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Expression of CD83 in Healthy and Disturbed Murine Pregnancy

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SUMMARY

Introduction

Gynaecologic immunologic research aims to answer an important question: How does the immune system manage to protect both mother and unborn child while not harming the semi-allogeneic and thus partially unaccustomed fetus? Several distinct adaptions in both the innate and adaptive immune system take place during pregnancy. Alterations in these processes can cause dramatic consequences like pregnancy loss. Here, molecules with immunomodulatory functions can provide possible treatment options. One molecule with the described features emerged as a candidate: The transmembrane molecule mCD83 as well as its soluble form, sCD83. As mCD83 overexpressing cells and cells from pregnant mice showed similar behaviour regarding interleukin-10 secretion and B-cell (BC) development, a contribution of mCD83 in immunologic pregnancy adaptions is possible. Additionally, the soluble form could be a future therapeutic agent in pregnancy disorders, regarding its already shown benefits in therapy of various autoimmune diseases in animal models.

The aim of this work is to evaluate the expression, release and regulation of CD83 in its membrane bound and soluble forms during normal and disturbed pregnancies in mouse models.

Methods

The semi-allogeneic pairing of two inbred stems, C57Bl6/J×BALB/c, results in healthy pregnancy and was used to investigate the expression in different stages of pregnancy. Pairing CBA/J females with DBA/2J males results in resorption of fetal units and represents a poor pregnancy outcome mating (PPOM). This model in comparison with CBA/J×BALB/c pairings (presenting a good pregnancy outcome mating (GPOM)) is a model for immunologic pregnancy disturbance. It was used to detect alterations in mCD83 expression and sCD83 release during disturbed pregnancy.

Results

During normal murine pregnancy, mCD83 expression increased with a peak on day 14 of pregnancy on B- and T-cells, while the amount of mCD83 positive cells was elevated at the end of pregnancy. PPOM mice showed higher mCD83 expression and mCD83 positive cell count on various lymphocyte subtypes in comparison to GPOM, while sCD83 levels were lower

in PPOM pregnancies. Splenocytes released sCD83 in cell culture, whereby the main part under unstimulated conditions was produced by BC. Progesterone treatment of splenocytes led to a dose dependent mCD83 upregulation on T-cells and reduced mCD83 expression as well as sCD83 release from BC. Culture of splenocytes with tissue inhibitor of metalloproteinases 1 (TIMP1) resulted in elevated sCD83 release and mCD83 expression on BC. Progesterone reduced TIMP1 expression on BC *in vitro*.

Conclusions

mCD83 expression and sCD83 release showed various alterations during normal murine pregnancy as well as when comparing PPOM with GPOM. Noticeable are in particular a higher mCD83 expression on splenic BC on day 14 of pregnancy. In BC from PPOM, mCD83 expression is higher than on BC from GPOM, while PPOM mice show a lower sCD83 serum level, hinting a problem in the shedding mechanism during PPOM.

Progesterone regulates mCD83 expression on BC via TIMP1 and a yet unknown proteinase, resulting in degradation of mCD83 with lower mCD83 expression and sCD83 release. Here, the resulting expression level may vary depending on the BC surroundings and cell compartmentation.

The results thereby suggest a CD83 involvement in pregnancy and encourage further research on mCD83 expression at the feto-maternal interface as well as sCD83 in human blood and tissue. Especially the sCD83 alterations are of clinical interest, indicating the molecule as potential therapeutical option for pregnancy disturbances.

ABBREVIATIONS

BC	B-cell
BCR	B-cell receptor
CD	Cluster of differentiation
DC	Dendritic cell
ELIZA	Enzyme-linked immunosorbent assay
FO	Follicular-zone B-cell
FOXP3	Forkhead-box-protein 3
GPOM	Good pregnancy outcome mating
Ig	Immunoglobulin
LPS	Lipopolysaccharide
mCD83	Membrane-bound form of CD83
mRNA	Messenger ribonucleic acid
MZ	Marginal-zone B-cell
np	Non-pregnant
PCR	Polymerase-chain-reaction
PIBF	Progesterone-induced-blocking factor 1
PMA	Phorbol-12-myristat-13-acetat
PPOM	Poor pregnancy outcome mating
RNA	Ribonucleic acid
sCD83	Soluble form of CD83
TC	T-cell
TH2	Type-2 helper T-cell
TIMP1	Tissue inhibitor of metalloproteinases 1
Treg	Regulatory T-cell
TN	Transitional-zone B-cell

1 Introduction

1.1 Immunology and pregnancy

Every pregnancy demands multiple adaptions including hormones, circulatory system, metabolism and the immune system. The latter is a crucial point, as it is now responsible for the health of two individuals and must accept the semi-allogeneic fetus without impairing the protection against pathogens.

Known adaptions occur both in the innate and the adaptive immune system and include, for example, alterations in the antibody profiles and cytokine milieu. The first stage of pregnancy is predominantly pro-inflammatory. The following and largest period is characterized by higher secretions of anti-inflammatory interleukins as well as a preference for fast-acting type-2 helper T-cell (TH2)-reactions. Finally, pro-inflammatory reactions characterize the initiation of labor [1–5].

The causes of several pregnancy disturbances, like preeclampsia, preterm birth or recurrent pregnancy loss are still not fully identified. In recurrent pregnancy loss for example, established causes like uterine or genetic defects or chronic endometriosis can only be found in less than a half of the cases [6].

In this context, there is a link between pregnancy disorders and a misregulation in the complex immunological adaptions during pregnancy [2,7]. Infections, for example, are a trigger leading to disturbed immunoreactions and high cytokine releases. But even without bacterial infection, sterile inflammation can cause severe complications like preterm labor [2,8–10].

Here, the investigation of the mechanisms behind the establishment of the described “immunological balance” in healthy pregnancy as well as possible disturbances paves the way for possible therapeutic tools. Thus, the examination of molecules with immunomodulatory functions is a major interest in gynaecologic immunologic research. One molecule, which shows a variety of functions in different immune cells as well as therapeutic potential in autoimmune diseases, is the transmembrane molecule CD83 (mCD83).

1.2 CD83

mCD83 was first described in 1992 as HB-15 on human dendritic cell (DC) subsets as well as on activated lymphocytes [11], and soon obtained a significant role as DC maturation marker [12,13]. It is a transmembrane glycoprotein of the immunoglobulin (Ig) superfamily with a single extracellular Ig-like domain and a cytoplasmatic domain [11]. mCD83 has been found in

various species [14,15], including mice, where the murine form shares 63 % amino acid identity with the human variant of the protein [15].

In addition to its use as an activation marker on DC, mCD83 was discovered to have a crucial role in human DC dependent T-cell (TC) activation [16–18]. In different studies on the other hand, CD83 expression levels on antigen presenting cells had no influence on their ability in activating $CD8^+$ TC and just a slight positive correlation with the activation of $CD4^+$ TC [19]. As CD83 deficient DC show no problems in TC stimulation, this contribution might not be crucial [19]. However, some viruses impact CD83 function on DC, thereby profiting from reduced TC reaction [20–22].

Studies revealed that the lack of mCD83 on murine DC represents higher inflammatory potential, resulting for example in increased autoimmune inflammation but also in better protection against bacteria [23–25]. Knockout of CD83 on DC in mice led to reduced peripheral Treg numbers and impaired Treg function [25]. The role of mCD83 on DC is described as important in immune regulation as well as in the prevention of overshooting immune reactions [24,25].

mCD83 can also be detected on TC, B-cells (BC) as well as on murine thymic epithelial cells [11,26–28]. The latter is crucial for murine TC development, as CD83 knockout mice showed a severely reduced amount of peripheral $CD4^+$ TC [28].

TC display different properties, depending on their CD83 expression. While overexpression leads to forkhead-box-protein 3 (FOXP3) upregulation, which is characteristic for regulatory TC (Treg) [29], silencing of the molecule impairs TC functions like proliferation or cytokine release [30]. Transgenic induced mCD83 expression on murine TC on the other hand enhances their interferon and interleukin production [31].

Regarding splenic BC, two major subtypes differ in their strategies to react against pathogenic threats: Follicular zone B-cells (FO) produce high-affinity antibodies, while marginal zone B-cells (MZ) are considered pre-activated and show a rapid response with low-affinity antibodies [32–36]. mCD83 overexpression on BC results in reduced maturation of FO but higher numbers of MZ. The absence of mCD83 on BC on the other hand decreases the MZ maturation significantly [37]. On BC, mCD83 co-localizes with the B-cell receptor (BCR) and reduces its

sensitivity [38]. Reduced BCR sensitivity may be linked to a preference of BC for developing into MZ [34].

mCD83 expressing DC as well as lymphocytes release a soluble form of the protein (sCD83) [39]. The amount of sCD83 depends on the mCD83 level of the cell, suggesting that sCD83 is released from mCD83 by shedding [39]. A different explanation and pathway for sCD83 production is alternative splicing [40]. The soluble form shows anti-inflammatory properties in *in vitro* studies: High sCD83 doses resulted in cytoskeletal disturbances in DC, impairing their ability to cluster with TC, which is essential for TC activation [41]. Furthermore, sCD83 has a confirmed anti-inflammatory therapeutic effect in various mouse models of autoimmune diseases and allograft transplantation [42–44].

1.3 CD83 and pregnancy

One example for adaptions in normal murine pregnancies is a preference for MZ over FO maturation [33,45]. The link to the molecule mCD83 arose when studies revealed its capability in influencing the BC maturation in the spleen as described above. In short, mCD83 expression on BC leads to a BC distribution similar to the one observed in pregnancies.

Another connection between pregnancy and mCD83 concerns the production of the anti-inflammatory interleukin-10 (IL-10): mCD83 overexpressing BC show high IL-10 production after lipopolysaccharide (LPS) stimulation; and IL-10 is elevated and crucial in murine pregnancy [46,47].

Considering the influence of mCD83 expression on lymphocyte function and the immunomodulatory capacity of sCD83, a contribution to tolerance during pregnancy can be postulated.

1.4 Mouse models in gynaecologic research

To investigate the causes, processes and consequences of disturbed pregnancies, various mouse models are used. One is the pairing of CBA/J females with males of the oldest inbreed strain, the DBA/2J; in comparison to BALB/c males. Pairing CBA/J with DBA/2J mice results in resorption of 10-80% of the formed feto-placental units [48,49].

In the 1980s, this model was extensively used to study the theory of acquired immunosuppression during pregnancy: It was believed that healthy pregnancy required immunosuppression, and that CBA/J×DBA/2J paired mothers lack it [48]. In any case, the exact

cause of the higher resorption rate is still unidentified, although various immunological alterations have been described.

These include infiltration of natural killer cells as well as other leukocytes, including TC and BC [48,50]. Especially TC got into focus as studies showed altered TC function in CBA/J×DBA/2J pairings [48,51].

However, recent data show effects far beyond a plain lack of immunosuppression. Higher oxidative stress as well as disturbed DNA-methylation are discussed [52,53]. Additionally, changes in the vascularisation are described, resulting in a disturbed blood supply for the trophoblast. Vascularisation deficits are not limited to the placenta, there is evidence for a generalised vascular problem in the mother, resulting for example in kidney diseases. Those findings indicate that thrombosis and ischaemia, resulting in inflammation, are part of the process leading to the resorptions [54,55]. Thus, the mechanisms are not simply explainable with primary immune system dysregulation [48].

Despite this, the model is still frequently used for immune-mediated, pro-inflammatory disturbances during pregnancy, especially recurrent pregnancy loss and preeclampsia [54]. Another model in gynaecologic research is for example the intravenous or intrauterine application of LPS in pregnant mice resulting in preterm birth or abortion [56,57].

Of course, research is not limited to pregnancy disturbances but covers adaptions during healthy pregnancy as well. Here, the most widely used inbred strain in research [58], the C57Bl6/J, is frequently used. The C57Bl6 strain was created in 1921 and brought to the Jackson Laboratory in 1948, thereby creating the C57Bl6/J substrain [59]. Because syngeneic pairings of inbred strains do not mimic the semi-allogeneic challenge that occurs in human pregnancies, experiments are frequently conducted using a pairing of a female and a male from different inbred strains, like C57Bl6/J×BALB/c [45,60,61].

2 Objectives

For the last few years, the immunologic research has placed a strong focus on molecules with immunomodulatory characteristics. In contrast to the classical immunosuppressives, they convey, as the name says, modulatory effects, without the disadvantage of a generalized immunosuppression.

The transmembrane molecule mCD83 as well as its soluble form emerged as promising therapeutic tools to treat inflammatory disorders.

To investigate the possible use of the molecule in immunological mediated pregnancy disturbances, an understanding of the functional properties of the molecule during normal as well as disturbed pregnancy is crucial.

Thus, the analysis of the expression of mCD83 on Lymphocytes as well as the release of sCD83 and its regulation during pregnancy was the object of the underlying publications.

3 Materials and methods

The experiments were conducted according to the methods described in the published manuscripts [62,63]. In brief, mCD83 expression on immune cells from lymphoid organs of pregnant or non-pregnant mice was evaluated.

3.1 Animals and cell preparation

Two mouse models were used: First, the expression of mCD83 was examined in different states of normal murine pregnancy in a C57Bl6/J×BALB/c pairing [45,61]. Then, a model for pregnancy disturbance was used to determine possible CD83 influences on pregnancy outcome. This model compares the pairing of CBA/J females with either BALB/c males, representing a good pregnancy outcome mating (GPOM) or DBA/2J males for poor pregnancy outcome mating (PPOM) [48,55].

After pairing, female mice were checked for vaginal plug regularly. Plug observation was declared as day 0 of pregnancy. Mice were sacrificed on either day 7, 14 or 18 of pregnancy. Non pregnant mice were sacrificed as controls. Blood as well as spleen, thymus and the uterus draining paraaortal and inguinal lymph nodes were collected. Single-cell suspensions were obtained from the taken organs. Additionally, single-cell suspensions were further used for isolation and depletion of specific lymphocyte subtypes.

3.2 Cell stimulation

Single cell suspensions, isolated BC and BC or TC depleted lymphocytes were cultured and stimulated in various settings as described in the publications. Stimulation was performed with LPS, Phorbol-12-myristat-13-acetat (PMA), ionomycin, pregnancy hormones and recombinant Tissue inhibitor of metalloproteinases 1 (TIMP1) or anti-TIMP1. After stimulation, the cells were separated from the supernatants via centrifugation.

3.3 Evaluation of mCD83 expression and sCD83 release

Fresh single-cell suspensions as well as stimulated cells were examined using flow cytometry. After gating for BC, TC and DC as well as subtypes, the mCD83 expression and portion of $mCD83^+$ cells of each group were investigated.

Enzyme-linked immunosorbent assays (ELIZA) were used to determine sCD83 levels in murine serum and cell supernatants after stimulation.

Experiments with C57Bl6/J×BALB/c pairing were conducted and analysed by me, while GPOM versus PPOM experiments were conducted by Jens Ehrhardt, Anne Tüngler and myself and analysed by Damián Muzzio, Rebekka Einenkel and myself.

4 Results

4.1 mCD83 on splenocytes during normal and disturbed murine pregnancy

First, the expression of mCD83 was evaluated during murine pregnancy. The analysis of splenocytes from C57Bl6/J×BALB/c paired mice showed an upregulation of mCD83 on TC, BC as well as on BC subtypes (Transitional-Zone B-Cells (TN), MZ, FO) on day 14 of pregnancy compared to non-pregnant (np) C57Bl6/J mice *(Figure 1B-C,* [63]*)*. Polymerase-chain-reaction (PCR) analysis of freshly isolated BC further confirmed an elevation of CD83 messenger ribonucleic acid (mRNA) on day 14 of pregnancy compared to np mice *(Figure 1E,* [63]*)*.

To evaluate a possible role of CD83 in pregnancy outcome, a new experiment was performed using the mouse model of systemic inflammation-induced pregnancy disturbance as described above. Since the expression of mCD83 peaked at day 14 during normal murine pregnancy in C57Bl6/J×BALB/c pairings, CBA/J mice were also sacrificed at this state of pregnancy. Results showed a higher mCD83 expression on BC as well as their subtypes MZ and FO in PPOM mice at day 14 of pregnancy compared to GPOM *(Figure 1B,* [62]*)*.

In addition to the analysis of the mCD83 expression, the percentages of mCD83 expressing cells among BC, TC and DC where investigated. During normal murine pregnancy, these percentages increase in BC, TC and regulatory $CD4^+CD25^+$ TC from day 7 to day 18. In DC on the other hand, a decrease was shown especially in the middle of pregnancy *(Figure 1F,* [63]*)*.

PPOM pairing led to significantly higher mCD83 percentages among BC, MZ, DC and TC when compared to GPOM on day 14 *(Figure 1C,* [62]*)*.

To sum up, during normal murine pregnancy the mCD83 expression peaks on day 14 of pregnancy on BC and TC, while the amount of mCD83 positive cells is elevated at the end of pregnancy. PPOM mice show higher mCD83 expression and mCD83 positive cell count on various lymphocyte subtypes in comparison to GPOM pairings.

4.2 mCD83 on lymphocytes from thymus and lymph nodes during normal and disturbed murine pregnancy

The investigation of mCD83 expression in lymphatic tissues revealed an upregulation of mCD83 on BC from paraaortic lymph nodes on day 7 of normal pregnancy. Inguinal lymph nodes showed a significant decrease of mCD83 positive DC in all stages of pregnancy compared to np mice. No differences were detectable in thymic tissue *(Figure 2A-D,* [63]*)*.

PPOM mice had higher mCD83 expression on DC from inguinal and paraaortal lymph nodes and thymus. Thymic TC showed higher mCD83 expression but a significantly lower rate of mCD83 positive cells in PPOM mice *(Figure 1B-C,* [62]*)*.

4.3 sCD83 levels in sera during normal and disturbed murine pregnancy

The soluble form of CD83, sCD83, is measurable in the blood serum. During normal murine pregnancy, no significant changes in sCD83 serum levels were detectable. However, data showed a trend towards higher sCD83 levels in pregnancy: 57.7% of mice at day 18 had serum levels over 1,1 pg/mL while only 16,7% of np mice exceeded that level *(Figure 2E,* [63]*)*. Mice with PPOM showed lower sCD83 concentrations on day 14 of pregnancy compared to mice with GPOM *(Figure 3A,* [62]*)*.

4.4 mCD83 and sCD83 upon *ex vivo* stimulation

mCD83 levels were additionally evaluated after a 48-hour stimulation of cultured splenic lymphocytes with LPS, PMA and ionomycin. The experiment showed a higher upregulation of mCD83 as well as higher mCD83 mRNA concentrations in BC derived from mice at the end of pregnancy compared to BC from np mice. Analyses of sCD83 in the supernatants revealed higher concentrations at the end of pregnancy *in vitro (Figure 3C-E,* [63]*)*.

When lymphocytes derived from PPOM vs GPOM pairings on day 14 were stimulated *in vitro*, no significant alteration of mCD83 expression between both groups of mice could be observed. The investigation of sCD83 levels in supernatants from PPOM pairings showed no significant changes in comparison to GPOM pairings *(Figure 2A-B,* [62]*)*.

To sum up, at the end of pregnancy BC react with higher mCD83 expression to proinflammatory stimulation and splenocytes release higher amounts of sCD83.

4.5 Source of sCD83 *in vitro*

Taking into account that sCD83 was measurable in the supernatants of stimulated and non-stimulated cell cultures, the aim was to investigate its sources. Therefore, splenic lymphocytes were depleted from either BC or TC before *in vitro* stimulation. In non-stimulated cell-cultures results showed a significantly decreased sCD83 concentration after BC depletion, while TC depletion had no effects on sCD83 release. Upon cell stimulation with LPS, PMA and ionomycin, TC as well as BC depletion resulted in a significantly reduced sCD83 concentration *(Figure 3F-G,* [63]*)*.

4.6 Influence of pregnancy related hormones on CD83 *in vitro*

Hormones are crucial regulators in pregnancy maintenance, especially estradiol and progesterone. Estradiol stimulation did not influence lymphocytic mCD83 expression *in vitro*. *(Figure 3B,* [63]*)*. Progesterone on the other hand showed a dose-dependent effect on mCD83 expression on TC derived from C57Bl6/J mice. An upregulation of mCD83 expression on DC and BC was only achievable using supraphysiologically high concentrations of progesterone *(Figure 2A,* [63]*)*.

The experiment was later repeated with lymphocytes derived from np CBA/J mice, using lower progesterone concentrations physiologically found in mice [64]. This setting led to a dose-dependent decrease in mCD83 expression on BC *(Figure 3D,* [62]*)*. Additionally, the sCD83 levels in supernatants from both splenocytes and isolated BC was determined after progesterone treatment. Here, progesterone significantly reduced the sCD83 release from splenocytes as well as BC *(Figure 3B,* [62]*)*.

4.7 New model of mCD83 membrane degradation

Progesterone treatment reduced both mCD83 on the cell surface and sCD83 release. sCD83 is most likely produced by mCD83 shedding [39]. Therefore, a plausible mechanism leading to reduced availability of both forms is the degradation of mCD83. Here progesterone may serve as a regulator.

A specific proteinase for the degradation of mCD83 is not known yet, but tissue inhibitor of metalloproteinases 1 (TIMP1) is a known inhibitor of various proteinases and can be regulated by progesterone [65,66].

In order to investigate a possible role of TIMP1, lymphocytes were cultured with LPS, PMA and ionomycin plus either recombinant TIMP1 or a blocking-antibody against TIMP1. Treatment with TIMP1 resulted in higher sCD83 concentration in splenocyte supernatants and higher mCD83 expression on BC, while anti-TIMP1 induced a significant decrease in mCD83 expression *(Figure 4B-C,* [62]*)*.

Finally, splenic lymphocytes were stimulated with Progesterone and TIMP1 expression was measured. Progesterone stimulation resulted in decreased TIMP-1 expression on BC *(Figure 4A,* [62]*).*

5 Discussion

Pregnancy requires various adaptions from the mother's body. In addition to alterations of the metabolism and the cardiovascular system, especially the immune system is required to fulfil an important task: it must undergo complex adaptions for the maintenance of the semi-allogeneic fetus as well as the mother's and unborn child's well-being. The mechanisms behind these adaptions are one of the major interests in gynaecological immunologic research. They provide a basis for therapeutical options in immunological problems during pregnancy as, for example, recurrent pregnancy loss.

BC undergo vast adaptions during pregnancy [33,45,67]. The starting point for the work objective of CD83 involvement in pregnancy originated from the observation that mCD83 overexpressing BC show properties similar to those of BC in murine pregnancy: Both show a MZ over FO maturation preference and release elevated amounts of IL-10 [37,45–47]. This led to the question whether BC or other immune cells upregulate mCD83 during pregnancy, and whether they serve as a source of the anti-inflammatory sCD83.

5.1 mCD83 and BC in pregnancy

Indeed, splenic BC as well as their subtypes (MZ, FO and TN) showed an mCD83 upregulation during normal murine pregnancy. Considering previous findings concerning mCD83 expression on BC, this upregulation suggests an immunosuppressive role of the molecule in pregnancy. However, most experiments concerning mCD83 expression on BC, like the aforementioned enhanced IL-10 secretion upon stimulation, were conducted using mice or cells that artificially overexpress CD83 [46]. As this may not mimic a physiologic situation, the results from these experiments might not fully translate into normal BC function. Nevertheless, there is evidence for a regulative role of mCD83 on BC, in which the molecule

supports the cell receiving inhibitory signals precluding a BC population over-stimulation [68,69].

Thus, the increase of mCD83 expression and mCD83 expressing cells during pregnancy indicate a possible role of the protein in the complicated maintenance of an immunological balance in pregnancy. Considering these modulations and their possible role in pregnancy maintenance, the mCD83 expression was measured in the aforementioned model for murine pregnancy disturbance.

As an increment of mCD83 was observed during the course of normal pregnancies, the opposite scenario was expected in PPOM mice. However, PPOM mice showed an even higher mCD83 expression on splenic BC than GPOM mice. At first glance this negates the theory of mCD83 serving as an important immunosuppressive molecule for good pregnancy outcome in this model. However, it has to be taken into account that several cell stimuli can lead to an mCD83 upregulation on cells, including cytokines [70]. The mCD83 upregulation in PPOM mice might be explained through the proinflammatory setting with elevated cytokine levels [55]. Additionally, the upregulation might as well be an auto-regulatory attempt of the mice's immune system in saving the pregnancy. Finally, a reduced cleavage of sCD83 from the membrane-bound form could result in mCD83 accumulation. An indication for this theory is the significantly reduced sCD83 level detectable in the sera of PPOM mice. This suggests a defective sCD83 shedding during PPOM and is further discussed under section 5.4.

As already mentioned, BC from pregnant mice show an MZ over FO preference [45]. The exact cause of how this adaption is generated is not uncovered yet [33]. As mCD83 overexpressing BC show a similar behaviour [37], an involvement of the molecule seemed possible and supported the interest in researching CD83 in the context of pregnancy. The highest expression of mCD83 was measurable on day 14 of pregnancy, while the peak in MZ/FO ratio as well as in total MZ is at the end of pregnancy [45]. While PPOM pregnancies fail this adaption and show significantly reduced MZ numbers [33], they express more mCD83 in comparison to GPOM. Thus, no positive correlation between mCD83 expression and MZ preference during pregnancy was found, making an involvement of mCD83 in this adaption unlikely.

5.2 mCD83 and TC in pregnancy

mCD83 is expressed on TC. Studies linked it with enhanced TC activation as well as interleukin production [31]. Later studies associated $mCD83^+$ TC with a Treg-like phenotype [26,29], respectively showed a correlation with mCD83 expression on TC and their differentiation into Treg [71]. Those TC have the ability to supress proliferation and cytokine release of activated $mCD83^-$ TC [26]. Experiments with knockout of mCD83 on Treg resulted in reduced immune tolerance and reduced cell stability, underlining the importance of the molecule for Treg regulation [24,72].

During normal murine pregnancy, splenic $CD4^+$ TC showed a significant increase in mCD83 expression in the middle of pregnancy. This could be an indication for higher Treg differentiation. The data fits with findings showing an increase of splenic Treg likewise on day 14 of pregnancy [73].

Though mCD83 expression was increased at day 14 of pregnancy, the percentage of $mCD83^+$ cells among $CD4^+$ TC and $CD4^+CD25^+$ Treg showed a significant increase only at day 18 of pregnancy compared to day 7. It has to be taken into account, that surface expression of mCD83 on unstimulated TC is rather rare, occurring in just 1-2% of unstimulated $CD25^-$ or $CD25^+$ TC [29]. Here, additional investigations regarding mCD83 function on TC as well as mCD83 expression on TC or Treg subtypes during pregnancy are needed. As Treg differentiation is an important feature during pregnancy adaptions and malfunctions are linked with pregnancy disturbances [74,75], mCD83 expression especially on Treg should further be investigated in PPOM mice.

5.3 mCD83 and DC in pregnancy

During normal murine pregnancy no significant alterations were measurable in mCD83 expression on $33D1^+/DCIR2^+$ DC[1], but the overall percentage of mCD83 expressing cells in the $33D1^+/DCIR^+$ cell population dropped in pregnant mice in spleen and inguinal lymph nodes. In PPOM pregnancy, higher mCD83 expression was detected in DC from thymus and lymph nodes. With mCD83 being a maturation marker on DC, this portrays a higher DC maturation

[1] 33D1 is a monoclonal antibody reacting with DCIR2, a surface marker on murine dendritic cell subpopulations in tissues from spleen, lymph nodes and thymus. If DCIR2 owns further biological activity is not known yet [93].

in PPOM mice. These findings are in concordance with other studies revealing a more mature DC phenotype in the PPOM model [76,77].

During pregnancy, various adaptions in the DC populations occur. Those affect the peripheral lymphatic system, but above all the fetomaternal interface being the direct contact point between mother and fetus [78]. $CD83^+$ DC are detectable in human decidual tissue and show a higher quantity in woman with recurrent miscarriage [79]. There is a correlation between higher abortion rates and more mature DC [76]. To further elucidate the role of mCD83 on DC in pregnancy a deeper analysis of DC subtypes as well as of decidual or placental DC is necessary. Additionally, these results encourage further studies concerning mCD83 functions to disclose the role of $mCD83^+$ DC in pregnancy.

5.4 sCD83

sCD83 has become a major research topic for its immunosuppressive functions and potential therapeutical options [80]. During pregnancy, PPOM mice revealed significantly lower sCD83 serum levels in comparison to GPOM. During normal murine pregnancy, no significant changes in the serum levels were detectable, but the results revealed a slight tendency towards higher sCD83 levels in pregnant mice. In 2020, Huo *et al.* performed a similar experiment using BALB/c mice proving a significant increase of sCD83 in the sera of pregnant mice. They furthermore confirmed an association of bad pregnancy outcome and lower sCD83 serum levels in an additional mouse model, using LPS-induced stress and abortion [56].

The soluble molecule is most likely produced by shedding of the membrane-bound protein [39]. Since mCD83 expression on splenic BC increased in PPOM pregnancy while sCD83 sera levels decreased, the pathogenic mechanism concerns the shedding.

In cell culture, splenocytes from late pregnant mice revealed to release higher amounts sCD83 in normal murine pregnancy. No differences were shown in the PPOM model, but a comparison is difficult as PPOM mice were sacrificed in the middle of pregnancy, so later changes are possible and need further investigation.

Various cell types have the ability to release sCD83 [39]. An in-depth investigation of cultured splenocytes showed drastically reduced sCD83 levels after BC depletion, suggesting BC to be the major source of sCD83 in an unstimulated state. Isolated BC released about the same amount of sCD83 as cultured splenocytes did. Nevertheless, upon stimulation, isolated BC showed significantly lower sCD83 release in comparison to all splenocytes. That emphasizes

BC being not the only source for sCD83 upon cell stimulation. As depletion of BC in this setting still led to a highly significant sCD83 reduction, BC pose as a major source of sCD83 upon stimulation.

Missing cell-cell-interactions could also account for the reduced sCD83 release of BC in comparison to all splenocytes after stimulation. The fact that TC depletion led to a significant reduction of sCD83 release marks TC as possible interaction partners for sCD83 release in BC upon stimulation, though a sCD83 release by the TC itself is not impossible. Purified TC showed no sCD83 release in culture in previous studies [39]. Other studies described a small release of sCD83 from sorted $CD4^+CD83^+$TC only upon stimulation [26], which is in line with the findings of the underlying publication.

Taking this and the previous findings on splenic BC into account, the high mCD83 expression on splenic BC during PPOM pregnancy could be a consequence of disturbed sCD83 release, leading to lower sCD83 serum levels. This theory is supported by the findings of BC being a main source of sCD83 production.

Considering the decreased sCD83 serum concentration in PPOM pregnancy and the anti-inflammatory properties of the molecule, a test of possible beneficial consequences of sCD83 administration during pregnancy is conceivable. sCD83 showed already therapeutical benefits in animal models for various autoimmune diseases like autoimmune encephalomyelitis or arthritis [43,81]. Promising results were gained in transplantation therapy as well. The survival of corneal grafts, for example, enhances with sCD83 pre-incubation of the graft [82]. And indeed, *Huo et al.* treated pregnant mice with recombinant, porcine sCD83. The group was able to show significantly lower abortions and embryo resorption rates in the LPS-induced abortion model. This treatment led to various immunologic alterations, which are considered significant for healthy pregnancy like enhanced IL-10 levels and increase of Treg numbers [56].

5.5 The role of pregnancy related hormones in the regulation of CD83

A detailed understanding of immune mediators as well as their functions and regulations helps not only to understand the mechanics behind the changes occurring during immunological processes like pregnancy, but also helps in the development of potential medical treatments. In case of CD83, numerous regulations are yet to be unveiled, so more in-depth studies are essential.

In pregnancy, especially hormones contribute to various changes of the mother's immune system, making them candidate regulators of CD83 during pregnancy. Excepting the analysis of mCD83 as DC maturation marker after DC stimulation with pregnancy hormones [83], the influence of pregnancy related hormones on mCD83 expression has not been studied up till the underlying publications.

In *in vitro* stimulation, unphysiologically high progesterone doses led to an upregulation of mCD83 on BC and DC. It is worth mentioning, that higher progesterone concentrations can be reached in different compartments of the body. In murine ovarian venous plasma, progesterone levels up to 3096 ng/mL are described during pregnancy [84], whereas the concentration in peripheral plasma reached 112,7 ng/mL [85]. Nevertheless, in the accomplished in vitro experiments, progesterone had to be increased to 50.000 ng/mL to show the aforementioned upregulation of mCD83 on BC.

Lower, physiological doses [64] decreased mCD83 expression on BC and lowered the amount of sCD83 in the cell cultures supernatants. Thus, progesterone limits mCD83 availability on BC. On TC, progesterone induced a dose-dependent increase of mCD83 expression. Here is a correlation between the highest progesterone levels and mCD83 expression on splenic TC being both around day 14 of pregnancy.

Progesterone develops its effects mainly via the intracellular progesterone receptor, which is existent in lymphocytes. Lymphocytes show a higher sensitivity to progesterone during pregnancy [86]. Those lymphocytes produce the so called progesterone-induced-blocking factor 1 (PIBF), a protein mediating various effects in pregnancy like the establishment of a TH2-dominant cytokine pattern [87].

In the course of normal murine pregnancy, the progesterone concentration in the blood increases (with a significant drop on day 10), reaching its peak at day 15, followed by a decline [85]. Low progesterone levels are associated with miscarriage in humans and rodents [87–89].

Those findings led to various trials and studies of therapeutic progesterone administration in pregnancy disturbances like recurrent pregnancy loss or threatened miscarriage. Results vary between no significant benefit and improved pregnancy outcome [90–92].

Although CD83 can be regulated by progesterone, the hormonal influence cannot explain, at least on BC, the alterations in mCD83 occurring during pregnancy. In TC on the other hand,

there is a correlation and thereby possible link between the dose-dependent effect on TC, as maximum mCD83 expression as well as progesterone concentration occur approximately at the same time.

In a next step it would be important to investigate the findings of this research *in vivo.* Here, many factors including cell-cell contacts and cell compartmentation are likely to modify the effects of progesterone on mCD83.

5.6 New pathways in CD83 regulation

Progesterone treatment reduced mCD83 expression as well as sCD83 release *in vitro.* A possible explanation for these findings is the degradation of mCD83 by a proteinase. TIMP1 is an inhibitor of many proteinases, expressed on BC and regulated by Progesterone [65,66].

In vitro experiments were performed to test a link between CD83 and TIMP1: TIMP1 treatment led to higher sCD83 release from splenocytes and mCD83 expression on BC, while inhibition of TIMP1 reduced mCD83 expression. These findings indicate TIMP1 being able to impede a not yet unveiled proteinase from mCD83 degradation of the cell surface. It is the first time a CD83 regulation through TIMP1 is described, opening the way to further experiments with TIMP1 controlled proteinases.

To further link progesterone in the chain, the influence of progesterone on TIMP1 expression on BC was tested. Progesterone downregulates TIMP1 on BC, which, in concordance with the findings above, should led to lower mCD83 expression:

Progesterone↑ → TIMP1↓ → Proteinase↑ → mCD83↓

To sum up, progesterone reduces mCD83 availability on BC and sCD83 release from splenocytes via a downregulation of TIMP1 *in vitro.*

In vivo, on the other hand, those cells find themselves in complex and changing compartmentations. Progesterone does not solely affect the BC but also its environment. Here, studies showed an induction of TIMP1 by progesterone, for example on uterine fibroblasts and human endometrial stromal cells [65,66]. Progesterone induced TIMP1 produced by those cells may lead to higher TIMP1 concentrations, and thus higher mCD83 expression on BC *in vivo* depending on the cell surroundings (Figure 1).

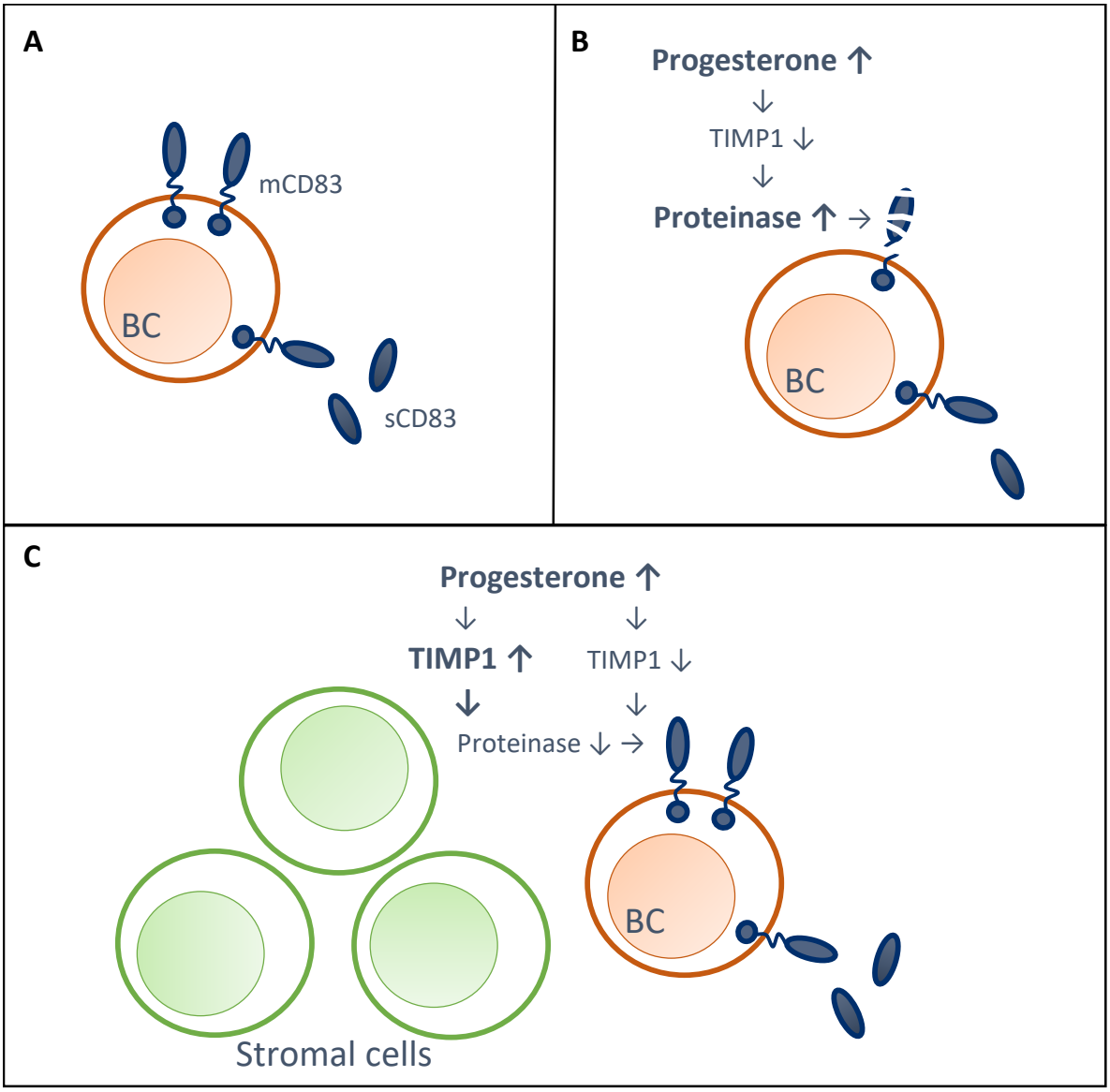

Figure 1 Schematic depiction of mCD83 expression and sCD83 release on BC depending on BC surroundings. (A) BC expressing mCD83 and releasing sCD83. (B) Isolated BC under the influence of progesterone. Progesterone down-regulates TIMP1 on BC, resulting in degradation of mCD83 by lacking proteinase inhibition and thereby lower sCD83 release. This scenario might occur in peripheral BC contributing to local inflammation. (C) BC surrounded by stromal cells (for example splenic or uterine stroma). Progesterone induced TIMP1-production from the stroma cells compensates the TIMP1-reduction on the BC, resulting in inhibition of proteinases and prevention of mCD83 degradation.

6 Limitations and conclusions

The underlying publications provided the first insight in CD83 and pregnancy. Thus, they delivered valuable information especially regarding the protein's expression in normal and disturbed murine pregnancy.

A direct comparison between the data of the two publications is difficult, since they were acquired in two different mouse models. As described in the introduction, the PPOM mouse model is not fully understood yet, making it challenging to interpret the data and to transfer them into human pregnancies. However, the main focus of the studies was to evaluate whether mCD83 expression changes during murine pregnancy and in an exemplary mouse model for pregnancy disturbance. And indeed, the molecule is expressed differentially during the stages of pregnancy on different lymphocytic cell types.

Noticeable is above all the higher mCD83 expression in the middle of normal pregnancy on splenic BC subtypes and TC. PPOM pregnancies revealed to express more mCD83 on splenic BC in comparison to GPOM while displaying lower sCD83 sera levels.

On top of that, new insights into the protein itself were shown, which led to new theories in pregnancy regulations with CD83 participation:

Firstly, sCD83 is released from lymphocytes, especially BC, and detectable in the serum. In PPOM pregnancy significantly reduced sCD83 sera levels hint a problem in sCD83 release.

Secondly, mCD83 expression and sCD83 release from BC is regulated by progesterone via TIMP1. Here, the compartmentation of the BC may eventually contribute to the mCD83 expression and sCD83 release by TIMP1 expression on surrounding cells.

Altogether, the collected data in connection with the immunoregulative functions of sCD83 points to a potential therapeutical use of sCD83 in pregnancy disturbances. Indeed, in a later work published in 2020, porcine recombinant sCD83 was used as treatment in an LPS-induced abortion model in mice, revealing a significantly reduced abortion rate [56].

Although a good set of methods was used to assess CD83 expression and release (flow cytometry, quantitative PCR, ELISA), future studies could profit from high-throughput ribonucleic acid (RNA)-sequencing to gain information about a wider spectrum of relevant cells. Also, in further research projects, the analysis of mCD83 expression on uterine cells and the feto-maternal interface will provide valuable insights into the local involvement of this

molecule in pregnancy wellbeing. Furthermore, information is needed concerning sCD83 dynamics and function in human pregnancy. Here, as PPOM mice showed lower sCD83 sera levels, the analysis of for example sCD83 in sera of woman with healthy pregnancies and pregnancy disturbances like recurrent pregnancy loss, premature birth or preeclampsia is a useful and achievable beginning.

7 References

[1] T.G. Wegmann, H. Lin, L. Guilbert, T.R. Mosmann, Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon?, Immunol. Today. 14 (1993) 353–356. https://doi.org/10.1016/0167-5699(93)90235-D.

[2] P. Chatterjee, V.L. Chiasson, K.R. Bounds, B.M. Mitchell, Regulation of the Anti-Inflammatory Cytokines Interleukin-4 and Interleukin-10 during Pregnancy, Front. Immunol. 5 (2014) 253. https://doi.org/10.3389/fimmu.2014.00253.

[3] P. Hsu, R.K.H. Nanan, Innate and adaptive immune interactions at the fetal-maternal interface in healthy human pregnancy and pre-eclampsia, Front. Immunol. 5 (2014) 125. https://doi.org/10.3389/fimmu.2014.00125.

[4] H. Lin, T.R. Mosmann, L. Guilbert, S. Tuntipopipat, T.G. Wegmann, Synthesis of T helper 2-type cytokines at the maternal-fetal interface., J. Immunol. 151 (1993) 4562–4573.

[5] N.M. Shah, A.A. Herasimtschuk, A. Boasso, A. Benlahrech, D. Fuchs, N. Imami, M.R. Johnson, Changes in T Cell and Dendritic Cell Phenotype from Mid to Late Pregnancy Are Indicative of a Shift from Immune Tolerance to Immune Activation, Front. Immunol. 8 (2017) 1138. https://doi.org/10.3389/FIMMU.2017.01138.

[6] H. El Hachem, V. Crepaux, P. May-Panloup, P. Descamps, G. Legendre, P.E. Bouet, Recurrent pregnancy loss: Current perspectives, Int. J. Womens. Health. 9 (2017) 331–345. https://doi.org/10.2147/IJWH.S100817.

[7] R.J. Kuon, T. Strowitzki, C. Sohn, V. Daniel, B. Toth, Immune profiling in patients with recurrent miscarriage, J. Reprod. Immunol. 108 (2015) 136–141. https://doi.org/10.1016/j.jri.2015.01.007.

[8] B.H. Yoon, R. Romero, J. Bin Moon, S.S. Shim, M. Kim, G. Kim, J.K. Jun, Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes, Am. J. Obstet. Gynecol. 185 (2001) 1130–1136. https://doi.org/10.1067/mob.2001.117680.

[9] I. Christiaens, D.B. Zaragoza, L. Guilbert, S.A. Robertson, B.F. Mitchell, D.M. Olson, Inflammatory processes in preterm and term parturition, J. Reprod. Immunol. 79

(2008) 50–57. https://doi.org/10.1016/j.jri.2008.04.002.

[10] S.E. Lee, R. Romero, H. Jung, C.W. Park, J.S. Park, B.H. Yoon, The intensity of the fetal inflammatory response in intraamniotic inflammation with and without microbial invasion of the amniotic cavity, Am. J. Obstet. Gynecol. 197 (2007) 294.e1-294.e6. https://doi.org/10.1016/j.ajog.2007.07.006.

[11] L.J. Zhou, R. Schwarting, H.M. Smith, T.F. Tedder, A novel cell-surface molecule expressed by human interdigitating reticulum cells, Langerhans cells, and activated lymphocytes is a new member of the Ig superfamily., J. Immunol. 149 (1992) 735–42.

[12] L.J. Zhou, T.F. Tedder, CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 2588–2592. https://doi.org/10.1073/pnas.93.6.2588.

[13] L.J. Zhou, T.F. Tedder, Human blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily., J. Immunol. 154 (1995) 3821–35.

[14] Y. Ohta, E. Landis, T. Boulay, R.B. Phillips, B. Collet, C.J. Secombes, M.F. Flajnik, J.D. Hansen, Homologs of CD83 from Elasmobranch and Teleost Fish, J. Immunol. 173 (2004) 4553–4560. https://doi.org/10.4049/jimmunol.173.7.4553.

[15] C.J. Twist, D.R. Beier, C.M. Disteche, S. Edelhoff, T.F. Tedder, The mouse Cd83 gene: Structure, domain organization, and chromosome localization, Immunogenetics. 48 (1998) 383–393. https://doi.org/10.1007/s002510050449.

[16] M. Kruse, O. Rosorius, F. Krätzer, D. Bevec, C. Kuhnt, A. Steinkasserer, G. Schuler, J. Hauber, Inhibition of CD83 cell surface expression during dendritic cell maturation by interference with nuclear export of CD83 mRNA, J. Exp. Med. 191 (2000) 1581–1589. https://doi.org/10.1084/jem.191.9.1581.

[17] E. Zinser, N. Turza, A. Steinkasserer, CNI-1493 mediated suppression of dendritic cell activation in vitro and in vivo, Immunobiology. 209 (2004) 89–97. https://doi.org/10.1016/j.imbio.2004.04.004.

[18] C. Aerts-Toegaert, C. Heirman, S. Tuyaerts, J. Corthals, J.L. Aerts, A. Bonehill, K. Thielemans, K. Breckpot, K. Breckpot, CD83 expression on dendritic cells and T cells: Correlation with effective immune responses, Eur. J. Immunol. 37 (2007) 686–695.

https://doi.org/10.1002/eji.200636535.

[19] B. Kretschmer, K. Lüthje, S. Ehrlich, A. Osterloh, M. Piedavent, B. Fleischer, M. Breloer, CD83 on murine APC does not function as a costimulatory receptor for T cells, Immunol. Lett. 120 (2008) 87–95. https://doi.org/10.1016/J.IMLET.2008.07.004.

[20] C.S. Heilingloh, L. Grosche, M. Kummer, P. Mühl-zürbes, L. Kamm, M. Scherer, M. Latzko, T. Stamminger, A. Steinkasserer, The Major Immediate-Early Protein IE2 of Human Cytomegalovirus Is Sufficient to Induce Proteasomal Degradation of CD83 on Mature Dendritic Cells, Front. Microbiol. 8 (2017) 119. https://doi.org/10.3389/fmicb.2017.00119.

[21] M. Kruse, O. Rosorius, F. Kratzer, G. Stelz, C. Kuhnt, G. Schuler, J. Hauber, A. Steinkasserer, Mature Dendritic Cells Infected with Herpes Simplex Virus Type 1 Exhibit Inhibited T-Cell Stimulatory Capacity, J. Virol. 74 (2000) 7127–7136. https://doi.org/10.1128/jvi.74.15.7127-7136.2000.

[22] G. Morrow, B. Slobedman, A.L. Cunningham, A. Abendroth, Varicella-Zoster Virus Productively Infects Mature Dendritic Cells and Alters Their Immune Function, J. Virol. 77 (2003) 4950–4959. https://doi.org/10.1128/JVI.77.8.4950.

[23] J.M. Bates, K. Flanagan, L. Mo, N. Ota, J. Ding, S. Ho, S. Liu, M. Roose-Girma, S. Warming, L. Diehl, Dendritic cell CD83 homotypic interactions regulate inflammation and promote mucosal homeostasis, Mucosal Immunol. 8 (2015) 414–428. https://doi.org/10.1038/mi.2014.79.

[24] L. Grosche, I. Knippertz, C. König, D. Royzman, A.B. Wild, E. Zinser, H. Sticht, Y.A. Muller, A. Steinkasserer, M. Lechmann, The CD83 Molecule – An Important Immune Checkpoint, Front. Immunol. 11 (2020) 721. https://doi.org/10.3389/fimmu.2020.00721.

[25] A.B. Wild, L. Krzyzak, K. Peckert, L. Stich, C. Kuhnt, A. Butterhof, C. Seitz, J. Mattner, N. Gröner, M. Gänsbauer, M. Purtak, D. Soulat, T.H. Winkler, L. Nitschke, E. Zinser, A. Steinkasserer, CD83 orchestrates immunity toward self and non-self in dendritic cells, JCI Insight. 4 (2019) e126246. https://doi.org/10.1172/jci.insight.126246.

[26] S. Kreiser, J. Eckhardt, C. Kuhnt, M. Stein, L. Krzyzak, C. Seitz, C. Tucher, I. Knippertz, C.

Becker, C. Günther, A. Steinkasserer, M. Lechmann, Murine CD83-positive T cells mediate suppressor functions in vitro and in vivo, Immunobiology. 220 (2015) 270–279. https://doi.org/10.1016/j.imbio.2014.08.005.

[27] B. Kretschmer, S. Kühl, B. Fleischer, M. Breloer, Activated T cells induce rapid CD83 expression on B cells by engagement of CD40, Immunol. Lett. 136 (2011) 221–227. https://doi.org/10.1016/j.imlet.2011.01.013.

[28] Y. Fujimoto, L.L. Tu, A.S. Miller, C. Bock, M. Fujimoto, C. Doyle, D.A. Steeber, T.F. Tedder, CD83 expression influences CD4+ T cell development in the thymus, Cell. 108 (2002) 755–767. https://doi.org/10.1016/S0092-8674(02)00673-6.

[29] S. Reinwald, C. Wiethe, A.M. Westendorf, M. Breloer, M. Probst-Kepper, B. Fleischer, A. Steinkasserer, J. Buer, W. Hansen, CD83 Expression in CD4 + T Cells Modulates Inflammation and Autoimmunity , J. Immunol. 180 (2008) 5890–5897. https://doi.org/10.4049/jimmunol.180.9.5890.

[30] L.L. Su, H. Iwai, J.T. Lin, C.G. Fathman, The Transmembrane E3 Ligase GRAIL Ubiquitinates and Degrades CD83 on CD4 T Cells, J. Immunol. 183 (2009) 438–444. https://doi.org/10.4049/jimmunol.0900204.

[31] M. Wolenski, S.O. Cramer, S. Ehrlich, C. Steeg, B. Fleischer, A. von Bonin, Enhanced Activation of CD83-Positive T Cells *, Scand. J. OfImmunology. 58 (2003) 306–311.

[32] D. Allman, S. Pillai, Peripheral B cell subsets., Curr. Opin. Immunol. 20 (2008) 149–157. https://doi.org/10.1016/j.coi.2008.03.014.

[33] D.O. Muzzio, K.B. Ziegler, J. Ehrhardt, M. Zygmunt, F. Jensen, Marginal zone B cells emerge as a critical component of pregnancy well-being, Reproduction. 151 (2016) 29–37. https://doi.org/10.1530/REP-15-0274.

[34] A. Cerutti, M. Cols, I. Puga, Marginal zone B cells: virtues of innatelike antibody-producing lymphozytes, Nat. Rev. Immunol. 13 (2013) 118–132. https://doi.org/10.1016/j.pestbp.2011.02.012.Investigations.

[35] H. Chu, A. Awasthi, G.C. White II, M. Chrzanowska-Wodnicka, S. Malarkannan, Rap1b Regulates B Cell Development, Homing, and T Cell-Dependent Humoral Immunity 1 HHS Public Access, J Immunol. 181 (2008) 3373–3383.

[36] A.M. Oliver, F. Martin, J.F. Kearney, IgMhighCD21high lymphocytes enriched in the splenic marginal zone generate effector cells more rapidly than the bulk of follicular B cells., J. Immunol. 162 (1999) 7198–7207.

[37] K. Lüthje, B. Kretschmer, B. Fleischer, M. Breloer, CD83 regulates splenic B cell maturation and peripheral B cell homeostasis, Int. Immunol. 20 (2008) 949–960. https://doi.org/10.1093/intimm/dxn054.

[38] M. Uhde, S. Kuehl, U. Richardt, B. Fleischer, A. Osterloh, Differential regulation of marginal zone and follicular B cell responses by CD83, Int. Immunol. 25 (2013) 507–520. https://doi.org/10.1093/intimm/dxt021.

[39] B.D. Hock, M. Kato, J.L. McKenzie, D.N.J. Hart, A soluble form of CD83 is released from activated dendritic cells and B lymphocytes, and is detectable in normal human sera, Int. Immunol. 13 (2001) 959–967. https://doi.org/10.1093/intimm/13.7.959.

[40] D. Dudziak, F. Nimmerjahn, G.W. Bornkamm, G. Laux, Alternative Splicing Generates Putative Soluble CD83 Proteins That Inhibit T Cell Proliferation, J. Immunol. 174 (2005) 6672–6676. https://doi.org/10.4049/jimmunol.174.11.6672.

[41] N. Kotzor, M. Lechmann, E. Zinser, A. Steinkasserer, The soluble form of CD83 dramatically changes the cytoskeleton of dendritic cells, Immunobiology. 209 (2004) 129–140. https://doi.org/10.1016/j.imbio.2004.04.003.

[42] J.F. Xu, B.J. Huang, H. Yin, P. Xiong, W. Feng, Y. Xu, M. Fang, F. Zheng, C.Y. Wang, F.L. Gong, A limited course of soluble CD83 delays acute cellular rejection of MHC-mismatched mouse skin allografts, Transpl. Int. 20 (2007) 266–276. https://doi.org/10.1111/j.1432-2277.2006.00426.x.

[43] E. Zinser, M. Lechmann, A. Golka, M.B. Lutz, A. Steinkasserer, Prevention and treatment of experimental autoimmune encephalomyelitis by soluble CD83, J. Exp. Med. 200 (2004) 345–351. https://doi.org/10.1084/jem.20030973.

[44] J. Eckhardt, S. Kreiser, M. Döbbeler, C. Nicolette, M.A. Debenedette, I.Y. Tcherepanova, C. Ostalecki, A.J. Pommer, C. Becker, C. Günther, E. Zinser, T.W. Mak, A. Steinkasserer, M. Lechmann, Soluble CD83 ameliorates experimental colitis in mice, Nature. 7 (2014) 1006–1018. https://doi.org/10.1038/mi.2013.119.

[45] D.O. Muzzio, R. Soldati, J. Ehrhardt, K. Utpatel, M. Evert, A.C. Zenclussen, M. Zygmunt, F. Jensen, B Cell Development Undergoes Profound Modifications and Adaptations During Pregnancy in Mice, Biol. Reprod. 91 (2014) 1–11. https://doi.org/10.1095/biolreprod.114.122366.

[46] B. Kretschmer, K. Lüthje, A.H. Guse, S. Ehrlich, F. Koch-Nolte, F. Haag, B. Fleischer, M. Breloer, CD83 modulates B cell function in vitro. Increased IL-10 and reduced Ig secretion by CD83Tg B cells, PLoS One. (2007) 755. https://doi.org/10.1371/journal.pone.0000755.

[47] S. Fest, A.C. Zenclussen, F. Jensen, Regulatory B10 Cells Restore Pregnancy Tolerance in a Mouse Model 1, Biol. Reprod. 89 (2013) 1–7. https://doi.org/10.1095/biolreprod.113.110791.

[48] E.A. Bonney, S.A. Brown, To drive or be driven: The path of a mouse model of recurrent pregnancy loss, Reproduction. 147 (2014) 153–167. https://doi.org/10.1530/REP-13-0583.

[49] M.S. Hamilton, B.L. Hamilton, Environmental influences on immunologically associated spontaneous abortion in CBA/J mice, J. Reprod. Immunol. 11 (1987) 237–241. https://doi.org/10.1016/0165-0378(87)90060-X.

[50] R.L. Gendron, M.G. Baines, Infiltrating decidual natural killer cells are associated with spontaneous abortion in mice, Cell. Immunol. 113 (1988) 261–267. https://doi.org/10.1016/0008-8749(88)90025-1.

[51] X.-Y. Zhu, Y.-H. Zhou, M.-Y. Wang, L.-P. Jin, M.-M. Yuan, D.-J. Li, Blockade of CD86 Signaling Facilitates a Th2 Bias at the Maternal-Fetal Interface and Expands Peripheral CD4+CD25+ Regulatory T Cells to Rescue Abortion-Prone Fetuses, Biol. Reprod. 72 (2005) 338–345. https://doi.org/10.1095/BIOLREPROD.104.034108.

[52] M.L. Zenclussen, I. Anegon, A.Z. Bertoja, C. Chauveau, K. Vogt, K. Gerlof, A. Sollwedel, H.D. Volk, T. Ritter, A.C. Zenclussen, Over-expression of heme oxygenase-1 by adenoviral gene transfer improves pregnancy outcome in a murine model of abortion, J. Reprod. Immunol. 69 (2006) 35–52. https://doi.org/10.1016/J.JRI.2005.10.001.

[53] L.Y. Brown, E.A. Bonney, R.S. Raj, B. Nielsen, S. Brown, Generalized Disturbance of

DNA Methylation in the Uterine Decidua in the CBA/J × DBA/2 Mouse Model of Pregnancy Failure, Biol. Reprod. 89 (2013) 120–121. https://doi.org/10.1095/BIOLREPROD.113.113142.

[54] A. Ahmed, J. Singh, Y. Khan, S. V. Seshan, G. Girardi, A new mouse model to explore therapies for preeclampsia, PLoS One. 5 (2010) e13663. https://doi.org/10.1371/journal.pone.0013663.

[55] D.A. Clark, G. Chaouat, P.C. Arck, H.W. Mittruecker, G.A. Levy, Cytokine-dependent abortion in CBA x DBA/2 mice is mediated by the procoagulant fgl2 prothrombinase [correction of prothombinase]., J. Immunol. 160 (1998) 545–549.

[56] S. Huo, F. Wu, J. Zhang, X. Wang, W. Li, D. Cui, Y. Zuo, M. Hu, F. Zhong, Porcine soluble CD83 alleviates LPS-induced abortion in mice by promoting Th2 cytokine production, Treg cell generation and trophoblast invasion, Theriogenology. 157 (2020) 149–161. https://doi.org/10.1016/j.theriogenology.2020.07.026.

[57] M.A. Elovitz, C. Mrinalini, M.D. Sammel, Elucidating the early signal transduction pathways leading to fetal brain injury in preterm birth, Pediatr. Res. 59 (2006) 50–55. https://doi.org/10.1203/01.pdr.0000191141.21932.b6.

[58] The Jackson Laboratory, C57BL/6J - B6 Strain Details, (2021). https://www.jax.org/strain/000664 (accessed September 10, 2021).

[59] Janvier Labs, C57BL/6JRj Mouse - Model characteristics, (2019). https://www.janvier-labs.com/en/fiche_produit/c57bl-6jrj_mouse/ (accessed September 10, 2021).

[60] M. Dorsch, I. Wittur, W. Garrels, Efficiency of timed pregnancies in C57BL/6 and BALB/c mice by mating one male with up to four females, Lab. Anim. 54 (2020) 461–468. https://doi.org/10.1177/0023677219897687.

[61] T. Shima, Y. Sasaki, M. Itoh, A. Nakashima, N. Ishii, K. Sugamura, S. Saito, Regulatory T cells are necessary for implantation and maintenance of early pregnancy but not late pregnancy in allogeneic mice, J. Reprod. Immunol. 85 (2010) 121–129. https://doi.org/10.1016/j.jri.2010.02.006.

[62] R. Einenkel, K.R.H. Packhäuser, J. Ehrhardt, A. Tüngler, M. Zygmunt, D.O. Muzzio, CD83 is locally regulated and differentially expressed in disturbed murine pregnancy,

Reproduction. 158 (2019) 323–333. https://doi.org/10.1530/REP-19-0171.

[63] K.R.H. Packhäuser, G. Roman-Sosa, J. Ehrhardt, D. Krüger, M. Zygmunt, D.O. Muzzio, A kinetic study of CD83 reveals an upregulation and higher production of sCD83 in lymphocytes from pregnant mice, Front. Immunol. 8 (2017) 486. https://doi.org/10.3389/fimmu.2017.00486.

[64] H. Hashimoto, T. Eto, K. Endo, G. Itai, T. Kamisako, H. Suemizu, M. Ito, Comparative study of doses of exogenous progesterone administration needed to delay parturition in Jcl:MCH(ICR) mice, Exp. Anim. 59 (2010) 521–524. https://doi.org/10.1538/expanim.59.521.

[65] A.L. Hampton, G. Nie, L.A. Salamonsen, Progesterone analogues similarly modulate endometrial matrix metalloproteinase-1 and matrix metalloproteinase-3 and their inhibitor in a model for long-term contraceptive effects, Mol. Hum. Reprod. 5 (1999) 365–371. https://doi.org/10.1093/MOLEHR/5.4.365.

[66] K. Imada, A. Ito, Y. Itoh, H. Nagase, Y. Mori, Progesterone increases the production of tissue inhibitor of metalloproteinases-2 in rabbit uterine cervical fibroblasts, FEBS Lett. 341 (1994) 109–112. https://doi.org/10.1016/0014-5793(94)80250-5.

[67] D. Muzzio, A.C. Zenclussen, F. Jensen, The Role of B Cells in Pregnancy: The Good and the Bad, Am. J. Reprod. Immunol. 69 (2013) 408–412. https://doi.org/10.1111/aji.12079.

[68] M. Breloer, B. Kretschmer, K. Lüthje, S. Ehrlich, U. Ritter, T. Bickert, C. Steeg, S. Fillatreau, K. Hoehlig, V. Lampropoulou, B. Fleischer, CD83 is a regulator of murine B cell function in vivo, Eur. J. Immunol. 37 (2007) 634–648. https://doi.org/10.1002/eji.200636852.

[69] M. Breloer, B. Fleischer, CD83 regulates lymphocyte maturation, activation and homeostasis., Trends Immunol. 29 (2008) 186–94. https://doi.org/10.1016/j.it.2008.01.009.

[70] D.Y. Li, C. Gu, J. Min, Z.H. Chu, Q.J. Ou, Maturation induction of human peripheral blood mononuclear cell-derived dendritic cells, Exp. Ther. Med. 4 (2012) 131–134. https://doi.org/10.3892/etm.2012.565.

[71] L. Chen, S. Guan, Q. Zhou, S. Sheng, F. Zhong, Q. Wang, Continuous expression of CD83 on activated human CD4+ T cells is correlated with their differentiation into induced regulatory T cells, Mol. Med. Rep. 12 (2015) 3309–3314. https://doi.org/10.3892/mmr.2015.3796.

[72] M. Doebbeler, C. Koenig, L. Krzyzak, C. Seitz, A. Wild, T. Ulas, K. Baßler, D. Kopelyanskiy, A. Butterhof, C. Kuhnt, S. Kreiser, L. Stich, E. Zinser, I. Knippertz, S. Wirtz, C. Riegel, P. Hoffmann, M. Edinger, L. Nitschke, T. Winkler, J.L. Schultze, A. Steinkasserer, M. Lechmann, CD83 expression is essential for Treg cell differentiation and stability, JCI Insight. 3 (2018) 1–16. https://doi.org/10.1172/jci.insight.99712.

[73] Q. Gao, M. Li, S. Zhang, R. Zhou, J. Wu, Z. Li, The effect of spleen on the change of Treg cells during mouse pregnancy, J. Med. Coll. PLA. 28 (2013) 273–280. https://doi.org/10.1016/S1000-1948(13)60043-X.

[74] M. Kwiatek, T. Geca, A. Krzyzanowski, A. Malec, A. Kwaśniewska, Peripheral dendritic cells and CD4+CD25 +Foxp3+ regulatory T cells in the first trimester of normal pregnancy and in women with recurrent miscarriage, PLoS One. 10 (2015) 1–14. https://doi.org/10.1371/journal.pone.0124747.

[75] T.T. Jiang, V. Chaturvedi, J.M. Ertelt, J.M. Kinder, D.R. Clark, A.M. Valent, L. Xin, S.S. Way, Regulatory T Cells: New Keys for Further Unlocking the Enigma of Fetal Tolerance and Pregnancy Complications, J. Immunol. 192 (2014) 4949–4956. https://doi.org/10.4049/jimmunol.1400498.

[76] S.M. Blois, U. Kammerer, C.A. Soto, M.C. Tometten, V. Shaikly, G. Barrientos, R. Jurd, D. Rukavina, A.W. Thomson, B.F. Klapp, N. Fernández, P.C. Arck, Dendritic Cells: Key to Fetal Tolerance?, Biol. Reprod. 77 (2007) 590–598. https://doi.org/10.1095/BIOLREPROD.107.060632.

[77] S. Blois, M. Tometten, J. Kandil, E. Hagen, B.F. Klapp, R.A. Margni, P.C. Arck, Intercellular Adhesion Molecule-1/LFA-1 Cross Talk Is a Proximate Mediator Capable of Disrupting Immune Integration and Tolerance Mechanism at the Feto-Maternal Interface in Murine Pregnancies, J. Immunol. 174 (2005) 1820–1829. https://doi.org/10.4049/JIMMUNOL.174.4.1820.

[78] P. Bizargity, E.A. Bonney, Dendritic cells: a family portrait at mid-gestation,

Immunology. 126 (2009) 565. https://doi.org/10.1111/J.1365-2567.2008.02918.X.

[79] K. Askelund, H.S. Liddell, A.M. Zanderigo, N.S. Fernando, T.Y. Khong, P.R. Stone, L.W. Chamley, CD83+Dendritic Cells in the Decidua of Women with Recurrent Miscarriage and Normal Pregnancy, Placenta. 25 (2004) 140–145. https://doi.org/10.1016/S0143-4004(03)00182-6.

[80] Z. Li, X. Ju, P.A. Silveira, E. Abadir, W.H. Hsu, D.N.J. Hart, G.J. Clark, CD83: Activation marker for antigen presenting cells and its therapeutic potential, Front. Immunol. 10 (2019) 1312. https://doi.org/10.3389/fimmu.2019.01312.

[81] D. Royzman, D. Andreev, L. Stich, M. Rauh, T. Bäuerle, S. Ellmann, L. Boon, M. Kindermann, K. Peckert, A. Bozec, G. Schett, A. Steinkasserer, E. Zinser, Soluble CD83 Triggers Resolution of Arthritis and Sustained Inflammation Control in IDO Dependent Manner, Front. Immunol. 10 (2019) 633. https://doi.org/10.3389/fimmu.2019.00633.

[82] K. Peckert-Maier, A. Schönberg, A.B. Wild, D. Royzman, G. Braun, L. Stich, K. Hadrian, P. Tripal, C. Cursiefen, A. Steinkasserer, E. Zinser, F. Bock, Pre-incubation of corneal donor tissue with sCD83 improves graft survival via the induction of alternatively activated macrophages and tolerogenic dendritic cells, Am. J. Transplant. 00 (2021) 1–17. https://doi.org/10.1111/AJT.16824.

[83] E. Ivanova, D. Kyurkchiev, I. Altankova, J. Dimitrov, E. Binakova, S. Kyurkchiev, CD83+ monocyte-derived dendritic cells are present in human decidua and progesterone induces their differentiation in vitro, Am. J. Reprod. Immunol. 53 (2005) 199–205. https://doi.org/10.1111/j.1600-0897.2005.00266.x.

[84] G. Pointis, B. Rao, M.T. Latreille, T.M. Mignot, L. Cedard, Progesterone levels in the circulating blood of the ovarian and uterine veins during gestation in the mouse, Biol. Reprod. 24 (1981) 801–805. https://doi.org/10.1095/biolreprod24.4.801.

[85] S.M. Murr, G.H. Stabenfeldt, G.E. Bradford, I.I. Geschwind, Plasma Progesterone During Pregnancy in the Mouse, Endocrinology. 94 (1973) 1973–1975.

[86] J. Szekeres-Bartho, J. Hadnagy, A.S. Pacsa, The suppressive effect of progesterone on lymphocyte cytotoxicity: unique progesterone sensitivity of pregnancy lymphocytes, J. Reprod. Immunol. 7 (1985) 121–128. https://doi.org/10.1016/0165-0378(85)90066-X.

[87] R. Joachim, A.C. Zenclussen, B. Polgar, A.J. Douglas, S. Fest, M. Knackstedt, B.F. Klapp, P.C. Arck, The progesterone derivative dydrogesterone abrogates murine stress-triggered abortion by inducing a Th2 biased local immune response The progesterone derivative dydrogesterone abrogates murine stress-triggered abortion by inducing a Th2 biased local immu, Steroids. (2003) 931–940. https://doi.org/10.1016/j.steroids.2003.08.010.

[88] C.W. Ku, J.C. Allen, S.M. Lek, M.L. Chia, N.S. Tan, T.C. Tan, Serum progesterone distribution in normal pregnancies compared to pregnancies complicated by threatened miscarriage from 5 to 13 weeks gestation : a prospective cohort study, BMC Pregnancy Childbirth. 18 (2018) 360.

[89] R. Deanesly, Termination of early pregnancy in rats after ovariectomy is due to immediate collapse of the progesterone dependent decidua, J. Reprod. Fertil. 35 (1973) 183–186. https://doi.org/10.1530/jrf.0.0350183.

[90] A. Coomarasamy, A.J. Devall, J.J. Brosens, S. Quenby, M.D. Stephenson, S. Sierra, O.B. Christiansen, R. Small, J. Brewin, T.E. Roberts, R. Dhillon-Smith, H. Harb, H. Noordali, A. Papadopoulou, A. Eapen, M. Prior, G.C. Di Renzo, K. Hinshaw, B.W. Mol, M.A. Lumsden, Y. Khalaf, A. Shennan, M. Goddijn, M. van Wely, M. Al-Memar, P. Bennett, T. Bourne, R. Rai, L. Regan, I.D. Gallos, Micronized vaginal progesterone to prevent miscarriage: a critical evaluation of randomized evidence, Am. J. Obstet. Gynecol. 223 (2020) 167–176. https://doi.org/10.1016/J.AJOG.2019.12.006.

[91] A.J. Devall, A. Papadopoulou, M. Podesek, D.M. Haas, M.J. Price, A. Coomarasamy, I.D. Gallos, Progestogens for preventing miscarriage: a network meta-analysis, Cochrane Database Syst. Rev. (2021). https://doi.org/10.1002/14651858.CD013792.PUB2.

[92] G. Dante, V. Vaccaro, F. Facchinetti, Use of progestagens during early pregnancy, Facts, Views Vis. ObGyn. 5 (2013) 66.

[93] ThermoFisher Scientific, Dendritic Cell Marker DCIR2 Monoclonal Antibody (33D1), eBioscience™ - Product Details, (2021). https://www.thermofisher.com/antibody/product/Dendritic-Cell-Marker-DCIR2-Antibody-clone-33D1-Monoclonal/14-5884-82 (accessed September 11, 2021).

8 Publications

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8.1 A kinetic Study of CD83 Reveals an Upregulation and Higher Production of sCD83 in Lymphocytes from Pregnant Mice.

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A Kinetic Study of CD83 Reveals an Upregulation and Higher Production of sCD83 in Lymphocytes from Pregnant Mice

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For the normal development of pregnancy, a balance between immune tolerance and defense is crucial. However, the mechanisms mediating such a balance are not fully understood. CD83 is a transmembrane protein whose expression has been linked to anti-inflammatory functions of T and B cells. The soluble form of CD83, released by cleavage of the membrane-bound protein, has strong anti-inflammatory properties and was successfully tested in different mouse models. It is assumed that this molecule contributes to the establishment of immune tolerance. Therefore, we postulated that the expression of CD83 is crucial for immune tolerance during pregnancy in mice. Here, we demonstrated that the membrane-bound form of CD83 was upregulated in T and B cells during allogeneic murine pregnancies. An upregulation was also evident in the main splenic B cell subtypes: marginal zone, follicular zone, and transitional B cells. We also showed that there was an augmentation in the number of $CD83^+$ cells toward the end of pregnancy within splenic B and $CD4^+$ T cells, while $CD83^+$ dendritic cells were reduced in spleen and inguinal lymph nodes of pregnant mice. Additionally, B lymphocytes in late-pregnancy presented a markedly higher sensitivity to LPS in terms of CD83 expression and sCD83 release. Progesterone induced a dosis-dependent upregulation of CD83 on T cells. Our data suggest that the regulation of CD83 expression represents a novel pathway of fetal tolerance and protection against inflammatory threats during pregnancy.

Keywords: CD83, pregnancy, tolerance, B cells, T cells

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INTRODUCTION

Pregnancy is associated with several immune adaptations that allow the growth of a healthy semi-allogeneic fetus. These include a finely regulated shift from a Th1 and Th17 toward a Th2 tolerogenic response driven by anti-inflammatory molecules secreted by, among others, the "so-called" regulatory cells (1). Several cell types have been described with regulatory functions during pregnancy, including T (2) and B lymphocytes (3). Lymphocytes with regulatory properties allow the eradication of external threats and limit proinflammatory responses. In particular, the presence of regulatory cells at the maternal–fetal interface is crucial in mammals with invasive placentation (4). A potential dysregulation of regulatory cells may have several consequences on the human health and has been

associated with autoimmune diseases, cancer, chronic infections (5, 6), and pregnancy-related disorders, including preterm birth, preeclampsia, and recurrent pregnancy loss (7–10). $CD4^+CD25^+$ regulatory T cells and $CD19^+CD24^{hi}CD27^+$ regulatory B cells are increased during normal pregnancies, while some cases of pregnancy loss associate with a lack of this increment (8, 11). In mouse models for pregnancy disturbances, the transfer of regulatory lymphocytes rescues mice from normally occurring pregnancy loss (12, 13).

An increasing body of evidence links inflammation in the absence of infection to the onset of preterm labor (14–16). For this reason, inflammatory pathways emerged as therapeutic targets to handle certain cases of preterm labor (17–19). Immune cells apply several strategies to exert their immunomodulatory functions. These include the release of factors, such as cytokines and chemokines, and expression of membrane ligands, including suppressive ligands (LFA-1, CTLA-4) and apoptosis-related ligands (PD-L1, TRAIL) (20–23). To this group belongs the CD83, which is a transmembrane molecule of the immunoglobulin family, first described as an activation marker for dendritic cells (DCs) (24, 25).

In DCs, CD83 is mostly associated to the maturation state and functions as a costimulatory molecule for T cell activation, independently of the level of expression (26–29). By targeting activated DCs, anti-CD83 depleting antibodies can prevent human peripheral blood mononuclear cell-induced acute graft vs. host disease in SCID mouse recipients (30, 31). Although the mechanisms through which membrane-bound CD83 regulates immune responses are still to be elucidated, one of the proposed pathways is though negative regulation of MARCH1 and MARCH8. These E3 ubiquitin-protein ligases control the ubiquitination of MHCII molecules, impacting on their turnover on DCs and B cells (32, 33). On the other hand, mice with $CD83^{-/-}$ DCs develop exacerbated dextran sodium sulfate induced colitis (34). CD83 on DCs can also regulate cytokine production of immature DCs through cell–cell contact, resulting in reduced secretion of MCP-1 and IL-12p40 (34).

CD83 expression can be easily detected on activated T cells (35–37). While reports implicating silencing of CD83 expression on antigen-specific $CD4^+$ T cells showed an impaired proliferative capacity and reduced cytokine secretion, this may not apply to all $CD4^+$ T cell populations or it may represent a mechanism to avoid over-reactive $CD4^+$ T cells (38–40). Indeed, CD83 overexpression in murine naïve $CD4^+$ T cells induces regulatory T cell-specific FOXP3 expression and confers *in vivo* tolerogenic properties (39).

Studies focused on B cells also show a correlation of CD83 and B cell activation (41). CD83 co-localizes with the BCR as well as with the LPS receptor and regulates signal transduction pathways downstream both receptors (42). On BCR, CD83 acts reducing its sensitivity, for which it may prevent over-reaction of activated B cells. BCR sensitivity is also thought to be a determinant for the MZ vs. FO B cell developmental decision (43). This may explain the MZ over FO B cell preference in the maturation of CD83 overexpressing B cells mice (44). When B cells are transferred with CD83, LPS stimulation leads to high IL-10 production derived from MZ B cells. CD83 expression on B cells also predisposes FO B cells to cell death. CD83-expressing B cells also respond with reduced IgG production. Taken together, CD83 expression on B cells is associated to a MZ over a FO B cell preference and anti-inflammatory B cell responses. As well as in mice with CD83 over-expressing B cells, normal pregnancies display several B cell adaptations that include a preference for MZ over FO maturation and a higher B cell-derived IL-10 production (12, 45, 46). This phenomenon seems to be reflected in the antibody profile of pregnant mice by elevated MZ B cell-derived Ig types IgM, IgA, and IgG3 (43).

Beyond the influence of membrane CD83 on lymphocyte function, an anti-inflammatory role of soluble CD83 was depicted in different models of autoimmune diseases as well as allograft transplantation (47–53). Soluble CD83 is very likely generated either by shedding of the membrane-bound protein (54–56) or by alternative splicing of the transcript (57). Due to the growing line of evidence of the therapeutic potential of exogenous sCD83 in inflammatory and autoimmune diseases, we hypothesize that it can be a tolerogenic molecule that might support pregnancy as well.

In this work, we show that the expression of the membrane molecule CD83 as well as its soluble form, sCD83, is increased in the course of normal murine pregnancies. These results suggest that sCD83 may play a role in the maintenance of pregnancy.

MATERIALS AND METHODS

PBS, FBS, PenStrep, PMA, and RPMI1640 were purchased from Merck Millipore (Billerica, MA, USA) and estrogen, progesterone, and LPS from Sigma-Aldrich Chemie GmbH (Munich, Germany). Following antibodies were purchased from BD Bioscience (Heidelberg, Germany): CD83 (Michel-19); B220 (RA3-6B2); CD4 (RM4-4 and RM4-5); CD23 (B3B4); CD21 (7G6); CD19 (1D3); CD11c (HC3); CD25 (PC61); CD69 (H1.2F3); TNF (MP6-XT22); IFN (XMG1.2); IL-17 (TC11-18H10); FoxP3 (R16-715); IL-10 (JES5-16E3); and Ki-67 (B56), as well as Purified NA/LE CD3e (145-2C11). DC Marker DCIR2 (33D1), Brefeldin A, and anti-mouse CD83 purified antibody were purchased from eBioscience (San Diego, CA, USA) and isotype-control (Purified Rat IgG1k isotype) from BioLegend (San Diego, CA, USA).

Animals

C57Bl6/J female mice and BALB/c male mice were purchased from Charles River (Sulzfeld, Babavia, Germany) or Janvier Labs (Saint-Berthevin Cedex, France). BALB/c males were bred in our Central Service and Research Facility for Animals (ZSFV). The animals were kept co-housed in a 12L:12D cycle with food and water *ad libitum*. Eight- to twelve-week-old C57Bl6/J female mice were paired with BALB/c males and checked for vaginal plug every morning. Observation of plug was declared day 0 of pregnancy, and the female was separated from the male. Mice were sacrificed at day 7, 14, or 18 post plug (7, 14, and 18 dpp); spleen, paraaortic lymph nodes (PLN), inguinal lymph nodes (ILN), and thymus as well as serum were collected. We performed permanent matings and mice representative of all stages of pregnancy were available weekly. Non-pregnant C57Bl6/J female mice were randomly sacrificed used as control.

Cell Preparation

Single cell suspensions from paraaortic lymph nodes, inguinal lymph nodes, and thymus were obtained. The tissue was carefully squeezed through a 40-µm nylon cell strainer and washed with PBS. In case of spleen tissue, an erythrocyte lysis with 10 mL Lysis Buffer (0.89% NH4Cl, 0.1% KHCO3, 0.003% 0.5 M EDTA) was performed and stopped after 5 min with 3 mL FBS. After washing, the cell suspension was filtered a second time with a 40-µm cell strainer. The cell counts of the suspensions were determined using a Neubauer chamber.

Flow Cytometry

Flow cytometry was applied to evaluate the expression of CD83 on B, T, or DCs from spleen, thymus, and lymph nodes. Cell suspensions were first incubated with CD16/32 mAb Fc block (BD Pharmingen, Heidelberg, Germany) for 5 min. Staining with fluorochrome-labeled specific antibodies was performed for 30 min at 4°C in the dark. Samples from spleen were additionally stained with Fixable Viability Dye eFluor 780 (eBioscience, San Diego, CA, USA) for 30 min and later washed with FACS buffer (1% BSA, 0.1% NaN3, 0.955% PBS) before Fc blocking. Data were acquired on FACSCanto (BD Bioscience) and analyzed by using FlowJo software (FlowJo, LLC, Ashland, TN, USA). Information about gating strategies can be provided upon request.

Cell Stimulation

The 1×10^6 splenic lymphocytes were cultured 48 h in a total volume of 500 µL RPMI 1,640 culture medium supplemented with 10% FBS and antibiotics on 48-well flat-bottom suspension plates. The stimulation was performed with either 10 µg/mL LPS for 48 h and 50 ng/mL PMA as well as 500 ng/mL ionomycin for the last 5 h. In order to examine the influence of pregnancy related hormones on CD83 expression, the cells were stimulated with different concentrations of estrogen (5 pg/mL, 100 pg/mL, and 100 ng/mL) or progesterone (50 ng/mL; 500 ng/mL; 5,000 ng/mL; 50,000 ng/mL). After stimulation cells and supernatants were separated by 5 min centrifugation at $1{,}300 \times g$. The supernatants were stored at −80°C, and the cells were directly used for Flow Cytometry.

Cell Depletion

To determine which cells produce soluble CD83, splenic lymphocytes were depleted from $CD4^+$ or $CD19^+$ cells. CD19 depletion was performed using CD19 MicroBeads mouse (Miltenyi Biotec GmbH, Teterow, Germany). For CD4 depletion, a negative selection Mouse $CD4^+$ T-Cell Isolation kit was purchased from EasySep (STEMCELL Technologies, Vancouver, BC, Canada). In both cases, the supplier's instructions were followed; 500,000 cells were cultured with 250 µL culture medium and stimulated with LPS as described before.

ELISA

The levels of sCD83 in sera and supernatants were measured using an ELISA kit for CD83 (Cloud-Clone Corp., Houston, TX, USA). The serum samples were diluted 1:2 in PBS, while the supernatants were examined without dilution. The test was performed following the instruction manual.

Real-time PCR

Magnetic isolated splenic $CD19^+$ B cells were treated with TriFast peqGOLD (VWR, Radnor, PA, USA). RNA isolation, cDNA synthesis, and real-time PCR were performed as previously described (58). RNA concentration was evaluated spectrophotometrically using the NanoPhotometer PEARL (IMPLEN, Munich, Germany). Samples were amplified in duplicate and nontemplate samples were used as controls. Primer pairs were chosen to span an exon–exon junction and so avoid unwanted genomic DNA amplification. Real-time PCR was performed using SYBR Green (AB/Life Technologies, Darmstadt, Germany) in a 7300 Real-time PCR System (Applied Biosystems, Darmstadt, Germany) with β-actin as housekeeping gene. Primer sequences were the following: CD83 Fw: TGAAGGTGACAGGATGCCC; CD83 Rw: CTTGGGGAGGTGACTGGAAG; β-actin Fw: TGGAATCCTGTGGCATCCATGAAAC; Rw: TAAAACGCA GCTCAGTAACAGTCC. All primers were purchased from Invitrogen (Carlsbad, CA, USA).

Recombinant sCD83 Production

Cloning of the DNA Encoding for the Murine CD83 Ectodomain

pGRS-88: The gene fragment was amplified by PCR with the primers O-GRS-112 (aattatt*TCTAGA*GCCACCATGTCGCA AGGCCTCCAGCTCCTGTTTC) and O-GRS-113 (GCTCCT GTACTTCCTGAAAGTTGACTCTGTAG) with the Phusion polymerase (NEB, Ipswich, MA, USA) from RNA isolated from stimulated lymphocytes. The PCR product was digested with *Xba*I and phosphorylated with T4 polynucleotide kinase 3′ phosphatase minus (NEB, Ipswich, MA, USA) and ligated to the vector pGRS-12 (59) digested with *Xba*I (NEB, Ipswich, MA, USA) and *Afe*I (NEB, Ipswich, MA, USA).

Expression and Purification of the Murine CD83 Ectodomain

The CD83 ectodomain was purified from supernatant of transiently transfected eukaryotic cells essentially a previously described (59). In brief, HEK-293T cells transfected with the DNA mixed with branched polyethylenimine (PEI) (Sigma-Aldrich Chemie GmbH, Munich, Germany). Three days after transfection, the supernatant was collected and concentrated (Vivaflow system, cassette with membrane of MW CO 5,000 Da; Sartorius Stedim, Göttingen, Germany). The expression of sCD83 on transfected cells was controlled by immune fluorescence using anti-CD83 antibody. sCD83 functionality was checked by its ability to reduce the generation of $CD80^+$ bone marrow-derived DCs (60). We obtained a reduction of almost 50% of $CD80^+$ cells. Then the protein was purified using streptactin superflow high capacity slurry (IBA Biotech, Göttingen, Germany) according to the instructions of the manufacturer. The presence of the protein in each collected fraction was checked by SDS-PAGE (61) and staining with Instant Blue (Expedeon, San Diego, CA, USA). For all our experiments, we used sCD83 purified from the same supernatant.

sCD83 Effect on T Cells

The 96-Well plates were coated with CD3e (1 µg/mL) and incubated overnight at 4°C. T cells from murine lymph nodes were

isolated using a negative selection Mouse CD4+ T Cell Isolation kit (STEMCELL Technologies, Vancouver, BC, Canada). A total of 100,000 cells were incubated overnight in 100 µL supernatants (see Cell Stimulation) with 10% FBS. To evaluate an effect of sCD83, either an Anti-Mouse CD83 Purified Antibody or an Isotype-Control were added.

Additionally, T Cells were incubated overnight in 100 µL culture medium and stimulated with a recombinant sCD83 protein (2 and 200 ng/mL, "CD83-low" and "CD83-high," respectively). As control, the amino terminal domain of the Schmallenberg glycoprotein Gc, which was produced and isolated in the same manner (59), was used in equivalent concentrations. LPS, PMA, and ionomycin were added as described before.

For intracellular staining, Brefeldin A was added for the last 5 h. Cells were collected and stained with fixable viability dye eFluor 660 and later with specific membrane antibodies. After extracellular staining, cells were fixated with fixation/permeabilization kit (eBioscience, San Diego, CA, USA), for 20 min, permeabilized using permeabilization buffer (eBioscience, San Diego, CA, USA), and intracellular stained with specific antibodies for 30 min.

Statistical Analysis

Normality was assessed by D'Agostino and Pearson omnibus normality test. MFI-data, serum sCD83 ELISA, and PCR of stimulated lymphocytes were analyzed using Kruskal–Wallis test with Dunn's posttest. Hormones stimulations experiments as well as lymphocyte depletion test was analyzed by ANOVA and Dunnett's test. *In vitro* secretion of sCD83 by ELISA in stimulated and nonstimulated np and 18 dpp isolated splenocytes was analyzed using two-way-ANOVA with Bonferroni posttests. Further data were analyzed by ANOVA with Tuckey's posttest. Significant differences between groups were indicated with asterisks as follows: $*p < 0.05$; $**p < 0.01$; and $***p < 0.001$.

RESULTS

CD83 Expression Is Upregulated in B and T Lymphocytes but Not in DCs during Pregnancy

In order to characterize the dynamics of CD83 during the course of normal murine pregnancy, we analyzed CD83 expression at different time points of normal murine pregnancies. We evaluated the presence of CD83 on CD11c+ and DCIR2+ dendritic cells, CD4+ T cells, and different B-cell populations (**Figure 1A**).

In spleen, we found an upregulation of CD83 not only on B cells but also on CD4+ T cells at day 14 of pregnancy (1.76- and 3.18-fold increase, respectively, **Figures 1B,C**). A deeper analysis within the major splenic B cell populations—transitional (TN), marginal zone (MZ), and follicular zone (FO) B cells—showed that the CD83 upregulation at day 14 also occurs in all subsets (1.47-, 2.76-, and 1.80-fold increase, respectively, **Figures 1C,D**). This increase was also confirmed by analyzing mRNA levels of CD83 on fresh-isolated B cells (**Figure 1E**).

The analysis of the percentages of CD83+ cells depicted a significant increase of positive B and T cells (1.36- and 2.65-fold increase in B and CD4+ T cells, respectively, **Figure 1F**) from early (7 dpp) to the end of pregnancy (18 dpp). DCs, however, showed a reduction of the percentage of CD83+ cells during pregnancy, being statistically significant at day 14 of pregnancy (3.08 ± 0.40 in np vs. 1.08 ± 0.17 in 14 dpp, **Figure 2A**). After an in-depth analysis of T cell subsets, an increase in the percentage of CD83+ cells among regulatory CD4+CD25+ cells was observed (from 1.39 ± 0.21 at early pregnancy to 6.54 ± 1.52 at advanced pregnancy, **Figure 1F**).

An upregulation of CD83 was also found in B cells from the uterus-draining paraaortic lymph nodes (MFI: 33.10 ± 2.68 in 7 dpp vs. 20.54 ± 2.98 in np, **Figure 2A**). In ILN, though a similar tendency was observed, no significant differences were found among the groups (**Figure 2B**). Neither B- nor CD4+ T cells showed differences in the percentages of CD83+ cells in PLN or ILN (**Figures 2C,D**). Significantly lower percentages of CD83+ in DCs were also observed on ILN of all groups of pregnant mice (55.23 ± 5.58, 41.86 ± 4.40, 48.7 ± 5.58, 44.28 ± 5.72 in np, 7, 14, and 18 dpp respectively, **Figure 2B**). No significant differences were found on either the CD83 expression or the percentage of CD83+ cells in any cell type in thymus (data not shown).

In vivo levels of sCD83 were determined from serum samples (**Figure 2E**). No statistically significant changes in the mean levels of sCD83 were seen. Nevertheless, only 16.7% of the non-pregnant mice had sCD83 serum levels over 1,100 pg/mL (the mean value for all groups of mice), while this percentage was higher in pregnant mice (50.0% in mice at 7 dpp, 25.0% at 14 dpp, and 57.7% at 18 dpp).

Progesterone Induces Higher CD83 Expression

Sexual hormones estradiol and progesterone influence a number of immune and non-immune adaptations to pregnancy (62–64). We observed that CD83 expression during pregnancy had a similar pattern to progesterone levels, with a maximum at day 14 of pregnancy (65). To test if progesterone or estradiol influences CD83 expression, we isolated splenocytes and stimulated them with increasing concentrations of estradiol and progesterone. Progesterone but not estradiol upregulated CD83 expression on B cells and DCs (**Figure 3A**). T cells, on the other hand, reacted to progesterone stimulation more sensitively and upregulated CD83 expression in a dose-dependent manner (**Figure 3A**). Estrogen stimulation failed to induce changes in CD83 expression also on T cells (**Figure 3B**).

Lymphocytes from Advanced Pregnant Mice Release More sCD83

The upregulation of CD83 both in B and T cells (**Figure 1B**) and in response to progesterone (**Figure 3A**) prompted us to assess the expression pattern of cell surface and soluble CD83 in lymphocytes of pregnant mice upon stimulatory challenge. We found that B cells from advanced pregnancy express much higher levels of CD83 when stimulated with LPS, PMA, and ionomycin (a combination we shortened as LPI) (3.82 ± 0.85-fold increase in 18 dpp vs. 1.91 ± 0.21-fold increase in np, **Figure 3C**). We did not observe any significant effect on the T cells population we analyzed (**Figure 3C**). CD83 mRNA levels were also analyzed

in LPI-stimulated B cells. The B cells from mice with advanced pregnancies displayed an almost fourfold increase in the levels of CD83 mRNA when compared with the cells from np mice (**Figure 3D**).

Taking into account the *in vivo* levels of sCD83 observed in sera of pregnant vs. non-pregnant mice, we sought to better characterize sCD83 production. We used the supernatant of the cultured cells to assess sCD83 production by ELISA. In agreement with

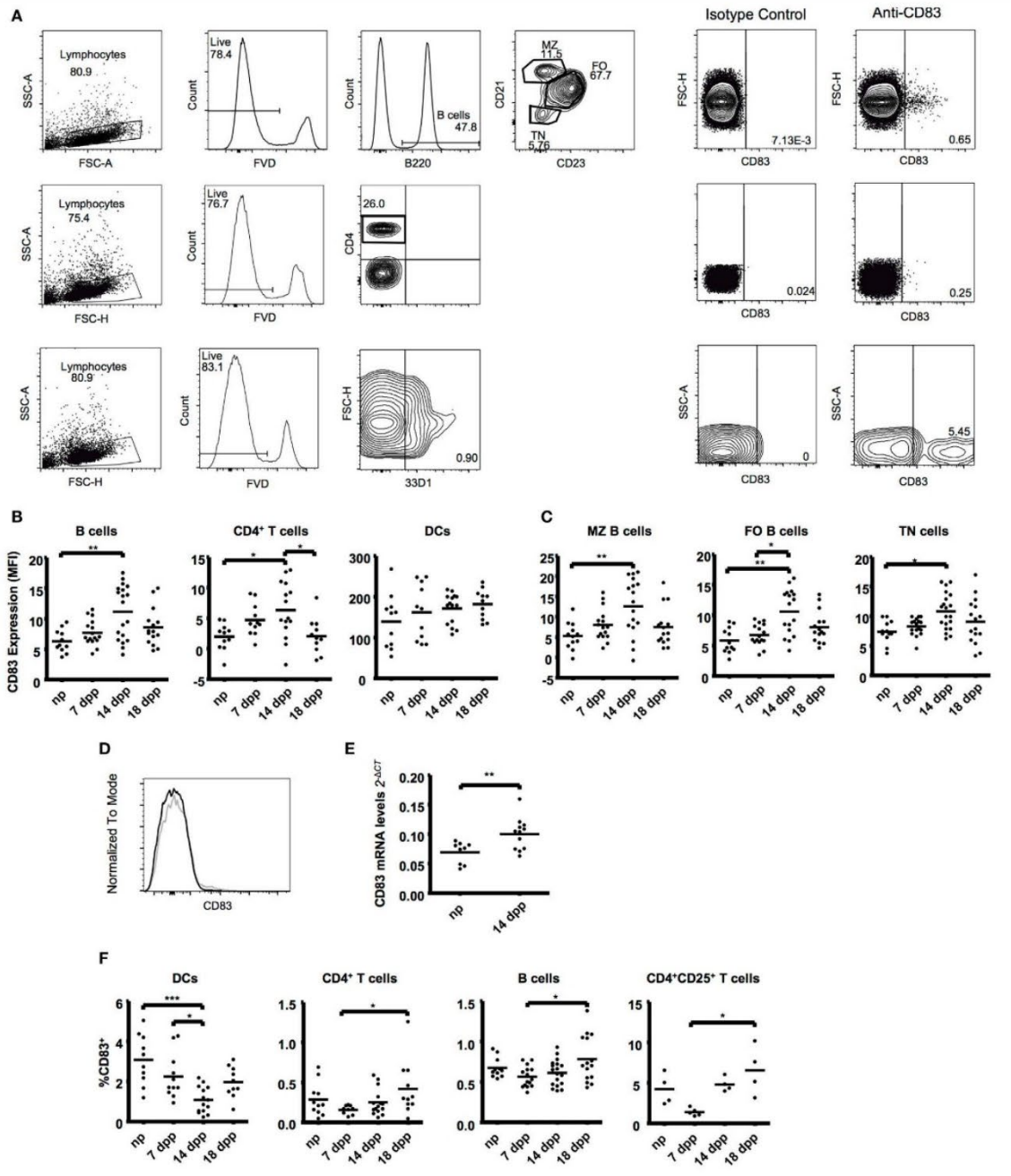

FIGURE 1 | Continued

FIGURE 1 | Continued
B cells (B220⁻), T cells (CD4⁻), and dendritic cells (33D1⁻) from fresh spleen tissue were examined in the course of normal murine pregnancies. **(A)** Representative plots showing gating strategies to CD83 expression (right). **(B)** Scatter dot plots show the median fluorescence intensity of CD83 within the respective cell types in live lymphocytes. **(C)** B220⁺ B cells were further subdivided in CD21/35^{hi}CD23^{low} marginal zone B cells (MZ), CD21/35^{int}CD23^{hi} follicular zone B cells (FO), and CD21/35⁻CD23⁻ transitional B cells (TN). Scatter dot plots show the mean fluorescence intensity of CD83. **(D)** Representative histogram overlapping CD83 expression on B cells of a non-pregnant mice (black line) or pregnant mice at day 14 (gray line). **(E)** Magnetic isolated splenic CD19⁺ B cells were analyzed for mRNA expression levels of CD83 by quantitative real-time PCR. FACS data were analyzed by Kruskal–Wallis test with Dunn's posttest. PCR was analyzed by Student's *t*-test. Significant differences are indicated (*$p \leq 0.05$, **$p \leq 0.01$). **(F)** Data show the percentage of CD83⁺ cells within the respective cell types in spleen. T cells were further characterized within the CD25⁺ population. Data show the percentage of CD83⁺ cells within CD25⁺ T cells. Data were analyzed by ANOVA with Tuckey's posttest. Significant differences are indicated (*$p \leq 0.05$, ***$p \leq 0.001$).

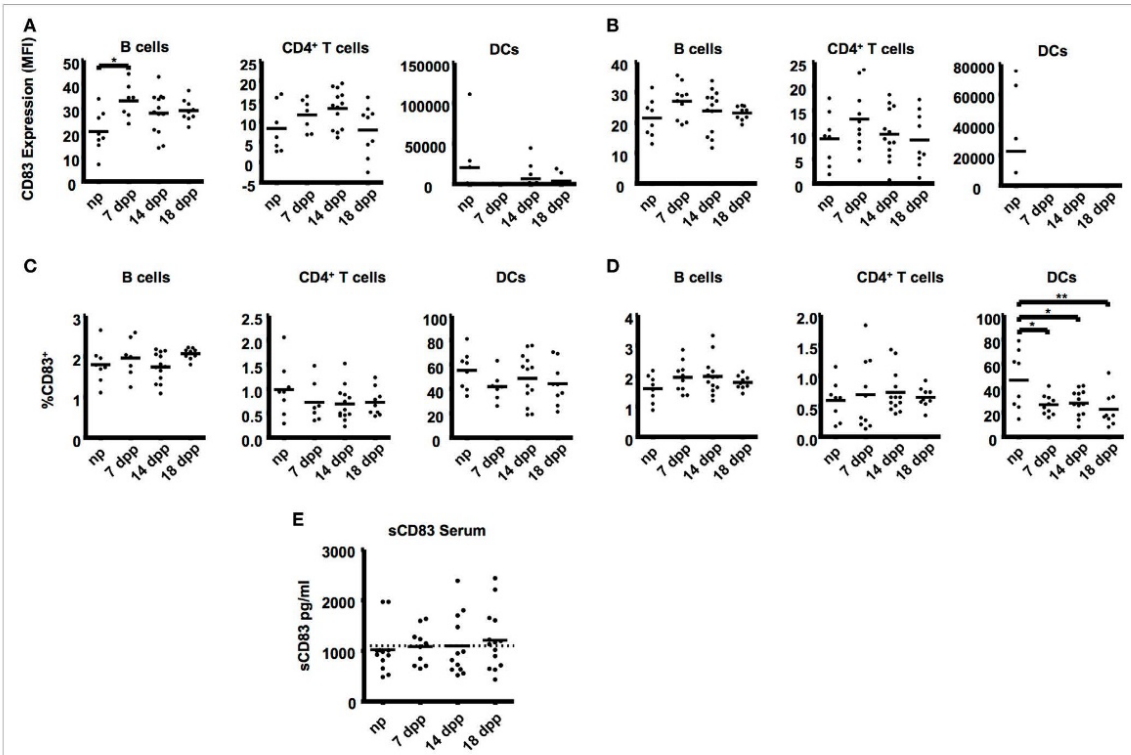

FIGURE 2 | B cells (B220⁻), T cells (CD4⁻), and dendritic cells (33D1⁻) from uterine-draining lymph nodes were examined in the course of pregnancy. **(A)** Scatter dot plots show the mean fluorescence intensity of CD83 within the respective cell types in paraaortic lymph nodes. **(B)** Scatter dot plots show the mean fluorescence intensity of CD83 within the respective cell types in inguinal lymph nodes. Data were analyzed by Kruskal–Wallis test with Dunn's posttest. Significant differences are indicated (*$p \leq 0.05$). **(C)** Percentage of CD83⁺ cells within the respective cell types in paraaortic lymph nodes **(C)** and inguinal lymph nodes **(D)**. Data were analyzed by ANOVA with Tuckey's posttest. Significant differences are indicated (*$p \leq 0.05$, **$p \leq 0.01$). **(E)** Sera of pregnant and non-pregnant mice were analyzed by ELISA to determine sCD83 levels during pregnancy. Data were analyzed by Kruskal–Wallis test with Dunn's posttest.

CD83 MFI and mRNA levels on B cells, we found that sCD83 was present in significantly higher concentrations in the supernatant of stimulated lymphocytes of advanced pregnant mice compared with the supernatant of lymphocytes of non-pregnant mice (1,793 ± 226.1 pg/mL in 18 dpp vs. 868.4 ± 124.9 pg/mL in np, **Figure 3E**). In summary, lymphocytes of late-pregnancy mice upregulate the expression of membrane CD83 and release more sCD83 upon stimulation than lymphocytes of non-pregnant mice do.

B Cells Are the Main Source of sCD83

Several lymphocyte populations present in the spleen express CD83 and have the potential to release sCD83. To identify the main source of splenic sCD83, we isolated splenocytes and further depleted CD19⁺ B and CD4⁺ T cells. Depleted splenocytes were stimulated to produce sCD83 as in **Figure 3C**, and levels of sCD83 in the supernatant of the cell culture were assessed by ELISA. Remarkably, CD19⁺ B cell depletion had the strongest negative effect on the basal release of sCD83 (220.4 ± 28.29 pg/mL

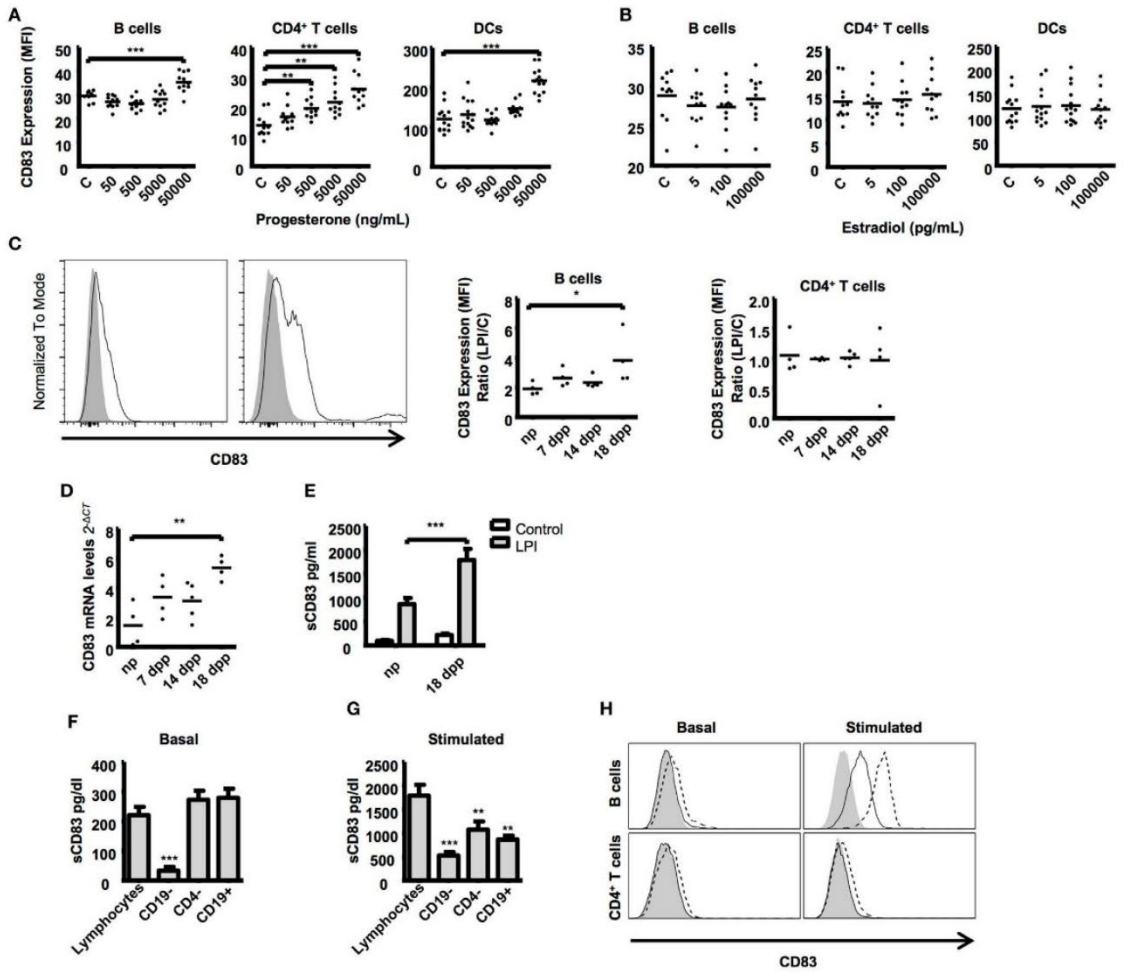

FIGURE 3 | Splenic lymphocytes from different stages of murine pregnancy were treated with key pregnancy hormones, progesterone and estrogen in increasing concentrations for 48 h. Cells cultured in medium alone served as control. Scatter dot plots show the CD83 expression on CD19+ B cells, CD4+ T cells. and CD11c+ dendritic cells after progesterone **(A)** or estrogen **(B)** stimulation. Data were analyzed by Kruskal–Wallis test with Dunn's posttest. Significant differences are indicated (**$p \leq 0.01$, ***$p \leq 0.001$). **(C)** Splenic lymphocytes from different stages of murine pregnancy were stimulated with LPS for 48 h plus PMA and ionomycin for the last 5 h. The histogram shows a representative stimulation from a non-pregnant mouse B cells (left) and a pregnant mouse at day 18 (right). Gray curves show basal CD83 expression, the black line represents CD83 expression after stimulation. Scatter dot plots show the quotient between CD83 MFI of LPS-stimulated and nonstimulated CD19+ B cells (left) and CD4+ T cells (right). **(D)** mRNA expression levels of CD83 by quantitative real-time PCR of stimulated lymphocytes relative to nonstimulated controls. **(E)** Bars compare the amount of sCD83 in the supernatant from LPS-stimulated lymphocytes from non-pregnant and pregnant mice from late-pregnancy stage and nonstimulated controls. Data were analyzed by two-way-ANOVA with Bonferroni posttests **(C)**. Significant differences are indicated (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$). **(F)** CD19- or CD4-depleted splenic lymphocytes and magnetic isolated splenic CD19+ B cells from mice at advanced pregnancies were cultured for 48 h. Data show the basal production of sCD83 in the supernatants in nonstimulated lymphocytes **(F)** or after stimulation with LPS, PMA, and ionomycin **(G)**. **(H)** Overlapping histogram display differences between nonstimulated (left) and stimulated (right) B cells (top) and CD4+ T cells (bottom). Filled curved represents the FMO for CD83, while filled and dashed lines show extracellular and intracellular CD83 staining, respectively. Data were analyzed with ANOVA and Dunnett's test to compare treatments against non-depleted controls. Significant differences are indicated (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

in total splenocytes vs. 28.48 ± 14.3 in CD19 depleted splenocytes, **Figure 3F**) as well as in the LPI induced release (1,793 ± 226.1 pg/mL in total splenocytes vs. 527.0 ± 75.52 pg/mL in CD19 depleted splenocytes, **Figure 3G**). Remarkably, there is no significant difference between purified CD19+ B cells release of sCD83 and total splenocytes (220.4 ± 28.29 pg/mL in total splenocytes vs. 279.4 ± 68.29 pg/mL in CD19+ B cells). However, upon stimulation, depletion of CD4+ T cells had as well a slightly detrimental effect (1,793 ± 226.1 pg/mL in total splenocytes vs. 1,077 ± 169.3 pg/mL in CD4 depleted splenocytes, **Figure 3G**);

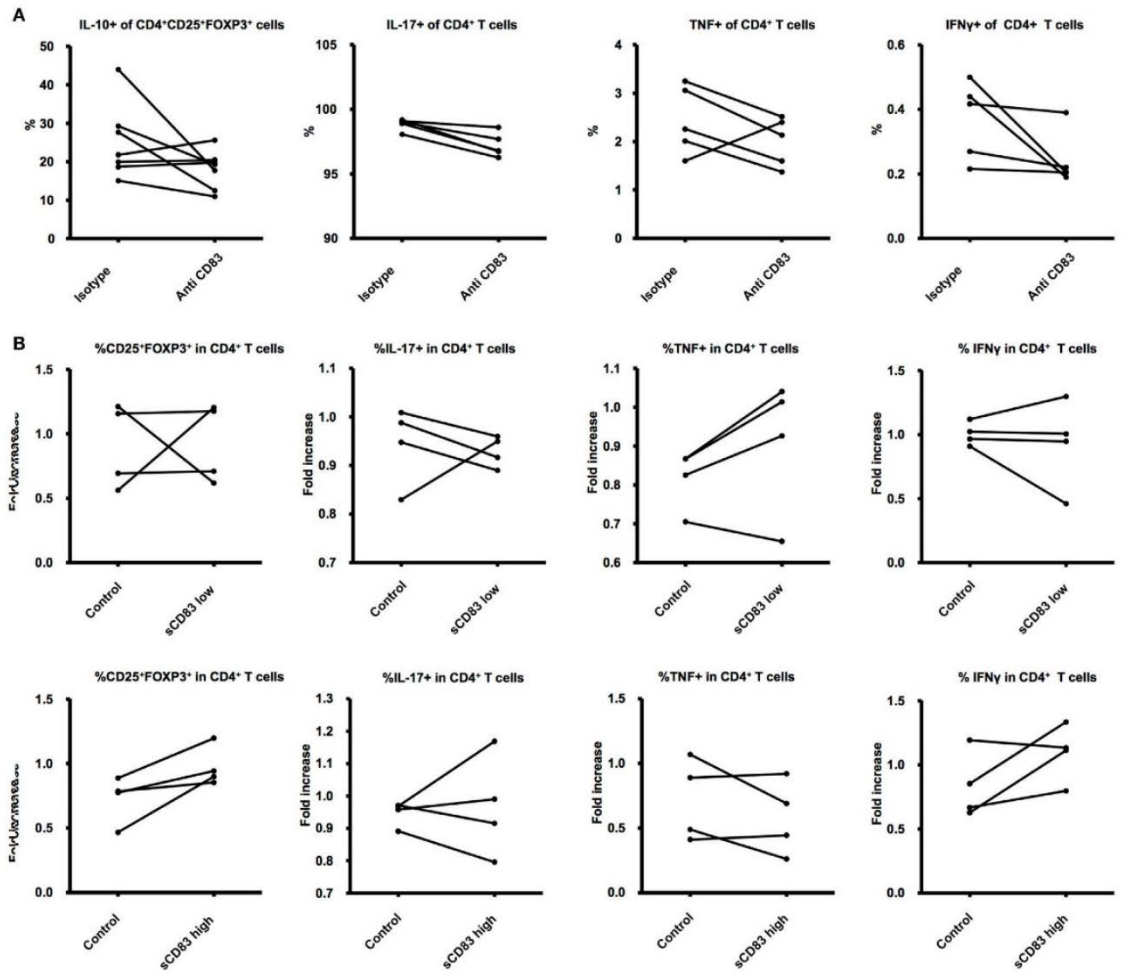

FIGURE 4 | Effects of sCD83 on T cells. **(A)** CD4+ T cells were isolated from lymph nodes and cultured 24 h with supernatant obtained from the experiments on (**Figure 1C**) and treated with a blocking antibody against CD83 or an isotype control. **(B)** CD4+ T cells were isolated from lymph nodes and cultured 24 h with LPS, PMA, ionomycin, and either recombinant sCD83 or a control protein in similar concentrations. The cells were later analyzed for expression of activation markers and associated intracellular and surface markers. Student's *t*-test was used to compare differences between the groups.

purified CD19+ B cells release of sCD83 is lower than that in total splenocytes (1,793 ± 452.2 pg/mL in total lymphocytes vs. 864.34 ± 176.8 pg/mL in CD19+ B cells), indicating that T cells might participate on the B cell activation for the release of sCD83. We also observed that under LPI stimulation, B cells produced display higher intracellular CD83 staining than do T cells under same conditions (**Figure 3H**). To sum up, B cells represent the main source of splenic sCD83 release upon LPI stimulation.

sCD83 Influence on T Cell Activity *In Vitro*

The soluble form of CD83 has been shown to possess *in vivo* as well as *in vitro* anti-inflammatory properties (47–53, 57). Taking into account the presence of sCD83 in the supernatants of stimulated splenocytes, we used the supernatant to stimulated naïve T Cells. In some experiments, we attempted to block sCD83 with a specific antibody against CD83. The presence of a blocking antibody in the cell culture failed to significantly reduce the cytokine expression of CD4+ T cells (**Figure 4A**).

sCD83 was previously shown to affect T-cell function via DCs, leading to higher percentages of CD4+CD25+Foxp3+ Tregs, lower production of proinflammatory cytokines, and increasing the release of immunosuppressive cytokines (47), all features that promote pregnancy wellbeing (2). To test the direct role of sCD83 on naïve CD4+ T cells, we generated recombinant sCD83 using methods already described (59). The recombinant protein was used to treat isolated CD4+ T cells. After 48 h of direct

stimulation, we were not able to show a direct effect of sCD83 on T cells (**Figure 4B**).

DISCUSSION

The fact that the immune system can mount a response to pathogen threats without compromising the health of a semi-allogeneic fetus has been a matter of major interest among reproductive biologists. We wondered if CD83 expression was one of the regulators of immune responses that accompany normal pregnancy.

In the case of B lymphocytes, CD83 overexpression induces an anti-inflammatory phenotype, by reduction of Ig secretion and induction of IL-10 secretion (66). Our data show an upregulation of membrane-bound CD83 on splenic and paraaortic lymph nodes B cells, which correlates with the increase of IL-10 producing B cells observed during healthy murine (12) and human pregnancies (8). We also found that B cells are especially sensitive at the end of pregnancy to inflammation triggers (PMA, LPS) in terms of CD83 upregulation and sCD83 secretion. Our data suggest that CD83 expression may be a key regulator which shapes B-cell function into a beneficial phenotype for late-pregnancy events, such as inflammation-triggered preterm birth (10).

We also found that B cells were the main source of the basal sCD83 release in the supernatant of cultured splenocytes. However, after stimulation, $CD4^+$ T cell depletion has a detrimental effect as well, indicating that T cells may cooperate in B-cell activation for subsequent sCD83 release. Kretschmer et al. previously showed that activated T cells support the CD83 upregulation on B cells via CD40-CD40L interactions (67).

Studies addressing CD83 expression on T cells have led to controversial results, which vary from effects on the stimulation of naïve and memory T cells (68), on the production of proinflammatory cytokines (38), or even correlating with immunosuppressive functions (39, 69). In our experiment, we showed that CD83 is upregulated on $CD4^+$ T cells during pregnancy and the proportion of $CD4^+CD25^+$ T cells that are positive for CD83 increases throughout the stages of pregnancy. CD83 expression particularly on $CD4^+CD25^+$ T cells is linked to immunosuppressive phenotype (39, 40). Additionally, our results are in agreement with the well-studied pregnancy-supporting role of regulatory T cells (70, 71).

Many of the changes we observed concerning CD83 expression on lymphocytes were significant from mid-pregnancy, when estradiol and progesterone are increasing in mice as well as in human pregnancies (65, 72). B as well as T lymphocytes express estrogen and progesterone receptors (73–76). Regulatory T cells, as well as other immune cells, are regulated by sex hormones during pregnancy (77, 78). In our experiments, we showed that progesterone had a dose-dependent effect on CD83 expression on $CD4^+$ T cells. We also observed progesterone-specific effects on CD83 expression on B Cells, but only by reaching high concentrations of the hormone in cell culture. When we used similar concentrations to serum levels observed in murine pregnancies, we were not able to show any significant differences in the CD83 expression on lymphocytes. We cannot exclude that progesterone may be involved in the changes observed on B cell CD83 expression during the course of pregnancy, since a relatively short stimulation was performed. On the other hand, our results support anti-inflammatory therapeutic use of progesterone that is based on high doses of the hormone to prevent preterm labor (79).

Having demonstrated the higher capacity of B lymphocytes from pregnant mice to release sCD83, we decided to test a direct effect of sCD83 on $CD4^+$ T cells *in vitro*. The mechanism of action of sCD83 has not been unveiled yet, but it has been proposed that may act through blocking of the natural ligand of CD83 (48). In our experiment involving the supernatant of stimulated lymphocytes, the blocking antibody that remained in the culture media may be altering the CD83/CD83-ligand interaction, leading to an apparent less T cell activation. In our attempt to block sCD83 to evidence more $CD4^+$ activation, we blocked membrane CD83 on effector $CD4^+$ T cells instead, leading to the opposite results.

Subsequently, we tested the direct effect of recombinant sCD83 on a $CD4^+$ T cell culture. We failed to show any significant change in $CD4^+$ T cell function. Previous reports showed that sCD83 influences T cell activity via induction of tolerogenic DCs (47, 60, 80). The higher expression of sCD83 we observed in lymphocytes from pregnant mice may have a favorable effect on tolerogenic DCs to coordinate innate as well as adaptive mechanisms to support implantation and progression of pregnancy (81).

A deeper understanding of the physiological functions of sCD83 during pregnancy might support the development of therapeutic tools for the treatment of pregnancy- and inflammation-related disorders.

ETHICS STATEMENT

Animal experiments were carried out according to institutional guidelines approved by the Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern (LALLF-MV; 7221.3-1-068/13 to DM). The experiments were conducted in conformity with the European Communities Council Directive 86/609/EEC.

AUTHOR CONTRIBUTIONS

KP performed experiments, analyzed data, and contributed to the elaboration of the manuscript. JE and DK performed experiments. GR-S designed and performed experiments and contributed to the writing of the manuscript. MZ contributed with reagents, the design of experiments, and the writing of the manuscript. DM conceived and designed the experiments, analyzed data, contributed with reagents, wrote the paper, and supervised the work.

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REFERENCES

1. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* (1993) 14:353–6. doi:10.1016/0167-5699(93)90235-D
2. Ruocco MG, Chaouat G, Florez L, Bensussan A, Klatzmann D. Regulatory T-cells in pregnancy: historical perspective, state of the art, and burning questions. *Front Immunol* (2014) 5:389. doi:10.3389/fimmu.2014.00389
3. Muzzio D, Zygmunt M, Jensen F. The role of pregnancy-associated hormones in the development and function of regulatory B cells. *Front Endocrinol* (2014) 5:39. doi:10.3389/fendo.2014.00039
4. Chaouat G. Reconsidering the Medawar paradigm placental viviparity existed for eons, even in vertebrates; without a "problem": why are Tregs important for preeclampsia in great apes? *J Reprod Immunol* (2015) 114:48–57. doi:10.1016/j.jri.2015.09.002
5. Candando KM, Lykken JM, Tedder TF. B10 cell regulation of health and disease. *Immunol Rev* (2014) 259:259–72. doi:10.1111/imr.12176
6. Piccirillo CA. Regulatory T cells in health and disease. *Cytokine* (2008) 43:395–401. doi:10.1016/j.cyto.2008.07.469
7. Craenmehr MHC, Heidt S, Eikmans M, Claas FHJ. What is wrong with the regulatory T cells and foetomaternal tolerance in women with recurrent miscarriages? *HLA* (2016) 87:69–78. doi:10.1111/tan.12737
8. Rolle L, Memarzadeh Tehran M, Morell-García A, Raeva Y, Schumacher A, Hartig R, et al. Cutting Edge: IL-10-producing regulatory B cells in early human pregnancy. *Am J Reprod Immunol* (2013) 70:448–53. doi:10.1111/aji.12157
9. Sasaki Y, Darmochwal-Kolarz D, Suzuki D, Sakai M, Ito M, Shima T, et al. Proportion of peripheral blood and decidual CD4(+) CD25(bright) regulatory T cells in pre-eclampsia. *Clin Exp Immunol* (2007) 149:139–45. doi:10.1111/j.1365-2249.2007.03397.x
10. Gomez-Lopez N, StLouis D, Lehr MA, Sanchez-Rodriguez EN, Arenas-Hernandez M. Immune cells in term and preterm labor. *Cell Mol Immunol* (2014) 11:571–81. doi:10.1038/cmi.2014.46
11. La Rocca C, Carbone F, Longobardi S, Matarese G. The immunology of pregnancy: regulatory T cells control maternal immune tolerance toward the fetus. *Immunol Lett* (2014) 162:41–8. doi:10.1016/j.imlet.2014.06.013
12. Jensen F, Muzzio D, Soldati R, Fest S, Zenclussen AC. Regulatory B10 cells restore pregnancy tolerance in a mouse model. *Biol Reprod* (2013) 89:90. doi:10.1095/biolreprod.113.110791
13. Zenclussen AC, Gerlof K, Zenclussen ML, Sollwedel A, Bertoja AZ, Ritter T, et al. Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. *Am J Pathol* (2005) 166:811–22. doi:10.1016/S0002-9440(10)62302-4
14. Shim S-S, Romero R, Hong J-S, Park C-W, Jun JK, Kim BL, et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. *Am J Obstet Gynecol* (2004) 191:1339–45. doi:10.1016/j.ajog.2004.06.085
15. Christiaens I, Zaragoza DB, Guilbert L, Robertson SA, Mitchell BF, Olson DM. Inflammatory processes in preterm and term parturition. *J Reprod Immunol* (2008) 79:50–7. doi:10.1016/j.jri.2008.04.002
16. Lee SE, Romero R, Jung H, Park CW, Park JS, Yoon BH. The intensity of the fetal inflammatory response in intraamniotic inflammation with and without microbial invasion of the amniotic cavity. *Am J Obstet Gynecol* (2007) 197:294.e1–294.e6. doi:10.1016/j.ajog.2007.07.006
17. Keelan JA. Pharmacological inhibition of inflammatory pathways for the prevention of preterm birth. *J Reprod Immunol* (2011) 88:176–84. doi:10.1016/j.jri.2010.11.003
18. MacIntyre DA, Sykes L, Teoh TG, Bennett PR. Prevention of preterm labour via the modulation of inflammatory pathways. *J Matern Fetal Neonatal Med* (2012) 25(Suppl 1):17–20. doi:10.3109/14767058.2012.666114
19. Sykes L, MacIntyre DA, Teoh TG, Bennett PR. Anti-inflammatory prostaglandins for the prevention of preterm labour. *Reproduction* (2014) 148:R29–40. doi:10.1530/REP-13-0587
20. Klinker MW, Lundy SK. Multiple mechanisms of immune suppression by B lymphocytes. *Mol Med* (2012) 18:123–37. doi:10.2119/molmed.2011.00333
21. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* (2010) 236:219–42. doi:10.1111/j.1600-065X.2010.00923.x
22. Sakaguchi S, Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T. Regulatory T cells: how do they suppress immune responses? *Int Immunol* (2009) 21:1105–11. doi:10.1093/intimm/dxp095
23. Sojka DK, Huang Y-H, Fowell DJ. Mechanisms of regulatory T-cell suppression – a diverse arsenal for a moving target. *Immunology* (2008) 124:13–22. doi:10.1111/j.1365-2567.2008.02813.x
24. Zhou LJ, Tedder TF. Human blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily. *J Immunol* (1995) 154:3821–35.
25. Zhou LJ, Tedder TF. CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. *Proc Natl Acad Sci U S A* (1996) 93:2588–92. doi:10.1073/pnas.93.6.2588
26. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol* (2002) 2:116–26. doi:10.1038/nri727
27. Fujimoto Y, Tu L, Miller AS, Bock C, Fujimoto M, Doyle C, et al. CD83 expression influences CD4+ T cell development in the thymus. *Cell* (2002) 108:755–67. doi:10.1016/S0092-8674(02)00673-6
28. Wolenski M, Cramer SO, Ehrlich S, Steeg C, Grossschupff G, Tenner-Racz K, et al. Expression of CD83 in the murine immune system. *Med Microbiol Immunol* (2003) 192:189–92. doi:10.1007/s00430-003-0179-9
29. García-Martínez LF, Appleby MW, Staehling-Hampton K, Andrews DM, Chen Y, McEuen M, et al. A novel mutation in CD83 results in the development of a unique population of CD4+ T cells. *J Immunol* (2004) 173:2995–3001. doi:10.4049/jimmunol.173.5.2995
30. Wilson J, Cullup H, Lourie R, Sheng Y, Palkova A, Radford KJ, et al. Antibody to the dendritic cell surface activation antigen CD83 prevents acute graft-versus-host disease. *J Exp Med* (2009) 206:387–98. doi:10.1084/jem.20070723
31. Seldon TA, Pryor R, Palkova A, Jones ML, Verma ND, Findova M, et al. Immunosuppressive human anti-CD83 monoclonal antibody depletion of activated dendritic cells in transplantation. *Leukemia* (2016) 30:692–700. doi:10.1038/leu.2015.231
32. Liu H, Jain R, Guan J, Vuong V, Ishido S, La Gruta NL, et al. Ubiquitin ligase MARCH 8 cooperates with CD83 to control surface MHC II expression in thymic epithelium and CD4 T cell selection. *J Exp Med* (2016) 213(9):1695–703. doi:10.1084/jem.20160312
33. Bannard O, McGowan SJ, Ersching J, Ishido S, Victora GD, Shin J-S, et al. Ubiquitin-mediated fluctuations in MHC class II facilitate efficient germinal center B cell responses. *J Exp Med* (2016) 213(6):993–1009. doi:10.1084/jem.20151682
34. Bates JM, Flanagan K, Mo L, Ota N, Ding J, Ho S, et al. Dendritic cell CD83 homotypic interactions regulate inflammation and promote mucosal homeostasis. *Mucosal Immunol* (2015) 8:414–28. doi:10.1038/mi.2014.79
35. Wolenski M, Cramer SO, Ehrlich S, Steeg C, Fleischer B, Von Bonin A. Enhanced activation of CD83-positive T cells. *Scand J Immunol* (2003) 58:306–11. doi:10.1046/j.1365-3083.2003.01303.x
36. Cramer SO, Trumpfheller C, Mehlhoop U, Moré S, Fleischer B, von Bonin A. Activation-induced expression of murine CD83 on T cells and identification of a specific CD83 ligand on murine B cells. *Int Immunol* (2000) 12:1347–51. doi:10.1093/intimm/12.9.1347
37. McKinsey TA, Chu Z, Tedder TF, Ballard DW. Transcription factor NF-kappaB regulates inducible CD83 gene expression in activated T lymphocytes. *Mol Immunol* (2001) 37:783–8. doi:10.1016/S0161-5890(00)00099-7
38. Su LL, Iwai H, Lin JT, Fathman CG. The transmembrane E3 ligase GRAIL ubiquitinates and degrades CD83 on CD4 T cells. *J Immunol* (2009) 183:438–44. doi:10.4049/jimmunol.0900204
39. Reinwald S, Wiethe C, Westendorf AM, Breloer M, Probst-Kepper M, Fleischer B, et al. CD83 expression in CD4+ T cells modulates inflammation and autoimmunity. *J Immunol* (2008) 180:5890–7. doi:10.4049/jimmunol.180.9.5890
40. Kreiser S, Eckhardt J, Kuhnt C, Stein M, Krzyzak L, Seitz C, et al. Murine CD83-positive T cells mediate suppressor functions in vitro and in vivo. *Immunobiology* (2015) 220:270–9. doi:10.1016/j.imbio.2014.08.005
41. Breloer M, Kretschmer B, Lüthje K, Ehrlich S, Ritter U, Bickert T, et al. CD83 is a regulator of murine B cell function in vivo. *Eur J Immunol* (2007) 37:634–48. doi:10.1002/eji.200636852

42. Uhde M, Kuehl S, Richardt U, Fleischer B, Osterloh A. Differential regulation of marginal zone and follicular B cell responses by CD83. *Int Immunol* (2013) 25:507–20. doi:10.1093/intimm/dxt021
43. Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. *Nat Rev Immunol* (2013) 13:118–32. doi:10.1038/nri3383
44. Lüthje K, Kretschmer B, Fleischer B, Breloer M. CD83 regulates splenic B cell maturation and peripheral B cell homeostasis. *Int Immunol* (2008) 20:949–60. doi:10.1093/intimm/dxn054
45. Muzzio DO, Soldati R, Ehrhardt J, Utpatel K, Evert M, Zenclussen AC, et al. Cell development undergoes profound modifications and adaptations during pregnancy in mice. *Biol Reprod* (2014) 91:115–115. doi:10.1095/biolreprod.114.122366
46. Muzzio DO, Ziegler KB, Ehrhardt J, Zygmunt M, Jensen F. Marginal zone B cells emerge as a critical component of pregnancy well-being. *Reproduction* (2016) 151:29–37. doi:10.1530/REP-15-0274
47. Bock F, Rössner S, Onderka J, Lechmann M, Pallotta MT, Fallarino F, et al. Topical application of soluble CD83 induces IDO-mediated immune modulation, increases Foxp3+ T cells, and prolongs allogeneic corneal graft survival. *J Immunol* (2013) 191:1965–75. doi:10.4049/jimmunol.1201531
48. Xu J-F, Huang B-J, Yin H, Xiong P, Feng W, Xu Y, et al. A limited course of soluble CD83 delays acute cellular rejection of MHC-mismatched mouse skin allografts. *Transpl Int* (2007) 20:266–76. doi:10.1111/j.1432-2277.2006.00426.x
49. Zinser E, Lechmann M, Golka A, Lutz MB, Steinkasserer A. Prevention and treatment of experimental autoimmune encephalomyelitis by soluble CD83. *J Exp Med* (2004) 200:345–51. doi:10.1084/jem.20030973
50. Eckhardt J, Kreiser S, Döbbeler M, Nicolette C, DeBenedette MA, Tcherepanova IY, et al. Soluble CD83 ameliorates experimental colitis in mice. *Mucosal Immunol* (2014) 7:1006–18. doi:10.1038/mi.2013.119
51. Lan Z, Ge W, Arp J, Jiang J, Liu W, Gordon D, et al. Induction of kidney allograft tolerance by soluble CD83 associated with prevalence of tolerogenic dendritic cells and indoleamine 2,3-dioxygenase. *Transplantation* (2010) 90:1286–93. doi:10.1097/TP.0b013e3182007bbf
52. Starke C, Steinkasserer A, Voll RE, Zinser E. Soluble human CD83 ameliorates lupus in NZB/W F1 mice. *Immunobiology* (2013) 218:1411–5. doi:10.1016/j.imbio.2013.06.002
53. Ge W, Arp J, Lian D, Liu W, Baroja ML, Jiang J, et al. Immunosuppression involving soluble CD83 induces tolerogenic dendritic cells that prevent cardiac allograft rejection. *Transplantation* (2010) 90:1145–56. doi:10.1097/TP.0b013e3181f95718
54. Hock BD. A soluble form of CD83 is released from activated dendritic cells and B lymphocytes, and is detectable in normal human sera. *Int Immunol* (2001) 13:959–67. doi:10.1093/intimm/13.7.959
55. Hock BD, Haring LF, Steinkasserer A, Taylor KG, Patton WN, McKenzie JL. The soluble form of CD83 is present at elevated levels in a number of hematological malignancies. *Leuk Res* (2004) 28:237–41. doi:10.1016/S0145-2126(03)00255-8
56. Sénéchal B, Boruchov AM, Reagan JL, Hart DNJ, Young JW. Infection of mature monocyte-derived dendritic cells with human cytomegalovirus inhibits stimulation of T-cell proliferation via the release of soluble CD83. *Blood* (2004) 103:4207–15. doi:10.1182/blood-2003-12-4350
57. Dudziak D, Nimmerjahn F, Bornkamm GW, Laux G. Alternative splicing generates putative soluble CD83 proteins that inhibit T cell proliferation. *J Immunol* (2005) 174:6672–6. doi:10.4049/jimmunol.174.11.6672
58. Doster A, Schwarzig U, Zygmunt M, Rom J, Schütz F, Fluhr H. Unfractionated heparin selectively modulates the expression of CXCL8, CCL2 and CCL5 in endometrial carcinoma cells. *Anticancer Res* (2016) 36:1535–44.
59. Roman-Sosa G, Brocchi E, Schirrmeier H, Wernike K, Schelp C, Beer M. Analysis of the humoral immune response against the envelope glycoprotein Gc of Schmallenberg virus reveals a domain located at the amino terminus targeted by mAbs with neutralizing activity. *J Gen Virol* (2016) 97:571–80. doi:10.1099/jgv.0.000377
60. Lechmann M, Krooshoop DJEB, Dudziak D, Kremmer E, Kuhnt C, Figdor CG, et al. The extracellular domain of CD83 inhibits dendritic cell-mediated T cell stimulation and binds to a ligand on dendritic cells. *J Exp Med* (2001) 194:1813–21. doi:10.1084/jem.194.12.1813
61. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (1970) 227:680–5. doi:10.1038/227680a0
62. Beagley KW, Gockel CM. Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. *FEMS Immunol Med Microbiol* (2003) 38:13–22. doi:10.1016/S0928-8244(03)00202-5
63. Barkley MS, Geschwind II, Bradford GE. The gestational pattern of estradiol, testosterone and progesterone secretion in selected strains of mice. *Biol Reprod* (1979) 20:733–8. doi:10.1095/biolreprod20.4.733
64. Spencer TE, Bazer FW. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Front Biosci* (2002) 7:d1879–98. doi:10.2741/spencer
65. Chung E, Yeung F, Leinwand LA. Akt and MAPK signaling mediate pregnancy-induced cardiac adaptation. *J Appl Physiol* (2012) 112:1564–75. doi:10.1152/japplphysiol.00027.2012
66. Kretschmer B, Lüthje K, Guse AH, Ehrlich S, Koch-Nolte F, Haag F, et al. CD83 modulates B cell function in vitro: Increased IL-10 and reduced Ig secretion by CD83Tg B cells. *PLoS One* (2007) 2:e755. doi:10.1371/journal.pone.0000755
67. Kretschmer B, Kühl S, Fleischer B, Breloer M. Activated T cells induce rapid CD83 expression on B cells by engagement of CD40. *Immunol Lett* (2011) 136:221–7. doi:10.1016/j.imlet.2011.01.013
68. Aerts-Toegaert C, Heirman C, Tuyaerts S, Corthals J, Aerts JL, Bonehill A, et al. CD83 expression on dendritic cells and T cells: correlation with effective immune responses. *Eur J Immunol* (2007) 37:686–95. doi:10.1002/eji.200636535
69. Chen L, Guan S, Zhou Q, Sheng S, Zhong F, Wang Q. Continuous expression of CD83 on activated human $CD4^+$ T cells is correlated with their differentiation into induced regulatory T cells. *Mol Med Rep* (2015) 12:3309–14. doi:10.3892/mmr.2015.3796
70. Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT. Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. *Immunology* (2004) 112:38–43. doi:10.1111/j.1365-2567.2004.01869.x
71. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* (2004) 5:266–71. doi:10.1038/ni1037
72. Kumar P, Magon N. Hormones in pregnancy. *Niger Med J* (2012) 53:179–83. doi:10.4103/0300-1652.107549
73. Cunningham M, Gilkeson G. Estrogen receptors in immunity and autoimmunity. *Clin Rev Allergy Immunol* (2011) 40:66–73. doi:10.1007/s12016-010-8203-5
74. Dressing GE, Goldberg JE, Charles NJ, Schwertfeger KL, Lange CA. Membrane progesterone receptor expression in mammalian tissues: a review of regulation and physiological implications. *Steroids* (2011) 76:11–7. doi:10.1016/j.steroids.2010.09.006
75. Zhang L, Chang K-K, Li M-Q, Li D-J, Yao X-Y. Mouse endometrial stromal cells and progesterone inhibit the activation and regulate the differentiation and antibody secretion of mouse B cells. *Int J Clin Exp Pathol* (2014) 7:123–33.
76. Bommer I, Muzzio DO, Zygmunt M, Jensen F. Progesterone and estradiol exert an inhibitory effect on the production of anti-inflammatory cytokine IL-10 by activated MZ B cells. *J Reprod Immunol* (2016) 116:113–6. doi:10.1016/j.jri.2016.05.008
77. Mjösberg J, Svensson J, Johansson E, Hellström L, Casas R, Jenmalm MC, et al. Systemic reduction of functionally suppressive CD4dimCD25highFoxp3+ Tregs in human second trimester pregnancy is induced by progesterone and 17beta-estradiol. *J Immunol* (2009) 183:759–69. doi:10.4049/jimmunol.0803654
78. Polanczyk MJ, Hopke C, Vandenbark AA, Offner H. Estrogen-mediated immunomodulation involves reduced activation of effector T cells, potentiation of Treg cells, and enhanced expression of the PD-1 costimulatory pathway. *J Neurosci Res* (2006) 84:370–8. doi:10.1002/jnr.20881
79. Dodd J, Crowther CA. The role of progesterone in prevention of preterm birth. *Int J Womens Health* (2009) 1:73. doi:10.2147/IJWH.S4730
80. Prechtel AT, Turza NM, Theodoridis AA, Steinkasserer A. CD83 knockdown in monocyte-derived dendritic cells by small interfering RNA leads to a diminished T cell stimulation. *J Immunol* (2007) 178:5454–64. doi:10.4049/jimmunol.178.9.5454

81. Blois SM, Kammerer U, Soto CA, Tometten MC, Shaikly V, Barrientos G, et al. Dendritic cells: key to fetal tolerance? *Biol Reprod* (2007) 77:590–8. doi:10.1095/biolreprod.107.060632

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8.2 CD83 is locally regulated and differentially expressed in disturbed murine pregnancy.

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Reproduction (2019)

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CD83 is locally regulated and differentially expressed in disturbed murine pregnancy

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Abstract

Alterations in the immunologic balance during pregnancy have been associated with poor pregnancy outcomes. The underlying mechanisms are complex and mouse models delivered valuable information on inflammatory imbalance in disturbed pregnancies and served as model to test potential anti-inflammatory therapies. CD83 is a transmembrane protein (mCD83) with a soluble form (sCD83) which possesses strong anti-inflammatory properties. During murine pregnancy, upregulated mCD83 expression induces sCD83 release after *in vitro* stimulation with LPS, phorbol myristate acetate (PMA) and ionomycin. The release mechanism of sCD83 and its control are yet to be elucidated. In this study, the expression of mCD83 and sCD83 has been extensively studied in the CBA/J × DBA/2J mouse model of pro-inflammatory-mediated pregnancy disturbances. mCD83 was higher expressed on splenic B cells, uterus-draining lymph nodes T cells and dendritic cells from mice with poor pregnancy outcome (PPOM) compared to mice with good pregnancy outcome (GPOM). PPOM, however, was accompanied by lower sCD83 serum levels. *In vitro* treatment of splenic B cells with progesterone led to a reduction of TIMP1 expression, mCD83 expression and sCD83 release, while TIMP1 treatment had a positive effect on sCD83 availability. These results suggest that tissue and matrix components are involved in the regulation of CD83 in murine pro-inflammatory pregnancies.

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Introduction

During pregnancy, the maternal immune system is confronted by the presence of paternal antigens expressed by the semi-allogeneic fetus. In order to avoid fetal rejection, the maternal immune system undergoes several adaptations. Maternal tolerance is orchestrated by a number of different mechanisms regulated by pregnancy hormones and trophoblast to shift decidual immune cells to their regulatory pendants, affecting systemic immune responses as well. Consequently, pregnant women, although not considered immunosuppressed, respond differently for example to viral infections like influenza or auto-antigens compared to non-pregnant women (Piccinni *et al.* 2016, Racicot & Mor 2017).

One major challenge of the immune system during pregnancy is to provide protection against infections despite the tolerogenic shift that immune cells undergo at the fetomaternal interface. This phenomenon is achieved by the interplay of both leukocytes and soluble factors that contribute to finely balance of both immune aspects. An imbalance of pro- and anti-inflammatory components represents a risk that threatens different aspects of pregnancy health. As a result, pregnancy complications, like preterm birth, preeclampsia, intrauterine growth restriction (IUGR) and recurrent pregnancy loss arise (Raghupathy *et al.* 2012, Kuon *et al.* 2015, Harmon *et al.* 2016).

Mouse models contributed to an improved understanding of the ongoing processes during pregnancy. Thereby, it was shown that a general pro-inflammatory immune reaction, but not necessarily against paternal antigens, contributes to fetal resorption (Robertson *et al.* 2007). Moreover, new therapy approaches were first tested in mouse models, for example the adoptive transfer of leukocytes (DCs, B10 cells, Tregs) and soluble factors (IH-Ig, IL10, TGFB) which contribute to the essential immune balance and decreased fetal resorption (Clark 2016).

Recently, we suggested that the soluble form of the transmembrane molecule CD83 (sCD83) could represent an important factor contributing to pregnancy maintenance (Packhäuser *et al.* 2017). Anti-inflammatory properties of sCD83 have been extensively examined in mouse models of autoimmunity, allograft transplantation and inflammatory disease (Lan *et al.* 2010, Bock *et al.* 2013, Starke *et al.* 2013, Eckhardt *et al.* 2014, Lin *et al.* 2018). However, it remains unclear if sCD83 is generated by alternative splicing or from shedding of the transmembrane form (mCD83) by proteases, as in the case of other immune-related factors (TGFA, TNFA, TACI, etc.) (Murphy 2008, Hoffmann *et al.* 2015).

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Although the function of mCD83 still remains under elucidation, different roles have been proposed depending on the cell type expressing it. On dendritic cells (DCs), mCD83 marks mature cells and is necessary for a proper T cell activation and co-stimulation (Zhou & Tedder 1995, 1996, Prechtel & Steinkasserer 2007). mCD83 is also expressed by activated T cells. CD83 silencing in $CD4^+$ T cells in mice results in defective functions like antigen presentation, cytokine secretion and proliferation (Su *et al.* 2009). However, the overexpression of mCD83 in naive murine $CD4^+$ T cells results in FOXP3 upregulation characteristic for regulatory T cells (Reinwald *et al.* 2008).

mCD83 expression on B cells influences their maturation in the spleen, impacting on the marginal zone (MZ)/follicular zone (FO) B cell relative numbers (Luthje *et al.* 2008, Krzyzak *et al.* 2016). Furthermore, mCD83 expression on B cells is associated to B cell anti-inflammatory responses, including higher IL10 production (Kretschmer *et al.* 2007). Both, MZ/FO ratio and B cell-derived IL10 are increased in murine pregnancies and altered in pathological mouse models of pregnancy (Jensen *et al.* 2013, Muzzio *et al.* 2014*a*, 2016).

B cells upregulate mCD83 expression after stimulation with stimulatory agents such as lipopolysaccharide (LPS) and PMA. Additionally, direct cell contact plays an important role in the induction of mCD83 expression, as in the case of CD40/CD40L interactions between B cells and $CD4^+$ T cells (Kretschmer *et al.* 2011). In our previous work, we showed that splenic B cells from advanced pregnancies were the most reactive to LPS stimulation *in vitro*. *In vivo*, however, the highest mCD83 expression on splenic B cells during gestation was observed at mid-pregnancy. We postulate that the compartmentation of the B cells plays an important role regulating CD83 availability during pregnancy. To test our hypothesis, we used a mouse model of systemic inflammation-induced pregnancy disturbance.

On the one hand, we show that the pregnancy-related hormone progesterone controls matrix components that influence CD83 availability during pregnancy. On the other hand, during inflammatory pregnancies, splenic but not peripheral B cells upregulate CD83 expression. Moreover, despite the higher mCD83 on splenic B cells, lower sCD83 is detected in the sera of affected mice. Our data suggest that tissue-specific components influence sCD83 availability during normal and impaired pregnancy.

Material and methods

Animals

CBA/J ($H2^k$) female and DBA/J ($H2^d$) male mice were purchased from Charles River or Janvier Labs (Saint-Berthevin Cedex, France). BALB/c ($H2^d$) males were bred in our Central Service and Research Facility for Animals (ZSFV). The animals were kept co-housed in a 12-h light/dark cycle with free access to food and water. Eight- to twelve-week-old CBA/J female mice were either paired with BALB/c males, generating normal pregnancies ('good pregnancy outcome mice' (GPOM)) or with DBA/2J males to obtain immune-induced pathological pregnancies ('poor pregnancy outcome mice' (PPOM)) (Clark *et al.* 1986, Muzzio *et al.* 2014*b*, 2016). In this mouse model, fetal resorptions are consequence of an immune rejection against paternal antigens (Clark *et al.* 1986), associated to a failure in regulatory T cell (Treg) generation to compensate the pro-inflammatory cytokine boost driven by activated leukocytes (Clark *et al.* 2004). Mice were checked for vaginal plug every morning. Observation of a plug was declared as day 0 of pregnancy, and the female was separated from the male. Mice were killed at day 14 post plug (14 dpp); spleen, thymus, paraaortic and inguinal lymph nodes and blood were collected. Non-pregnant CBA/J female mice were randomly killed and used for *in vitro* experiments. Animal experiments were carried out according to institutional guidelines approved by the Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern (LALLF-MV; 7221.3-1-068/13 to DM). The experiments were conducted in conformity with the European Communities Council Directive 86/609/EEC.

Cell preparation

Single-cell suspensions from para-aortic lymph nodes, inguinal lymph nodes, and thymus were obtained as previously described (Packhäuser *et al.* 2017). The tissue was carefully pressed through a 40-µm nylon cell strainer and washed with PBS. In case of spleen tissue, an erythrocyte lysis with 10 mL Lysis Buffer (0.89% NH_4Cl, 0.1% $KHCO_3$, 0.003% EDTA) was performed and stopped after 5 min with 3 mL FBS. After washing, the cell suspension was filtered a second time with a 40-µm cell strainer. The cell counts of the suspensions were determined using a Neubauer chamber.

Flow cytometry

Flow cytometry was applied to evaluate the expression of CD83 on B, T, or DCs from spleen, thymus, and lymph nodes as described before (Packhäuser *et al.* 2017). Cell suspensions were first incubated with CD16/32 mAb Fc block (BD Pharmingen, Heidelberg, Germany) for 5 min. Staining with fluorochrome-labeled specific antibodies was performed for 30 min at 4°C in the dark. Samples from spleen were additionally stained with Fixable Viability Dye eFluor 780 (eBioscience) for 30 min and later washed with FACS buffer (1% BSA (Sigma-Aldrich), 0.1% NaN_3 (Carl Roth, Karlsruhe, Germany) in DPBS) before Fc blocking. For intracellular staining, fixation and permeabilization of the cells were accomplished with BD Perm/Wash and BD Cytofix/Cytoperm (BD Biosciences) according to manufacturer's instructions. Subsequently, cells were incubated with fluorochrome-labeled antibodies for 30 min at 4°C for intracellular staining. Following antibodies were purchased from BD Bioscience CD83 (Michel-19); B220 (RA3-6B2); CD4 (RM4-4 and RM4-5); CD23 (B3B4);

CD21 (7G6) and CD19 (1D3). Polyclonal goat IgG biotinylated anti-TIMP was purchased from R&D. DC Marker DCIR2 (33D1) and Streptavidin were purchased from eBioscience. Data were acquired on FACSCanto (BD Bioscience) and analyzed by using FlowJo software (FlowJo, LLC).

Cell stimulation

1×10^6 splenic lymphocytes or magnetic isolated splenic CD19+ B cells (CD19 MicroBeads mouse; Miltenyi Biotec GmbH) were cultured 48 h in a total volume of 500 µL RPMI 1640 culture medium supplemented with 10% FBS and antibiotics on 48-well flat-bottom suspension plates. The stimulation was performed with either 10 µg/mL LPS O111:B4 for 48 h and 50 ng/mL PMA as well as 500 ng/mL ionomycin (all from Sigma-Aldrich Chemie GmbH) for the last 5 h. In order to examine the influence of metalloproteinases on CD83 expression, cells were additionally stimulated with recombinant Mouse TIMP1 (100 ng/mL; BioLegend, San Diego, USA) or anti-TIMP1 (1 µg/mL; R&D Systems) from the beginning of the experiment for a total of 48 h. In order to examine the influence of progesterone on CD83 expression, the cells were stimulated 50 ng/mL or 500 ng/mL this pregnancy-related hormone (Sigma-Aldrich). After stimulation, cells and supernatants were separated by 5 min centrifugation at 300 ***g*** and the cells were directly used for flow cytometry or by 10 min at 8000 ***g*** to store the supernatants at −80°C and lyse cells with peqGOLD TriFast™ (VWR, Radnor PA, USA) for RNA extraction.

ELISA

The levels of sCD83 in sera and supernatants were measured using an ELISA kit for CD83 (Cloud-Clone Corp., Houston, TX, USA). The serum samples were diluted 1:2 in PBS, while the supernatants were examined without dilution. The test was performed following the instruction manual.

Real-time PCR

Magnetic isolated splenic CD19+ B cells were treated with TriFast peqGOLD (VWR, Radnor PA, USA). RNA concentration was spectrophotometrically assessed in a the NanoPhotometer PEARL (IMPLEN, Munich, Germany). RNA was reverse-transcribed applying High-Capacity cDNA Archive Kit (Applied Biosystems). For the qPCR, the samples were amplified in duplicate and non-template controls were included. Primer pairs were chosen to span an exon–exon junction to avoiding genomic DNA amplification. Real-time PCR was performed using Power SYBR® Green (AB/Life Technologies) in a 7300 Real-time PCR System (Applied Biosystems) with *Actb* as housekeeping gene. Primer sequences were the following: *Cd83* forward: TGAAGGTGACAGGATGCCC; *Cd83* reverse: CTTGGGGAGGTGACTGGAAG; *Actb* forward: TGGAATCCTGTGGCATCCATGAAAC; *Actb* reverse: TAAAACGCAGCTCAGTAACAGTCC. All primers were purchased from Invitrogen.

Statistical analysis

In vivo and *ex vivo* data comparing GPOM vs PPOM pregnancies were analyzed by unpaired Student's *t*-test. *In vitro* data comparing treatments and control were analyzed by paired Student's *t*-test or repeated-measures ANOVA with Dunnett's posttest where applicable.

Results

mCD83 is upregulated in PPOM in a cell- and compartment-dependent manner

We observed higher mCD83 expression on splenic B cells in PPOM as compared to GPOM (2.26 ± 0.27-fold increase; $P<0.001$; Fig. 1B). This was accompanied by higher percentages of mCD83+ B cells (1.74 ± 0.19 in GPOM vs 2.54 ± 0.22 in PPOM; $P<0.05$; Fig. 1C). Although an increment in the percentage of CD83+ cells could be observed within splenic DCs (8.31 ± 1.06 in GPOM vs 20.7 ± 2.62% in PPOM; $P<0.001$; Fig. 1C) and T cells (0.11 ± 0.01 in GPOM vs 0.19 ± 0.03% in PPOM; $P<0.05$; Fig. 1C), the overall mCD83 expression level was not significantly different. We also analyzed the expression of the main mature splenic B cell subsets, CD21/35^{hi}CD23^{low} marginal zone (MZ) and CD21/35^{int}CD23^{hi} follicular zone (FO) B cells. Both, FO and MZ B cells expressed significantly higher levels of mCD83 in PPOM than in GPOM mice (2.05 ± 0.24-fold increase in FO and 3.52 ± 0.75-fold increase in MZ B cells respectively; $P<0.001$; Fig. 1B). Within B cell subpopulations, mCD83+ percentages were significantly increased only in MZ B cells (1.60 ± 0.15 in GPOM vs 2.57 ± 0.21% in PPOM; $P<0.01$).

In contrast, no significant differences were found in the mCD83 expression on peripheral B cells of thymus and uterus-draining lymph nodes (Fig. 1B). In thymus, however, we observed that mCD83 was elevated in DCs as well in CD4+ T cells of PPOM mice as compared to GPOM (2.32 ± 0.18-fold increase in DC and 1.18 ± 0.02-fold increase in T cells; $P<0.001$ and $P<0.01$ respectively; Fig. 1B). The percentage of mCD83+ cells was, however, reduced in thymic CD4+ T cells (0.66 ± 0.08 in GPOM vs 0.44 ± 0.04% in PPOM; $P<0.05$; Fig. 1C). Both uterine-draining lymph nodes (inguinal (ILN) and paraaortic (PLN)) showed higher mCD83 expression on DCs in PPOM as compared to GPOM (2.07 ± 0.17 and 2.29 ± 0.23-fold increase respectively; $P<0.001$; Fig. 1B).

In summary, mCD83 was upregulated on different lymphocyte subpopulations of PPOM. There was a significant difference in the expression of mCD83 molecule in splenic B cells and dendritic cells from thymus and uterus-draining lymph nodes.

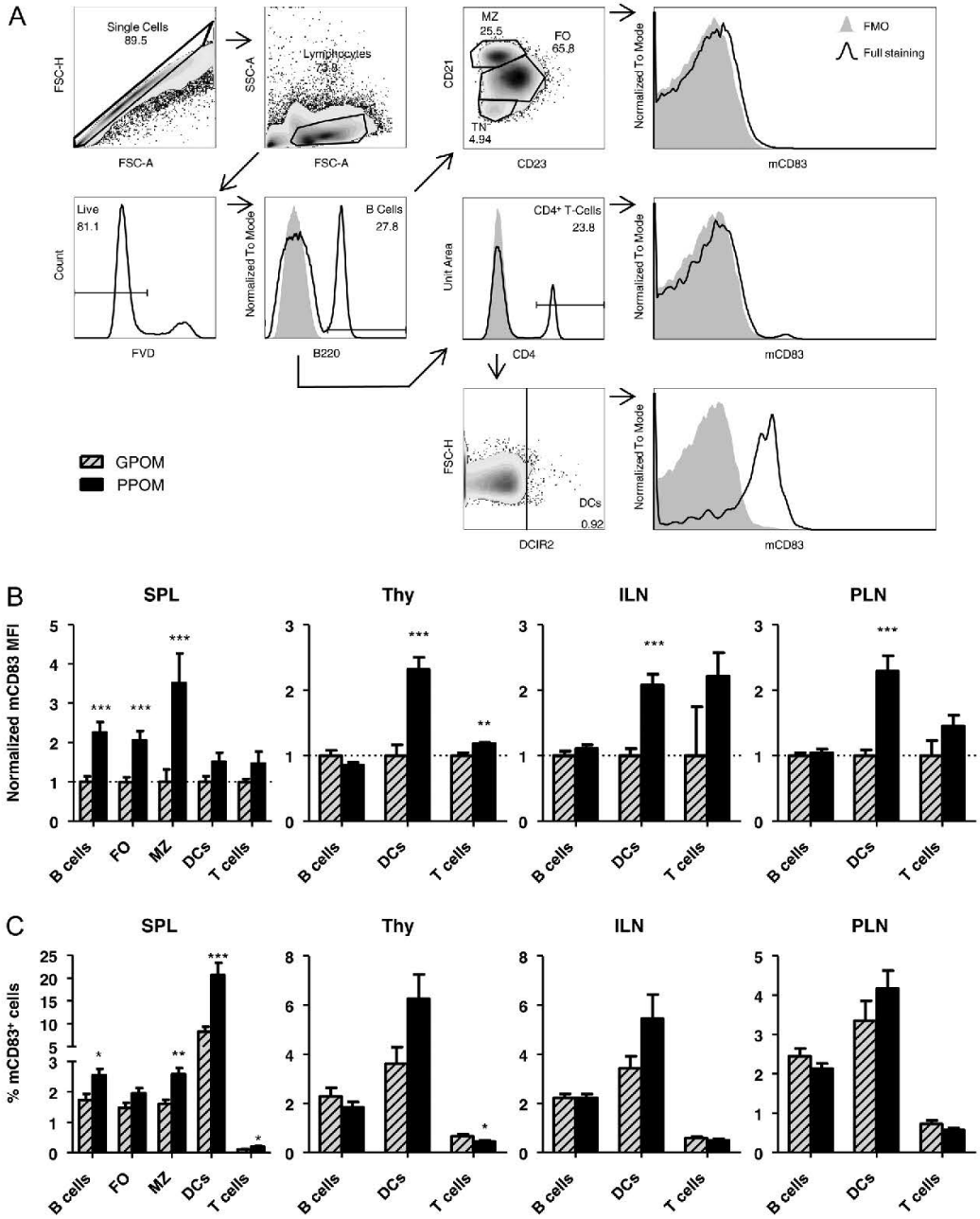

Figure 1 PPOM express higher mCD83 than GPOM in splenic but not in peripheral B cells *in vivo*. B cells (B220$^+$), T cells (CD4$^+$) and dendritic cells (DCIR2$^+$) from GPOM ($n=16$) were compared to cells from PPOM ($n=22$). (A) Representative plots show gating strategies for determination of mCD83 expression. In overlapping histograms (right), the gray area represents the unstained control and the black line the positive staining. Bars show the relative median fluorescence intensity (MFI) of mCD83 (B) or the percentage of mCD83$^+$ cells (C) in live B cells (B220$^+$), T cells (CD4$^+$) and dendritic cells (DCIR2$^+$) from spleen (SPL), thymus (THY), paraaortic (PLN) and inguinal (ILN) lymph nodes. In spleen, B220$^+$ B cells were further subdivided in CD21/35^{hi}CD23^{low} marginal zone B cells (MZ) and CD21/35^{int}CD23^{hi} follicular zone B cells (FO). All data were analyzed using Student's *t*-test ($n \geq 16$). Significant differences are indicated (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

Ex vivo *stimulation reveals no difference between PPOM and GPOM expression of mCD83 and sCD83*

Taking into account the discrepancy in terms of mCD83 expression between splenic and peripheral B cells, we moved forward to compare splenic B cell expression of mCD83 after *ex vivo* stimulation with inflammatory signals. Here, neither differences in the mCD83 expression nor in the sCD83 release could be depicted between the two groups of mice after 48-h stimulation (Fig. 2A and B).

A pregnancy-specific hormone influences sCD83 expression

In our previous work, we found splenic B cells to be a major source of sCD83 during pregnancy and we suggested that it may contribute to anti-inflammatory responses that support pregnancy maintenance (Packhäuser *et al.* 2017). In this experiment, we found that GPOM had higher serum levels of sCD83 than pro-inflammatory PPOM at day 14 of pregnancy (2308.65 ± 176.92 vs 1638.89 ± 76.3048 pg/mL respectively, Fig. 3A).

During pregnancy a variety of immune and non-immune adaptations take place regulated that is by the sexual hormones estradiol and progesterone. In our previous work, we found progesterone but not estradiol regulating mCD83 on splenic B cells, T cells and DCs.

Here, splenic lymphocytes or magnetically isolated $CD19^+$ splenic B cells from non-pregnant CBA/J female mice were stimulated with 500 ng/mL progesterone (the same order of magnitude as during murine pregnancies; Hashimoto *et al.* 2010). The treatment led to a significant reduction of sCD83 in the supernatants of the cell culture of splenocytes (374.40 ± 20.60 vs 303.69 ± 16.07 pg/mL) and B cells (590.63 ± 26.55 vs 508.95 ± 10.16 pg/mL) compared to the respective controls; $P < 0.05$; Fig. 3B.

To determine if the effect of progesterone was linked to a reduced production of CD83, we stimulated splenic lymphocytes with progesterone and/or LPS. We did not observe any significant changes in the levels of intracellular staining for CD83 (iCD83), although a significant reduction at the mRNA levels could be seen (from 1.472 ± 0.59 to 1.303 ± 0.52 after progesterone treatment; Fig. 3C).

The effect of progesterone on CD83 expression on resting lymphocytes was also assessed performing intra and extracellular staining of CD83. Progesterone treatment led to a reduction on both, mCD83 (216.75 ± 6.09 MFI for untreated controls, 199.67 ± 4.20 for 50 ng/mL progesterone and 183.84 ± 5.00 with 500 ng/mL, Fig. 3D) and iCD83 (241.8 ± 32.86 MFI for untreated controls, 229.6 ± 34.94 with 500 ng/mL progesterone, $P < 0.05$, $n = 7$, data not shown). The effect of progesterone on mCD83 was stronger than on iCD83 (0.849 ± 0.016 vs 0.937 ± 0.025). At the mRNA levels, progesterone did not have any significant effect on *Cd83* expression (Fig. 3D).

TIMP1 increases sCD83 availability ex vivo

Since the reduction of sCD83 in the supernatants of progesterone-treated B lymphocytes correlated with a reduction of mCD83 levels, we looked for mechanisms that would affect CD83 membrane turnover, decreasing mCD83 and sCD83 availability. Many membrane proteins are target of degradation by proteinases which are regulated by proteases inhibitors. The metallopeptidase inhibitor 1 (TIMP1) is expressed on B cells (Alter *et al.* 2003) and a known target of progesterone as well (Imada *et al.* 1994, Hampton *et al.* 1999). Using isolated lymphocytes, we were able to show that progesterone led to significantly decreased TIMP1 expression on splenic B cells (378.17 ± 12.97 after progesterone treatment vs

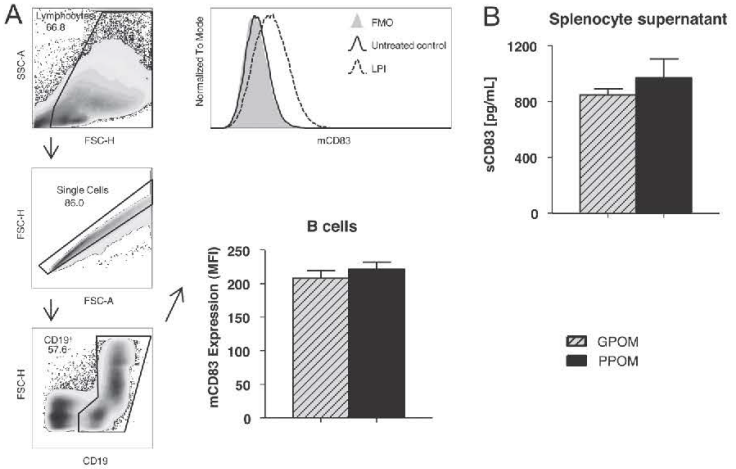

Figure 2 *Ex vivo* stimulation of splenic B cells reveals no difference in mCD83 expression between GPOM and PPOM. (A) Splenic lymphocytes were stimulated with LPS for 48 h with PMA and ionomycin for the last 5 h (LPI). Plots show a representative gating strategy for determination of mCD83 expression on $CD19^+$ B cells (left). The overlapping histograms display mCD83 expression of untreated (filled line) and LPI-stimulated B cells (dashed line). Gray area represents the FMO for CD83. Bars show the corresponding median fluorescence intensities (MFI) of mCD83 (bottom-right) on LPI-stimulated B cells. (B) Supernatants of the LPI-stimulated lymphocytes were analyzed by ELISA to determine sCD83 levels. All data were analyzed using Student's *t*-test (A: $n = 10$, B: $n = 9$).

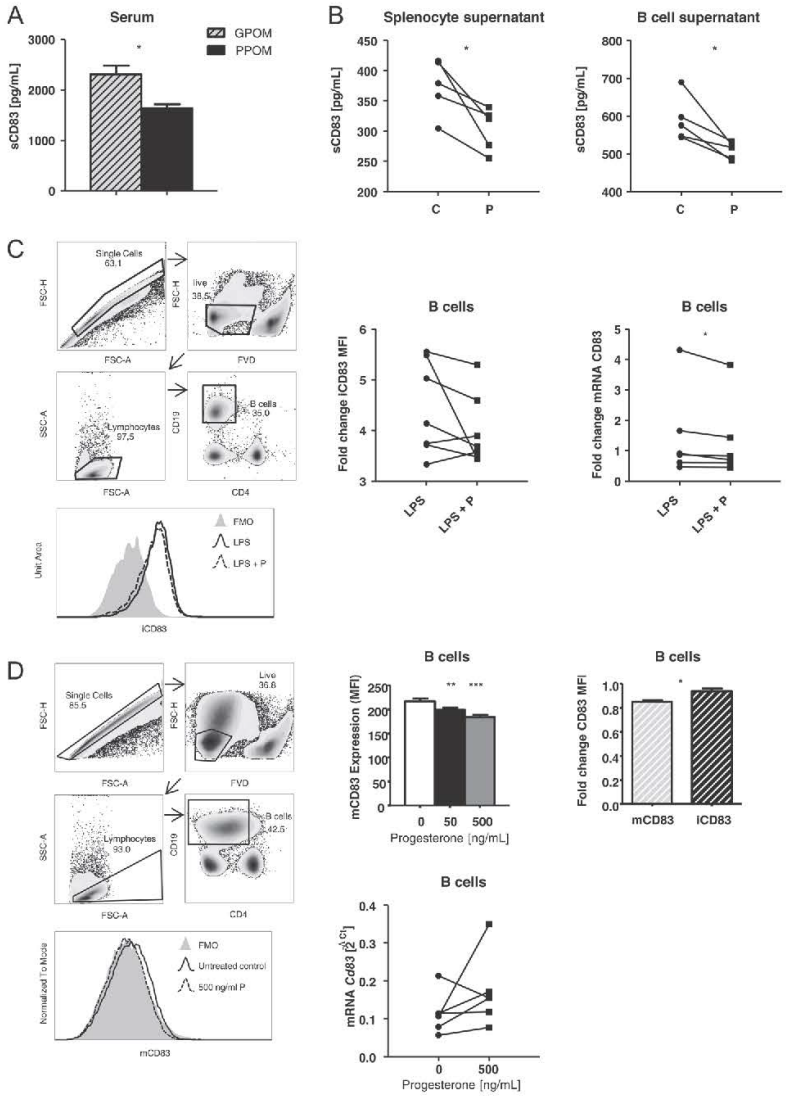

Figure 3 Progesterone reduces mCD83 and sCD83 availability *in vitro*. (A) Sera of pregnant mice were analyzed by ELISA to determine sCD83 levels. Data were analyzed using Student's *t*-test (GPOM $n=19$, PPOM $n=8$). Significant differences are indicated (*$P\leq0.05$). (B) Splenic lymphocytes or magnetically isolated CD19⁺ B cells from the spleen of non-pregnant CBA/J mice were cultured for 48 h with progesterone or with medium alone as control. Graph shows levels of sCD83 of supernatants determined by ELISA. Data were analyzed using paired *t*-test ($n=5$). Significant differences are indicated (*$P\leq0.05$). (C) Splenic lymphocytes from non-pregnant CBA/J mice were cultured for 48 h with LPS alone or with LPS and 500 ng/mL progesterone. PMA and ionomycin were added for the last 5 h. The plots show a representative gating strategy (left). Overlapping histograms (right) display differences in iCD83 between LPS (filled line) and LPS with progesterone (dashed line) stimulated CD19⁺ B cells. Gray area represents the FMO for CD83. Data represent the fold increase of iCD83 MFI (middle) or *Cd83* mRNA (right) in splenic CD19⁺ B cells over the corresponding untreated controls. Data were analyzed using paired *t*-test (flow cytometry: $n=7$; qPCR: $n=6$). (D) Splenic lymphocytes from non-pregnant CBA/J mice were cultured for 48 h with or without the presence of 50 or 500 ng/mL progesterone. The plots show a representative gating strategy (left). Overlapping histogram displays differences between with progesterone-stimulated B cells (dashed line) and untreated control (filled line). Gray area represents the FMO for CD83. Bars (top-middle) show the corresponding median fluorescence intensity (MFI) of mCD83 within CD19⁺ B cells (right-bottom). Bars (right) display the fold change of either mCD83 ($n=4$) or iCD83 ($n=7$) MFI after progesterone treatment. Graph (bottom) shows *Cd83* mRNA levels of control vs progesterone-treated B cells ($n=6$). Data from flow cytometry were analyzed by ANOVA and Dunnett's Multiple Comparison test to compare treatments against control (top-middle) or unparited *t*-test (right). Data from qPCR were analyzed by paired *t*-test ($n=6$). Significant differences are indicated (**$P\leq0.01$, ***$P\leq0.001$).

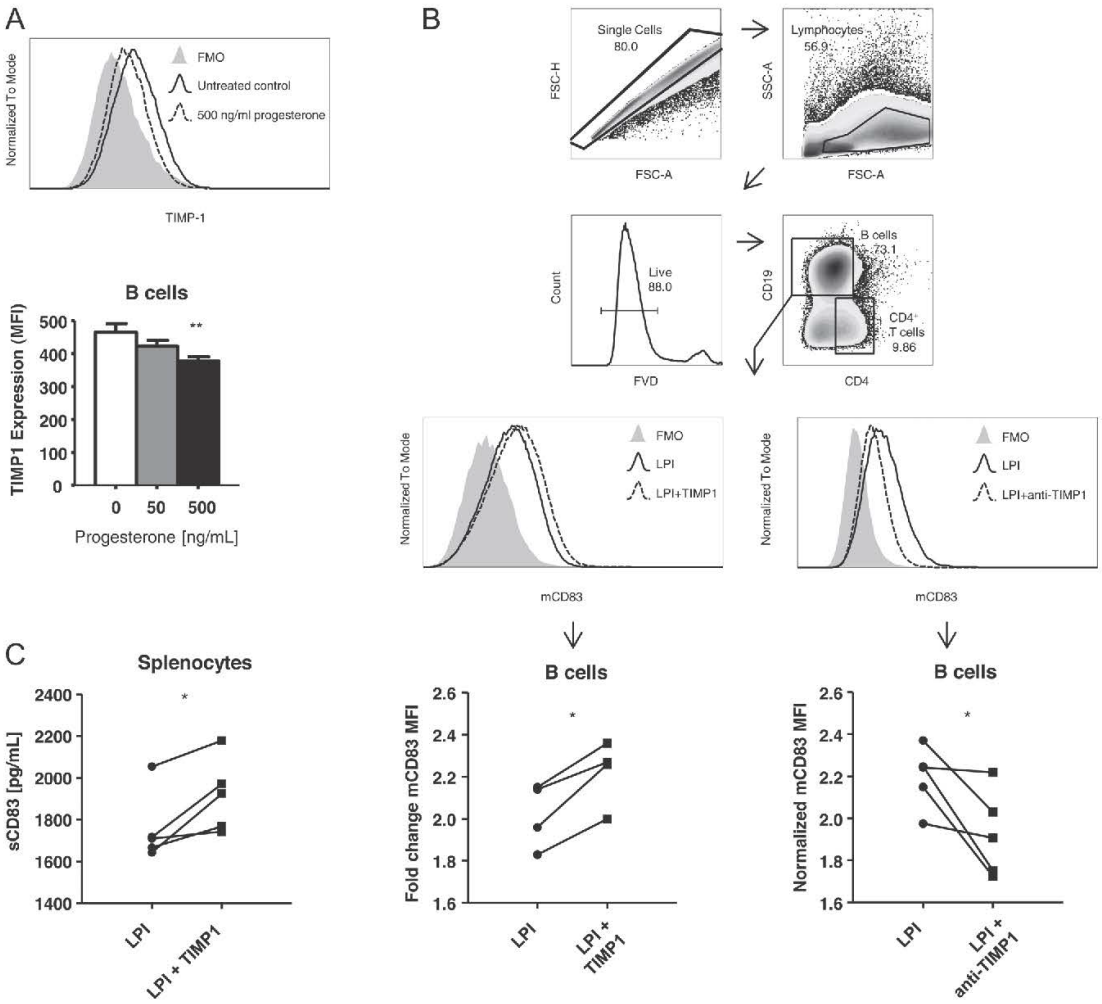

Figure 4 The metallopeptidase inhibitor TIMP1 increases mCD83 and sCD83 availability *in vitro*. (A) Splenic lymphocytes from non-pregnant CBA/J mice were cultured for 48 h with 50 or 500 ng/mL progesterone. Cells cultured in medium alone served as control. Overlapping histograms (top) display differences between progesterone-stimulated B cells (dashed line) and untreated control (filled line). Gray area represents the FMO for TIMP1. Bars show the median fluorescence intensity (MFI) of TIMP1 within CD19$^+$ B cells (bottom). Data were analyzed by ANOVA and Dunnett's multiple comparison test to compare treatments with controls ($n=4$). Significant differences are indicated (**$P \leq 0.01$). (B) Splenic lymphocytes were cultured for 48 h with LPS and for the last 5 h with PMA and ionomycin (LPI). Recombinant TIMP1 or anti-TIMP1 blocking antibody were added from the beginning of the experiment. Scatter dot plots show a representative gating strategy to CD19$^+$ B cells and CD4$^+$ T cells (top). Overlapping histograms display differences between TIMP1 (middle-left) or anti-TIMP1 (middle-right) expression on CD19$^+$ B cells (dashed lines) and the respective controls (filled line). Gray curve represents the FMO for mCD83. The bottom-left graph shows the fold increase of the corresponding MFI of mCD83 on CD19$^+$ B cells after stimulation with LPI and TIMP1 ($n=4$). The bottom-right graph represents the normalized MFI of mCD83 on CD19$^+$ B cells after stimulation with LPI and anti-TIMP1 ($n=5$). Data were analyzed using paired *t*-test. Significant differences are indicated (*$P \leq 0.05$). (C) Graph shows the amount of sCD83 in supernatants of LPI or LPI and TIMP1-treated splenocytes determined by ELISA. Data were analyzed using paired *t*-test ($n=5$). Significant differences are indicated (*$P \leq 0.05$).

465.92 ± 26.59 MFI in controls, respectively; $P<0.01$; Fig. 4A), supporting the idea of a protease involvement regulating CD83 availability.

To confirm that TIMP1 was involved in the changes of m- and sCD83 expression, we cultured splenic lymphocytes with LPS and either recombinant TIMP1 or a blocking antibody against TIMP1. TIMP1 treatment was able to elevate mCD83 expression (2.02 ± 0.08 in LPI vs 2.22 ± 0.08-fold increase with LPI and TIMP1, respectively; $P<0.05$, Fig. 4B), while anti-TIMP1 reduced

it (2.197 ± 0.07 in LPI vs 1.926 ± 0.09-fold increase with LPI and Anti-TIMP1, respectively; $P < 0.05$; Fig. 4B). Furthermore, TIMP1 treatment of splenocytes led to significantly increased sCD83 release (1917.66 ± 78.81 with LPS and TIMP1 vs 1758.61 ± 75.40 pg/mL for LPS-treated controls; $P < 0.05$; Fig. 4C).

In summary, TIMP1 effects on mCD83 inversely correlate with those observed with progesterone, favoring sCD83 availability.

Discussion

CBA/J × DBA/2J pregnancies (here referred as PPOM) are characterized by a failed maternal tolerance (Clark *et al.* 1980). The pro-inflammatory manifestations can be observed systemically in the mother and locally at the resorptive fetoplacental unit (Ahmed *et al.* 2010). Innate cells, including neutrophils, NK cells, macrophages and DCs have been found to be the main effectors of the resorptive phenotype (Bonney & Brown 2014).

mCD83 was first described as a maturation marker for DCs and its expression on DCs is linked to a higher pro-inflammatory potential (Aerts-Toegaert *et al.* 2007, Wilson *et al.* 2009, Pinho *et al.* 2014, Seldon *et al.* 2016). mCD83$^+$ DCs are present in human decidua and in high numbers associated with miscarriage (Askelund *et al.* 2004, Qian *et al.* 2015). Our data show that DCs from the uterus-draining lymph nodes in PPOM expressed higher levels of mCD83 than in GPOM, which supports the observations in human studies.

CD4$^+$ T cells can as well express mCD83, for which both anti- and pro-inflammatory roles were suggested, depending on the subpopulation of T cell that was studied (Wolenski *et al.* 2003, Reinwald *et al.* 2008). Although we did not analyze different CD4$^+$ T cell subsets in detail, we observed the significant differences between both groups of mating only in thymic T cells. As PPOM expressed higher levels of mCD83 than GPOM, we assume that mCD83 expression on thymic CD4$^+$ T cells is accompanying the pro-inflammatory T cell repertoire that was already described in those mice.

Similarly, B cells in spleen depicted significant differences in mCD83 expression. In our previous work, we showed that healthy murine pregnancy is accompanied by an upregulation of mCD83 expression on splenic B cells *in vivo*. Moreover, splenic B cells from pregnant mice acquire a higher capacity to upregulate mCD83 expression after stimulation with pro-inflammatory signals as LPS (Packhäuser *et al.* 2017). PPOM pregnancies are characterized by higher levels of pro-inflammatory cytokines, including TNFA and IFNG, which contribute to the disturbed pregnancy phenotype (Clark *et al.* 1998). These cytokines are as well inducers of mCD83 expression (Li *et al.* 2012, Lin *et al.* 2018). As expected for pro-inflammatory pregnancies, splenic B cells from PPOM displayed higher levels of mCD83 than in GPOM.

The fact that differences in mCD83 expression on B cells are absent in uterus-draining lymph nodes suggests that there may be mechanisms to limit CD83 expression in the periphery even during pro-inflammatory pregnancies. Our results suggest that compartmentation and matrix components seem to play a role regulating the CD83 biology. In fact, B cells in spleen are compartmentalized and they migrate upon activation (Pereira *et al.* 2010). In the splenic stroma, B cells interact with several cell types that exert a considerable influence on the B cell fate (Mueller & Germain 2009). We observed that PPOM, despite their higher mCD83 expression on different lymphocyte population, displayed lower serum levels of sCD83. It has been suggested that proteolytic shedding may lead to the release of sCD83 from its membrane-bound form (Hock *et al.* 2001). Then, a higher splenic mCD83 expression accompanied with lower sCD83 release suggests that if a protease is involved, its function diminished in PPOM (Fig. 5).

Consistent with this, we observed that in *ex vivo* stimulated splenic B cells, progesterone limits CD83 availability in a process which seems to be mediated by TIMP1. TIMP1 influenced CD83 availability, increasing mCD83 expression and sCD83 release after *ex vivo* stimulation of splenic B cells. Progesterone treatment, on the other hand, induced TIMP1 downregulation, reducing CD83 availability (Fig. 6).

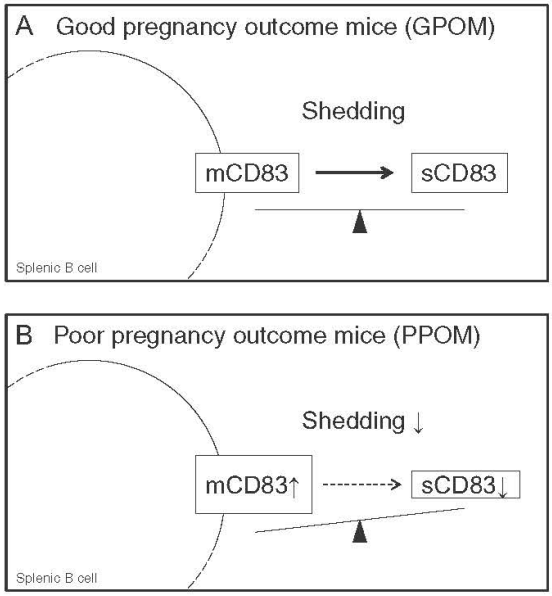

Figure 5 Schematic summary of CD83 shedding in GPOM and PPOM on splenic B cells. In GPOM (A), a balanced shedding of mCD83 provides the source of sCD83 in healthy pregnancies. In contrast, an imbalanced shedding of mCD83 in PPOM (B) results in reduced sCD83 release.

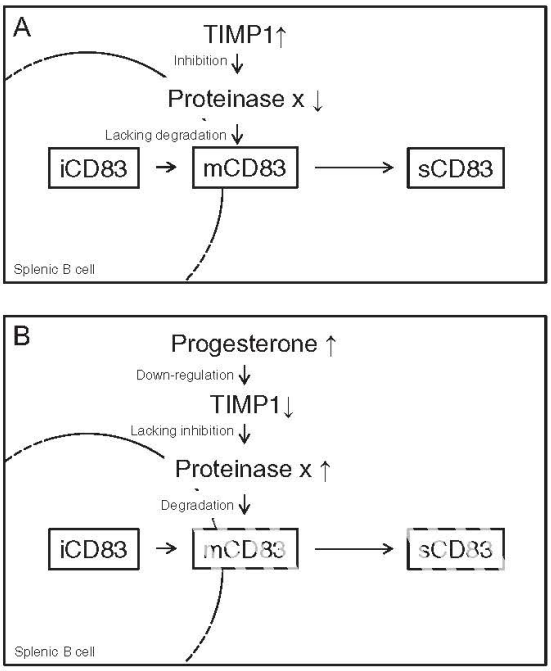

Figure 6 Schematic summary of the proposed progesterone-dependent regulation of CD83 availability by TIMP1. (A) TIMP1 inhibition of a proteinase that degrades mCD83 results in a higher mCD83 expression and therefore improved availability for sCD83 release. (B) Progesterone, acting through inhibition of B cell TIMP1 expression, facilitates the degradation of mCD83, reducing mCD83 expression and sCD83 release.

Progesterone may display contradictory roles depending on the target cell. In the case of TIMP1 production, for example, progesterone showed a negative effect on B lymphocytes but induces TIMP1 expression in uterine fibroblasts (Imada *et al.* 1994) and human endometrial stromal cells (Hampton *et al.* 1999). Then, if the influence of stromal-derived TIMP1 or metalloproteinases prevails over the local effect on B cell expressed TIMP1, we may observe the contrary phenotype in splenic B cells *in vivo* than in *ex vivo*-treated cells. So the effect that we observed on isolated lymphocytes may correspond to a peripheral/activated phenotype, as in the case of a local inflammatory process. In this case, activated B cells could leave their niches and act to contribute to a pro-inflammatory response during pregnancy minimizing the anti-inflammatory effect of sCD83 release.

In this scenario, progesterone would exert a regulatory role on sCD83 availability in B cells that had left the splenic compartment, which find no impediment in acting in a pro-inflammatory manner. Splenic B cell residents, on the other hand, remain as source of systemic sCD83 that may contribute to pregnancy maintenance.

As proposed for sCD83, certain soluble factors as TNFA, TGFA and TACI are derived by cleavage from their latent or the extracellular domains of their respective membrane proteins in a process that is mediated by metalloproteinases from the ADAM family (Murphy 2008, Hoffmann *et al.* 2015). These metalloproteinases can be produced by the same lymphocytes that express such factors or they can be released by cells of the stromal cells into the extracellular matrix. Metalloproteinases and their inhibitory molecules have critical roles during pregnancy, and their synthesis and release are under hormonal control. We found that while progesterone treatment reduces sCD83 release, ADAM10 expression is reduced and ADAM17 is unchanged in B cells (data not shown). Because this is associated to a reduced mCD83 expression and TIMP1 expression, it seems unlikely that these ADAM proteases are involved in the sCD83 release. The mechanisms of sCD83 release need further evaluation.

Overall, our data indicate that matrix components regulate mCD83 expression during pregnancy impacting on sCD83 availability. These components may be compromised in pro-inflammatory pregnancies, leading to altered sCD83 release.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

R E and K P performed experiments, analyzed data and contributed to the elaboration of the manuscript. J E and A T performed experiments and analyzed data. M Z contributed with reagents, the design of experiments and the writing of the manuscript. D M conceived and designed the experiments, analyzed data, contributed with reagents, wrote the paper and supervised the work.

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References

Aerts-Toegaert C, Heirman C, Tuyaerts S, Corthals J, Aerts JL, Bonehill A, Thielemans K & Breckpot K 2007 CD83 expression on dendritic cells and T cells: correlation with effective immune responses. *European Journal of Immunology* **37** 686–695. (https://doi.org/10.1002/eji.200636535)

Ahmed A, Singh J, Khan Y, Seshan SV & Girardi G 2010 A new mouse model to explore therapies for preeclampsia. *PLoS ONE* **5** e13663. (https://doi.org/10.1371/journal.pone.0013663)

Alter A, Duddy M, Hebert S, Biernacki K, Prat A, Antel JP, Yong VW, Nuttall RK, Pennington CJ, Edwards DR *et al*. 2003 Determinants of human B cell migration across brain endothelial cells. *Journal of Immunology* **170** 4497–4505. (https://doi.org/10.4049/jimmunol.170.9.4497)

Askelund K, Liddell HS, Zanderigo AM, Fernando NS, Khong TY, Stone PR & Chamley LW 2004 CD83(+)dendritic cells in the decidua of women with recurrent miscarriage and normal pregnancy. *Placenta* **25** 140–145. (https://doi.org/10.1016/S0143-4004(03)00182-6)

Bock F, Rossner S, Onderka J, Lechmann M, Pallotta MT, Fallarino F, Boon L, Nicolette C, DeBenedette MA, Tcherepanova IY *et al*. 2013 Topical application of soluble CD83 induces IDO-mediated immune modulation, increases Foxp3+ T cells, and prolongs allogeneic corneal graft survival. *Journal of Immunology* **191** 1965–1975. (https://doi.org/10.4049/jimmunol.1201531)

Bonney EA & Brown SA 2014 To drive or be driven: the path of a mouse model of recurrent pregnancy loss. *Reproduction* **147** R153–R167. (https://doi.org/10.1530/REP-13-0583)

Clark DA 2016 Mouse is the new woman? Translational research in reproductive immunology. *Seminars in Immunopathology* **38** 651–668. (https://doi.org/10.1007/s00281-015-0553-x)

Clark DA, McDermott MR & Szewczuk MR 1980 Impairment of host-versus-graft reaction in pregnant mice. II. Selective suppression of cytotoxic T-cell generation correlates with soluble suppressor activity and with successful allogeneic pregnancy. *Cellular Immunology* **52** 106–118. (https://doi.org/10.1016/0008-8749(80)90404-9)

Clark DA, Chaput A & Tutton D 1986 Active suppression of host-vs-graft reaction in pregnant mice. VII. Spontaneous abortion of allogeneic CBA/J x DBA/2 fetuses in the uterus of CBA/J mice correlates with deficient non-T suppressor cell activity. *Journal of Immunology* **136** 1668–1675.

Clark DA, Chaouat G, Arck PC, Mittruecker HW & Levy GA 1998 Cytokine-dependent abortion in CBA x DBA/2 mice is mediated by the procoagulant fgl2 prothrombinase [correction of prothombinase]. *Journal of Immunology* **160** 545–549.

Clark DA, Manuel J, Lee L, Chaouat G, Gorczynski RM & Levy GA 2004 Ecology of danger-dependent cytokine-boosted spontaneous abortion in the CBA x DBA/2 mouse model. I. Synergistic effect of LPS and (TNF-alpha+IFN-gamma) on pregnancy loss. *American Journal of Reproductive Immunology* **52** 370–378. (https://doi.org/10.1111/j.1600-0897.2004.00237.x)

Eckhardt J, Kreiser S, Dobbeler M, Nicolette C, DeBenedette MA, Tcherepanova IY, Ostalecki C, Pommer AJ, Becker C, Gunther C *et al*. 2014 Soluble CD83 ameliorates experimental colitis in mice. *Mucosal Immunology* **7** 1006–1018. (https://doi.org/10.1038/mi.2013.119)

Hampton AL, Nie G & Salamonsen LA 1999 Progesterone analogues similarly modulate endometrial matrix metalloproteinase-1 and matrix metalloproteinase-3 and their inhibitor in a model for long-term contraceptive effects. *Molecular Human Reproduction* **5** 365–371. (https://doi.org/10.1093/molehr/5.4.365)

Harmon AC, Cornelius DC, Amaral LM, Faulkner JL, Cunningham Jr MW, Wallace K & LaMarca B 2016 The role of inflammation in the pathology of preeclampsia. *Clinical Science* **130** 409–419. (https://doi.org/10.1042/CS20150702)

Hashimoto H, Eto T, Endo K, Itai G, Kamisako T, Suemizu H & Ito M 2010 Comparative study of doses of exogenous progesterone administration needed to delay parturition in Jcl:MCH(ICR) mice. *Experimental Animals* **59** 521–524. (https://doi.org/10.1538/expanim.59.521)

Hock BD, Kato M, McKenzie JL & Hart DN 2001 A soluble form of CD83 is released from activated dendritic cells and B lymphocytes, and is detectable in normal human sera. *International Immunology* **13** 959–967. (https://doi.org/10.1093/intimm/13.7.959)

Hoffmann FS, Kuhn PH, Laurent SA, Hauck SM, Berer K, Wendlinger SA, Krumbholz M, Khademi M, Olsson T, Dreyling M *et al*. 2015 The immunoregulator soluble TACI is released by ADAM10 and reflects B cell activation in autoimmunity. *Journal of Immunology* **194** 542–552. (https://doi.org/10.4049/jimmunol.1402070)

Imada K, Ito A, Itoh Y, Nagase H & Mori Y 1994 Progesterone increases the production of tissue inhibitor of metalloproteinases-2 in rabbit uterine cervical fibroblasts. *FEBS Letters* **341** 109–112. (https://doi.org/10.1016/0014-5793(94)80250-5)

Jensen F, Muzzio D, Soldati R, Fest S & Zenclussen AC 2013 Regulatory B10 cells restore pregnancy tolerance in a mouse model. *Biology of Reproduction* **89** 90. (https://doi.org/10.1095/biolreprod.113.110791)

Kretschmer B, Luthje K, Guse AH, Ehrlich S, Koch-Nolte F, Haag F, Fleischer B & Breloer M 2007 CD83 modulates B cell function in vitro: increased IL-10 and reduced Ig secretion by CD83Tg B cells. *PLoS ONE* **2** e755. (https://doi.org/10.1371/journal.pone.0000755)

Kretschmer B, Kuhl S, Fleischer B & Breloer M 2011 Activated T cells induce rapid CD83 expression on B cells by engagement of CD40. *Immunology Letters* **136** 221–227. (https://doi.org/10.1016/j.imlet.2011.01.013)

Krzyzak L, Seitz C, Urbat A, Hutzler S, Ostalecki C, Glasner J, Hiergeist A, Gessner A, Winkler TH, Steinkasserer A *et al*. 2016 CD83 modulates B cell activation and germinal center responses. *Journal of Immunology* **196** 3581–3594. (https://doi.org/10.4049/jimmunol.1502163)

Kuon RJ, Strowitzki T, Sohn C, Daniel V & Toth B 2015 Immune profiling in patients with recurrent miscarriage. *Journal of Reproductive Immunology* **108** 136–141. (https://doi.org/10.1016/j.jri.2015.01.007)

Lan Z, Lian D, Liu W, Arp J, Charlton B, Ge W, Brand S, Healey D, DeBenedette M, Nicolette C *et al*. 2010 Prevention of chronic renal allograft rejection by soluble CD83. *Transplantation* **90** 1278–1285. (https://doi.org/10.1097/TP.0b013e318200005c)

Li DY, Gu C, Min J, Chu ZH & Ou QJ 2012 Maturation induction of human peripheral blood mononuclear cell-derived dendritic cells. *Experimental and Therapeutic Medicine* **4** 131–134. (https://doi.org/10.3892/etm.2012.565)

Lin W, Buscher K, Wang B, Fan Z, Song N, Li P, Yue Y, Li B, Li C & Bi H 2018 Soluble CD83 alleviates experimental autoimmune uveitis by inhibiting filamentous actin-dependent calcium release in dendritic cells. *Frontiers in Immunology* **9** 1567. (https://doi.org/10.3389/fimmu.2018.01567)

Luthje K, Kretschmer B, Fleischer B & Breloer M 2008 CD83 regulates splenic B cell maturation and peripheral B cell homeostasis. *International Immunology* **20** 949–960. (https://doi.org/10.1093/intimm/dxn054)

Mueller SN & Germain RN 2009 Stromal cell contributions to the homeostasis and functionality of the immune system. *Nature Reviews: Immunology* **9** 618–629. (https://doi.org/10.1038/nri2588)

Murphy G 2008 The ADAMs: signalling scissors in the tumour microenvironment. *Nature Reviews: Cancer* **8** 929–941. (https://doi.org/10.1038/nrc2459)

Muzzio DO, Soldati R, Ehrhardt J, Utpatel K, Evert M, Zenclussen AC, Zygmunt M & Jensen F 2014a B cell development undergoes profound modifications and adaptations during pregnancy in mice. *Biology of Reproduction* **91** 115. (https://doi.org/10.1095/biolreprod.114.122366)

Muzzio DO, Soldati R, Rolle L, Zygmunt M, Zenclussen AC & Jensen F 2014b B-1a B cells regulate T cell differentiation associated with pregnancy disturbances. *Frontiers in Immunology* **5** 6. (https://doi.org/10.3389/fimmu.2014.00006)

Muzzio DO, Ziegler KB, Ehrhardt J, Zygmunt M & Jensen F 2016 Marginal zone B cells emerge as a critical component of pregnancy well-being. *Reproduction* **151** 29–37. (https://doi.org/10.1530/REP-15-0274)

Packhäuser KRH, Roman-Sosa G, Ehrhardt J, Kruger D, Zygmunt M & Muzzio DO 2017 A kinetic study of CD83 reveals an upregulation and higher production of sCD83 in lymphocytes from pregnant mice. *Frontiers in Immunology* **8** 486. (https://doi.org/10.3389/fimmu.2017.00486)

Pereira JP, Kelly LM & Cyster JG 2010 Finding the right niche: B-cell migration in the early phases of T-dependent antibody responses. *International Immunology* **22** 413–419. (https://doi.org/10.1093/intimm/dxq047)

Piccinni MP, Lombardelli L, Logiodice F, Kullolli O, Parronchi P & Romagnani S 2016 How pregnancy can affect autoimmune diseases progression? *Clinical and Molecular Allergy* **14** 11. (https://doi.org/10.1186/s12948-016-0048-x)

Pinho MP, Migliori IK, Flatow EA & Barbuto JA 2014 Dendritic cell membrane CD83 enhances immune responses by boosting intracellular calcium release in T lymphocytes. *Journal of Leukocyte Biology* **95** 755–762. (https://doi.org/10.1189/jlb.0413239)

Prechtel AT & Steinkasserer A 2007 CD83: an update on functions and prospects of the maturation marker of dendritic cells. *Archives of Dermatological Research* **299** 59–69. (https://doi.org/10.1007/s00403-007-0743-z)

Qian ZD, Huang LL & Zhu XM 2015 An immunohistochemical study of CD83- and CD1a-positive dendritic cells in the decidua of women with recurrent spontaneous abortion. *European Journal of Medical Research* **20** 2. (https://doi.org/10.1186/s40001-014-0076-2)

Racicot K & Mor G 2017 Risks associated with viral infections during pregnancy. *Journal of Clinical Investigation* **127** 1591–1599. (https://doi.org/10.1172/JCI87490)

Raghupathy R, Al-Azemi M & Azizieh F 2012 Intrauterine growth restriction: cytokine profiles of trophoblast antigen-stimulated maternal lymphocytes. *Clinical and Developmental Immunology* **2012** 734865. (https://doi.org/10.1155/2012/734865)

Reinwald S, Wiethe C, Westendorf AM, Breloer M, Probst-Kepper M, Fleischer B, Steinkasserer A, Buer J & Hansen W 2008 CD83 expression in CD4+ T cells modulates inflammation and autoimmunity. *Journal of Immunology* **180** 5890–5897. (https://doi.org/10.4049/jimmunol.180.9.5890)

Robertson SA, Care AS & Skinner RJ 2007 Interleukin 10 regulates inflammatory cytokine synthesis to protect against lipopolysaccharide-induced abortion and fetal growth restriction in mice. *Biology of Reproduction* **76** 738–748. (https://doi.org/10.1095/biolreprod.106.056143)

Seldon TA, Pryor R, Palkova A, Jones ML, Verma ND, Findova M, Braet K, Sheng Y, Fan Y, Zhou EY *et al*. 2016 Immunosuppressive human anti-CD83 monoclonal antibody depletion of activated dendritic cells in transplantation. *Leukemia* **30** 692–700. (https://doi.org/10.1038/leu.2015.231)

Starke C, Steinkasserer A, Voll RE & Zinser E 2013 Soluble human CD83 ameliorates lupus in NZB/W F1 mice. *Immunobiology* **218** 1411–1415. (https://doi.org/10.1016/j.imbio.2013.06.002)

Su LL, Iwai H, Lin JT & Fathman CG 2009 The transmembrane E3 ligase GRAIL ubiquitinates and degrades CD83 on CD4 T cells. *Journal of Immunology* **183** 438–444. (https://doi.org/10.4049/jimmunol.0900204)

Wilson J, Cullup H, Lourie R, Sheng Y, Palkova A, Radford KJ, Dickinson AM, Rice AM, Hart DN & Munster DJ 2009 Antibody to the dendritic cell surface activation antigen CD83 prevents acute graft-versus-host disease. *Journal of Experimental Medicine* **206** 387–398. (https://doi.org/10.1084/jem.20070723)

Wolenski M, Cramer SO, Ehrlich S, Steeg C, Fleischer B & von Bonin A 2003 Enhanced activation of CD83-positive T cells. *Scandinavian Journal of Immunology* **58** 306–311. (https://doi.org/10.1046/j.1365-3083.2003.01303.x)

Zhou LJ & Tedder TF 1995 Human blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily. *Journal of Immunology* **154** 3821–3835.

Zhou LJ & Tedder TF 1996 CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. *PNAS* **93** 2588–2592. (https://doi.org/10.1073/pnas.93.6.2588)

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EIDESSTATTLICHE ERKLÄRUNG

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Die Dissertation ist bisher keiner anderen Fakultät, keiner anderen wissenschaftlichen Einrichtung vorgelegt worden.

Ich erkläre, dass ich bisher kein Promotionsverfahren erfolglos beendet habe und dass eine Aberkennung eines bereits erworbenen Doktorgrades nicht vorliegt.

Greifswald, den 23.01.2022 K.Packhäuser

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