zytokine assoziiert war. Außerdem führte der Transfer von naiven, CD83-defizienten T-Zellen im Vergleich zum Transfer von Wildtyp T-Zellen zu einer stärkeren Kolitis, was eine wichtige Funktion der CD83 Expression auf Tkonv-Zellen zeigt.

Zusammenfassend konnten wir zeigen, dass die Expression von CD83 auf aktivierten Tkonv-Zellen durch Zell-intrinsische als auch -extrinsische Mechanismen die T-Zell Aktivierung negativ reguliert.

Auch wenn CD83 als klassischer Marker für reife DCs verwendet wird, wird die genaue Rolle von CD83 auf DCs noch immer kontrovers diskutiert. Wir konnten zeigen, dass CD83-defiziente DCs eine verminderte T-Zell stimulatorische Aktivität

aufweisen, was eine kostimulatorische Funktion von DC-exprimiertem CD83 nahelegt.

Zusammenfassend zeigt die Expression von CD83 auf T-Zellen eine inhibitorische Funktion, wohingegen die Expression von CD83 auf DCs mit einer eher kostimulatorischen Aktivität einhergeht. Aus diesem Grund sollte die Funktion von CD83 auf verschiedenen Immunzellen stets differenziert betrachtet werden.

8 References

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9 Index

9.1 Abbreviations

Abbreviation	
aa	Amino acid
ACD	Allergic contact dermatitis
AF	Alexa Fluor
AIDS	Acquired immune deficiency syndrome
APC	Allophycocyanin
APCs	Antigen presenting cells
BFA	Brefeldin
BV	Brilliant Violet
CD	Cluster of differentiation
CD	Crohn´s disease
CD83$^{-/-}$	CD83 general knockout mice
CD83cKO	CD83 conditional knockout mice
CD4KO	T cell-specific CD83 conditional knockout mice
CD4WT/CD11cWT	CD83 wildtype mice
CD11cKO	DC-specific CD83 conditional knockout mice
cDC	Conventional dendritic cell
cDNA	Complementary DNA
CDR	Complementary determining region
CHS	Contact hypersensitivity
CLR	C-type lectin receptor
cTEC	Cortical thymic epithelial cells
CTL	Cytotoxic T-lymphocyte
CTLA-4	Cytotoxic T-lymphocyte antigen-4
Cy	Cyanine
D	Diversity
DAMP	Damage associated molecular pattern
DC	Dendritic cell
dDC	Dermal dendritic cell
dLN	Draining lymph node
DN	Double negative

DNA	Deoxyribonucleic acid
DNFB	1-fluoro-2,4-finitrobenzene
dNTP	Deoxy-Nucleotide-Triphosphate
DP	Double positive
DSS	Dextran sodium sulfate
EAE	Experimental autoimmune encephalomyelitis
EDTA	Ethylenediaminetetraacetic acid
eGFP	Enhanced green florescence protein
EGTA	Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid
ELISA	Enzyme linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
FoxP3	Forkhead-box 3
FvD	Fixable viability dye
GATA3	GATA-binding protein 3
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HA	Hemagglutinin
HIV	Human immunodeficiency virus
H&E	Hematoxylin and eosin staining
HR	Hypersensitivity reaction
i.p.	Intraperitoneal
IBD	Inflammatory bowel disease
ICAM1	Intercellular adhesion molecule 1
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon-γ
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cell
IRF	Interferon regulatory factor
ITAM	Tyrosine-based activatory motifs
ITIM	Tyrosine-based inhibitory motifs
iTreg	Induced Treg

J	Joining
KO	Knock out
LAG3	Lymphocyte-activation gene 3
LANUV	State Agency for Nature, Environment and Consumer Protection
LTα	Lymphotoxin α
LFA1	Lymphocyte function-associated antigen
LN	Lymph nodes
LP	Lamina propria
LPS	Lipopolysaccharide
MACS	Magnetic activated cell sorting
MAPK	Mitogen-activated protein kinase
MD-2	Myeloid differentiation factor-2
MFI	Mean fluorescence intensity
MHCI/II	Major histocompatibility complex class I/II
mLN	Mesenteric lymph nodes
mRNA	Messenger RNA
NF-κB	Nuclear factor κ-light-chain-enhancer of activated B cells
NK cells	Natural killer cell
NLR	NOD-like receptor
nTreg	Natural Treg
PAMP	Pathogen associated molecular patern
PB	Pacific Blue
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein-1
pDC	Plasmacytoid Dendritic cell
PE	Phycoerythrin
PerCP	Peridinin chlorphyll protein
PFA	Paraformaldehyde
PMA	Phorbol-12-myristate-13-acetate
PRR	Pattern recognition receptor
Rag	Recombination activating gene
RLR	RIG-I-like receptor

RNA	Ribonucleic acid
RORγt	Retinoic acid receptor-related orphan receptor gamma-t
RT	Room temperature
sCD83	Soluble CD83
SCID	Severe combined immunodeficiency
SLAM	Signaling lymphocyte activation
T-bet	T-box–containing protein expressed in T cells
Tconv	Conventional T cell
TCR	T cell receptor
TF	Transcription factor
TGF-ß	Transforming growth factor ß
TH	T helper cell
TLR	Toll-like receptor
TNCB	1-chloro-2,3,4-trinitro-benzene
TNF.α	Tumor necrosis Factor-α
Treg	Regulatory T cell
TCR	T cell receptor
UC	Ulcerative colitis
V	Variable
WT	Wildetype
ZAP-70	Zeta-chain-associated protein kinase 70

9.2 List of figures

9.3 List of tables

10 Appendix

10.1 Acknowledgements

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10.2 Curriculum Vitae

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

Publications

Lapuente D, Storcksdieck Genannt Bonsmann M, Maaske A, Stab V, Heinecke V, **Watzstedt K**, Heß R, Westendorf AM, Bayer W, Ehrhardt C, Tenbusch M. *IL-1β as mucosal vaccine adjuvant: the specific induction of tissue-resident memory T cells improves the heterosubtypic immunity against influenza A viruses.* Mucosal Immunol. 2018 Jul;11(4):1265-1278.

Conference contributions

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Watzstedt K., Buer J., Hansen W., CD83 expression has a $CD4^+$ T cell intrinsic inhibitory function in vitro and in vivo. 15th Day of Research of the Faculty of Medicine, University Hospital Essen, Essen, Germany (2016).

Watzstedt K., Buer J., Hansen W., CD83 expression has a $CD4^+$ T cell intrinsic inhibitory function in vitro and in vivo. 46th Annual Meeting of the German Society for Immunology, Hamburg, Germany (2016).

Research grands

Poster award. 15th Day of Research of the Faculty of Medicine, University Hospital Essen, Essen, Germany (2016).

10.3 Declarations

Erklärung:

Hiermit erkläre ich, gem. § 7 Abs. (2) d) + f) der Promotionsordnung der Fakultät für Biologie zur Erlangung des Dr. rer. nat., dass ich die vorliegende Dissertation selbständig verfasst und mich keiner anderen als der angegebenen Hilfsmittel bedient, bei der Abfassung der Dissertation nur die angegeben Hilfsmittel benutzt und alle wörtlich oder inhaltlich übernommenen Stellen als solche gekennzeichnet habe.

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Katarina Liedtke

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Essen, den _____ _____

Wiebke Hansen