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Expression of the mucin-like glycoprotein CD24 and its ligand siglec-10 in placentas with acute and post SARS-CoV-2 infection

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ABSTRACT

CD24 is a mucin-like glycoprotein expressed on trophoblast cells and endothelial tissue of first and third trimester placentas. As an immune suppressor, CD24 may contribute to maternal immune tolerance to the growing fetus. CD24 is known to interact with the sialic acid-binding immunoglobulin-type lectins (Siglecs), specifically siglec-10. The aim of this study was to investigate the expression of both, CD24 and siglec-10 on placental tissue slides from acute covid patients, patients who survived a covid-19 infection and normal term controls. For the evaluation of CD24 & siglec-10 we used a total of 60 placentas, 10 acute covid-19 female, 10 acute covid-19 male, 10 post-covid-19 female, 10 post-covid-19 male, 10 female term controls and 10 male term controls. Immunohistochemical staining against CD24 and siglec-10 was performed and the expression of both markers was done with an immunoreactive score (IRS). Identity of CD24- or siglec-10 expressing cells was analyzed by double immune fluorescence analyses. The expression of CD24 is significantly downregulated on the extravillous trophoblast and on Hofbauer cells of female acute covid placentas. In the contrary, CD24 is significantly upregulated on male post-covid-19 Hofbauer cells. The CD24-ligand siglec-10 is significantly downregulated in post-covid-19 Hofbauer cells independently of fetal sex, whereas it shows significant higher expression in control female Hofbauer cells. CD24 and its ligand siglec-10 are differentially expressed in placentas of patients who survived a covid-19 infection. Surprisingly this effect is related to the fetal gender. Further investigation is necessary to analyze especially the imprinting effect of this infection.

1. Introduction

At the beginning of the COVID-19 pandemic, uncertainties about the virus and its dangers during pregnancy caused great uncertainty and fear, especially among pregnant women (Meister et al., 2023). In a number of studies this elevated fear was confirmed (Hagenbeck et al., 2020; Hagenbeck et al., 2023; Schaal et al., 2023; Schaal et al., 2022). Because pregnancy is accompanied with a general immunomodulation of the pregnant women but not necessarily an immune-compromised state, immune changes subject pregnant women to increased susceptibility to viral infection. During the covid-19 pandemic, pregnant women were more susceptible to serious illness (Accurti et al., 2022). Also, other studies showed that elevated mortality of pregnant women with covid-19 infection was observed (Accurti et al., 2022; Ahmad et al., 2022; Ambedkar et al., 2023).

New data suggest an increased risk of obstetric complications, including maternal complications, preterm labor, intrauterine growth restriction, hypertensive disorders, stillbirths, gestational diabetes and risk of neonatal developmental disorders (Nobrega et al., 2024; Celik et al., 2024; Bernad et al., 2023; Moza et al., 2023). Overall, there are still controversial concerns about the potential for vertical transmission to the fetus (Tosto et al., 2023).

CD24 is a small (27 amino acids) protein attached to the membrane via a glycosylphosphatidylinositol (GP-I) anchor (Kay et al., 1991; Rougon et al., 1991). It composed of several potential sites for N-and-O-linked glycosylation, rendering the molecule structurally similar to mucins (Aigner et al., 1997). CD24 binds to Siglec-10, and together they form a strong immune-suppression axis (Liu and Zheng,

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2007). Binding of the Siglec-10–CD24 axis was demonstrated to be an important immune check point for immune tolerance in mouse autoimmune models (Crocker et al., 2007; Pillai et al., 2012). Recently, CD24 was identified as an immune-modulator on cancer cells inhibiting the phagocytic potential of macrophages as was shown by Barkal et al. (Barkal et al., 2019).

Recombinant CD24, in the form of CD24-Fc was recently demonstrated to be a promising drug for blocking over-shooting immune reactions ("the cytokine storm") in SARS-2-Covid-19 infections(Song et al., 2022). CD24-containing exosomes are also in a clinical trial to reduce symptoms and severity of Covid-19 (Shapira et al., 2022), indicating a pharmacological link of immune suppression and CD24 in different diseases

In our previous studies we showed that the expression of placental CD24 in the first trimester is linked to the glandular epithelial cells of the uterine glands and to other decidual cells (Sammar et al., 2017). The protein was found to be co-expressed with Siglec-10 as it does in cancer cells (Barkal et al., 2019; Chen et al., 2023). The co-expression of the complex is widely localized to the close vicinity of the invasive extravillous trophoblasts, supporting placentation and suppressing the immune rejection of the invading trophoblasts by the maternal decidual cells(Sammar et al., 2017). the Using qRT-PCR analysis revealed a significant increase in CD24 expression from the first and early second trimester to term delivery. In cases of early and preterm preeclampsia, the mRNA level of CD24 is reduced compared to normal term delivery and especially compared to age-matched preterm delivery (Sammar et al., 2021).

In this study, we evaluated the CD24 and siglec-10 protein level in placental tissue from acute covid patients, patients who survived a covid-19 infection and normal term controls.

Because a systematic investigation of the CD24/Siglec-10 axis, this study aimed to investigate both CD24 as well as Siglec-10 in acute and post-covid-19 placental tissue with a specific attention to immune cell populations associated with covid-19 infection.

2. Materials and Methods

2.1. Study subjects

This study was approved by the ethics committee of the Ludwig-Maximilian-University (LMU) Munich, Germany in July 2021. The placental tissue of 60 placentas, 10 acute covid-19 female, 10 acute covid-19 male, 10 post-covid-19 female, 10 post-covid-19 male, 10 female term controls and 10 male term controls, who delivered in the University Hospital Augsburg in the years 2020–2022 were obtained and included into the study after written informed consent. The control group was matched to the covid-19 group in pregnancy week, fetal sex, and age of the mother +/- 5 years. In order to rule out confounders, healthy patients who fulfilled the following criteria were excluded from the study: preeclampsia, HELLP, intrauterine growth restriction, fertility treatment, signs for systemic inflammation in the blood other than covid-19, placentation disorders like placenta accreta/percreta/increta.

The placentas were preserved in buffered formalin immediately after delivery. The samples were then dissected from the central part of the placenta in the institute for pathology in the University Hospital Augsburg, containing decidua, extravillious and villious trophoblasts. After fixation in buffered formalin the samples were then embedded in paraffin and cut with a sliding microtome to 2–3µm slides. The study grouped the placenta based on fetal sex and COVID-19 infection (see Table 1).

The clinical details of the study population are shown in Table 1.

Table 1
Clinical details of the study population.

	Control (n = 20)	Acute Covid (n = 20)	Post Covid (n = 20)	p-value
	Male (n = 10)	Male (n = 10)	Male (n = 10)	
	Female (n = 10)	Female (n = 10)	Female (n = 10)	
Age at delivery	32,26 ± 3,49	31,26 ± 5,31	30,95 ± 4,14	p = 0,57
	32,00 ± 3,16	30,60 ± 5,76	31,50 ± 3,78	p = 0,66
	32,50 ± 3,92	31,67 ± 5,07	30,40 ± 4,60	p = 0,68
BMI (before pregnancy)	24,90 ± 4,81	25,43 ± 4,85	25,47 ± 5,18	p = 0,82
	25,96 ± 4,48	25,84 ± 4,64	25,75 ± 4,52	p = 0,99
	23,94 ± 5,12	24,98 ± 5,31	25,18 ± 6,00	p = 0,72
Gravidity	1,79 ± 1,08	2,32 ± 1,00	2,25 ± 1,33	p = 0,27
	1,78 ± 0,97	2,00 ± 0,82	2,10 ± 1,37	p = 0,94
	1,80 ± 1,23	2,67 ± 1,12	2,40 ± 1,35	p = 0,17
Parity	1,37 ± 0,50	2,05 ± 0,71	2,05 ± 1,28	p = 0,02
	1,44 ± 0,53	1,80 ± 0,63	2,10 ± 1,37	p = 0,55
	1,30 ± 0,48	2,33 ± 0,71	2,00 ± 1,25	p = 0,02
Gestational age at delivery	39,25 ± 1,92	39,20 ± 2,02	39,25 ± 2,10	p = 0,99
	39,40 ± 1,90	39,30 ± 2,11	39,40 ± 1,90	p = 1,00
	39,10 ± 2,03	39,10 ± 2,03	39,10 ± 2,38	p = 0,98
Birthweight, g	3178,95 ± 506,60	3268,68 ± 404,91	3165,00 ± 627,41	p = 0,96
	3343,78 ± 409,12	3279,50 ± 422,84	3375,00 ± 594,61	p = 0,49
	3030,60 ± 559,36	3256,67 ± 409,21	2955,00 ± 616,00	p = 0,80
APGAR 10 minutes	9,89 ± 0,32	9,74 ± 0,56	9,95 ± 0,22	p = 0,14
	9,78 ± 0,44	9,60 ± 0,70	10,00 ± 0,00	p = 0,19
	10,00 ± 0,00	9,89 ± 0,33	9,90 ± 0,32	p = 0,33
Umbilical artery pH	7,26 ± 0,11	7,27 ± 0,09	7,28 ±0,07	p = 0,58
	7,25 ± 0,11	7,25 ± 0,11	7,28 ± 0,07	p = 0,59
	7,26 ± 0,12	7,29 ± 0,06	7,28 ± 0,08	p = 0,89

2.2. Immunohistochemistry

In preparation for immunohistochemistry, the paraffin sections had to be deparaffinized with Roticlear® and afterwards bathed in 100 % ethanol. To stop the endogenous peroxidase activity, the samples were then incubated in 3 % H_2O_2 in methanol for 20 minutes and rehydrated in a descending alcohol gradient to distilled water. In the next step, the slices were put in a high-pressure cooker for 5 min using boiling sodium citrate buffer with pH 6.0 for antigen retrieval.

Subsequently the slices were treated for 5 min with a blocking solution (Reagent 1; ZytoChem Plus HRP Polymer System IgG kit (Mouse/Rabbit) by Zytomed) for saturating electrostatic charges. Then tissue sections were incubated for 16 hours at 4°C with Siglec-10, or 45 minutes at room temperature with CD24. After washing the slides with phosphate-buffered saline (PBS), the ZytoChem Plus HRP Polymer System IgG kit (Mouse/Rabbit) (Zytomed, Berlin, Germany) and liquid DAB+ (Diaminobenzidin) Substrate Chromogen System (Agilent Technologies, Santa Clara, USA) was used for visualization of the bound primary antibodies (brown staining). The slices were counterstained with Mayer 's acid hemalum for 2 min and stained blue for 5 min in tap

water. In the following step the samples were dehydrated in an ascending series of alcohol, then treated with Roticlear® and coverslipped with RotiMount (Carl Roth, Germany).

All antibodies which were used in this study are listed in Table 2.

For the evaluation of the quantity of antigen-presenting macrophages and Hofbauer cells the number of cells was counted in three image sections at a magnification with a 40x lens. The total number of cells was then calculated by summing the three areas. For the evaluation of the intensity and distribution patterns of the antigen expression in the extravillous trophoblast and the syncytiotrophoblast the semi-quantitative immunoreactive score of Remmele (IRS) (Remmele and Schicketanz, 1993; Remmele and Stegner, 1987) was used. The IRS is calculated by the multiplication of the grade of optical staining intensity (0=none, 1=weak, 2=moderate and 3=strong staining) and the percentage of positive staining cells (also divided into 4 categories: 0=no staining, 1= <10 % of the cells, 2=10–50 % of the cells, 3=51–80 % of the cells and 4=more than 80 % of the cells).

2.3. Immunofluorescence

The double immunofluorescence staining allowed us to characterize specific antigens simultaneously. The same formalin-fixed and paraffin-embedded samples were placed in Roticlear® for 20 min for deparaffinization. Subsequently, the sections were panned in ethanol in order of descending concentrations (100 %, 70 %, 50 %) and washed in distilled water. Unmasking of antigens was performed by a 5 min heat pretreatment in a pressure cooker with EDTA buffer, pH 9.0. After washing in distilled water and PBS for 4 min, incubation with immunofluorescence blocking buffer (Cell Signaling; USA) was performed to prevent unspecific staining. The solution was tipped off after 60 min and the primary antibodies were applied at 4°C for 16 hours or 45 minutes at 22°C in case of CD24. After a washing step with PBS the bound primary antibodies were detected at room temperature for 30 minutes by following secondary antibodies:

Table 2
Primary and secondary antibodies for immunohistochemistry/immunofluorescence.

Antibody	Isotype	Clone	Dilution	Source
Anti-CD68	Rabbit IgG	Monoclonal; clone D4B9C	1:1000	Cell Signaling, USA
Anti-CD163	Mouse IgG1	Monoclonal; clone OTI2G12	1:2000	Abcam, UK
Anti-CD24	Mouse IgG	Monoclonal; clone SWA11	1:1000	P. Altevogt, BZKF Heidelberg, Germany
Anti-CD3	Rabbit IgG	Monoclonal; clone 2GV6	Ready to use	Roche, Switzerland
Anti-CD15, Biotin	Mouse IgM, kappa	Monoclonal; clone HI98	1:150	Invitrogen, USA
Anti-CK7	Mouse IgG1, kappa	Monoclonal; clone OV-TL 12/30	1:200	Agilent, USA
Anti-Siglec−10	Mouse IgG2B	Monoclonal; clone 265817	1:100	R&D Systems, USA
Target Antibody	Detection antibody		Dilution	Source
Anti-CD24	Goat-anti-mouse IgG, Alexa Fluor 488		1:500	Thermo Fisher, USA
Anti-CD163	Goat-anti-mouse IgG, Cy5		1:100	Jackson Immunotech, USA
Anti-CD68	Goat-anti-rabbit IgG, Cy3		1:500	Jackson Immunotech, USA
Anti-CD3	Goat-anti-rabbit IgG, Cy3		1:500	Jackson Immunotech, USA
Anti-CD15, Biotin	Streptavidin, Cy5		1:900	Jackson Immunotech, USA
Anti-CK7	Goat-anti-mouse IgG, Cy5		1:100	Jackson Immunotech, USA

Following a further washing step in PBS sections were covered with True Black for 1 minute at 22°C to quench tissue autofluorescence and subsequently washed again. After drying the slides were cover slipped using DAPI containing fluorescence mounting medium (Vector Laboratories; USA).

2.4. Statistical Analyses

All statistical analyses were conducted using non-parametric tests due to the non-normal distribution of the data with SPSS 28 by IBM©. The Kruskal-Wallis test was used to compare CD24 and SIGLEC-10 expression between more than two groups, while the Mann-Whitney U test was employed to compare differences between two groups. Results were presented as median IRS scores. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Expression of CD24 in extravillous trophoblast cells

CD24 is expressed in both the nucleus and the cytoplasm of extravillous trophoblast cells (EVT) of normal control third trimester placentas. Although there was a difference in placentas of male offspring (Fig. 1A, median IRS score 5) and female offspring (Fig. 1D, median IRS score 4), these differences were not significant ($p > 0.05$). Significant differences were observed in CD24 expression in EVT between female control (Fig. 1D) and female outcome placentas of women with acute Covid-19 infection (Fig. 1E, median control IRS 4 versus median IRS of 2 in acute covid-19 infection placentas; $p = 0.0019$). There is no difference in the expression of CD24 between placentas of male control (Fig. 1A), male acute covid (Fig. 1B) and male post covid (Fig. 1C). The difference between acute covid placentas of male offspring (Fig. 1B) and female offspring (Fig. 1E) is not significant too. Taking a look to the post covid male placentas (Fig. 1C) and the post covid female placentas (Fig. 1F) we observed a difference between these two groups, but it is also not significant. The expression of CD24 in the EVT of female control placentas (Fig. 1D) and female post covid placentas (Fig. 1F) showed no significant differences. A summary of all staining results in EVT is presented in Fig. 1G.

3.2. Expression of CD24 in fetal Hofbauer cells inside placental villi

CD24 is expressed in both the nucleus and the cytoplasm of Hofbauer cells within villous tissue of normal control third trimester placentas. Although there was a difference in placentas of male offspring (Fig. 2A, median IRS score 4.5) and female offspring (Fig. 2D, median IRS score 5.5), these differences were not significant ($p > 0.05$). Significant differences were observed in CD24 expression in Hofbauer cells within villous tissue between female control (Fig. 2D) and female outcome placentas of women with acute Covid-19 infection (Fig. 2E, median control IRS 5.5 versus median IRS of 4 in acute covid-19 infection placentas; $p = 0.024$). The expression of CD24 in Hofbauer cells of male control placentas (Fig. 2A) and male covid placentas (Fig. 2B) showed no significant differences. In male placentas, there was a significant upregulation of CD24 expression in Hofbauer cells of post Covid-19 placentas (Fig. 2C, IRS $\approx$ 6; $p = 0.011$) compared to control Hofbauer cells (Fig. 2A, IRS = 4.5). The expression of CD24 in Hofbauer cells of female control placentas (Fig. 2D) and female post covid placentas (Fig. 2F) showed no significant differences.

A summary of all staining results in Hofbauer cells is presented in Fig. 2G.

3.3. Expression of SIGLEC-10 in endothelial cells inside placental villi

SIGLEC-10 is expressed in endothelial cells within villous tissue of normal control third trimester male (Fig. 3A) and female (Fig. 3D)

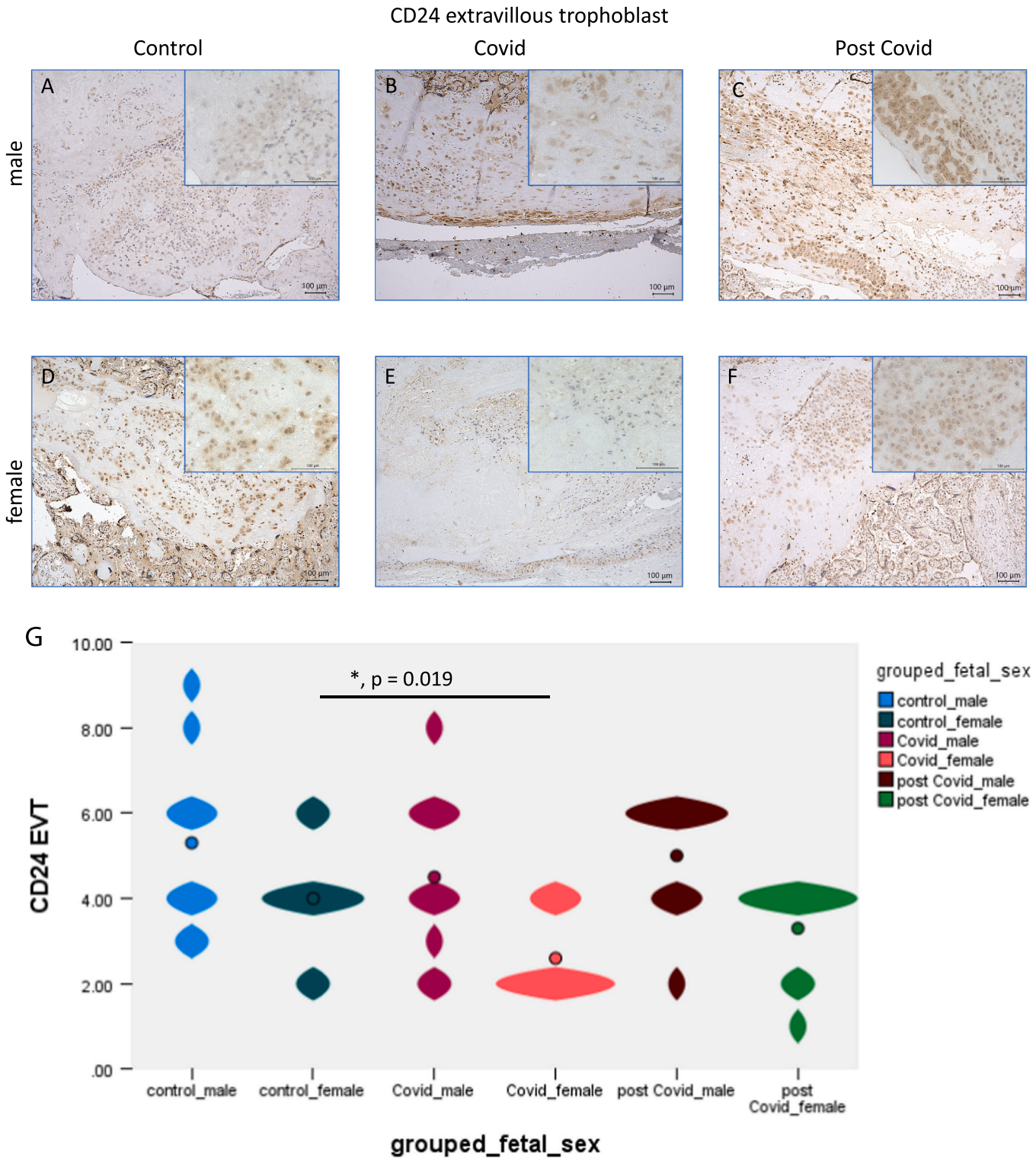

Fig. 1. Expression of CD24 was identified in extravillous trophoblast cells (EVTs) in healthy control placentas of male (A) female fetuses (D), magnification 10x and 40x insert. Unchanged expression is seen in acute covid-19 male EVT (B). Significantly reduced expression was observed in acute female covid-19 EVT (E), magnification 10x and 40x insert. Non-significant changes were observed in post-covid-19 male (C) and female EVT (F). A summary of the staining results is shown as violin plot in G. Significant differences are marked with an asterisk and the p-value is stated.

placentas. Significant differences were observed in SIGLEC-10 expression in endothelial cells within villous tissue between control (Fig. 3A, D), acute (Figs. 3B, 3E and post Covid-19 infection (Figs. 3C, 3F; median control IRS 1.75 versus median IRS of 1 in acute and 1.3 in post covid-19 infection placentas; $p = 0.044$). A summary of all staining results in endothelial cells is presented in Fig. 3G. Double immunofluorescence and CD31 as a marker for endothelial cells were used to proof the identity of SIGLEC-10 expressing cells within placental villi of all cases (Fig. 3H-M).

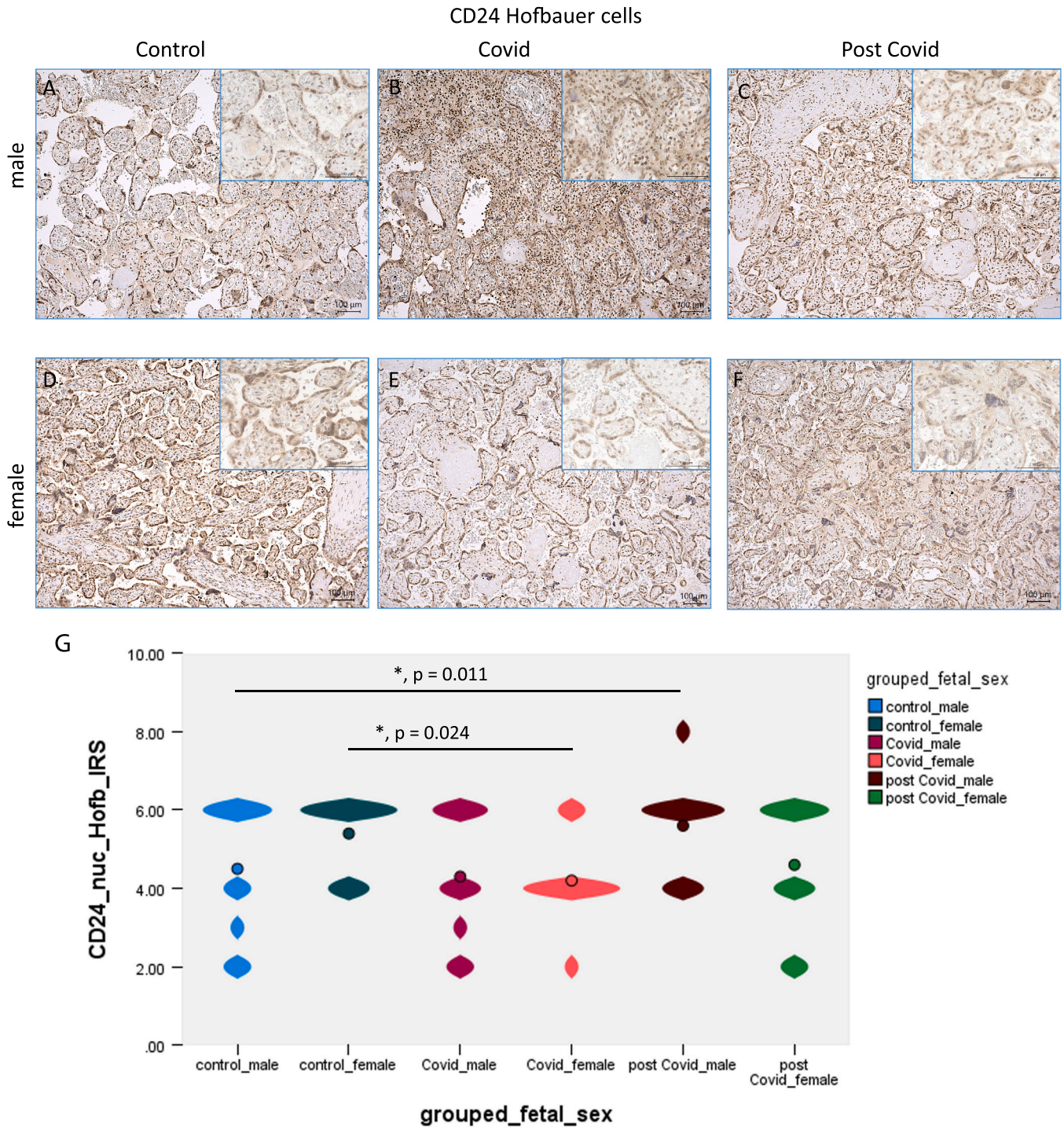

Fig. 2. CD24 is expressed in Hofbauer cells in high numbers within villous tissue in healthy control placentas of male (A) and female fetuses (D), magnification 10x and 40x insert. Unchanged expression is seen in acute covid-19 male Hofbauer cells (B). Significantly reduced expression of CD24 was observed in acute female covid-19 Hofbauer cells (E), magnification 10x and 40x insert. CD24 is expressed in moderate numbers of Hofbauer cells within villous tissue in healthy control placentas of male fetuses (A), magnification 10x and 40x insert. Significantly elevated expression of CD24 was observed in male post covid-19 Hofbauer cells (C), magnification 10x and 40x insert. Unchanged expression is seen in post-covid-19 female Hofbauer cells (F). A summary of the staining results is shown as violin plot in G. Significant differences are marked with an asterisk and the p-value is stated.

3.4. Identification of CD24 expressing cells in the decidua as extravillous trophoblast

Within the male control decidua we see an abundance of CD24-positive cells stained in green (Fig. 4A). Cytokeratin-7 (lower left corner, CK7, pink) was used as EVT specific marker. Single CD24 expression is shown in the upper right corner. Double expression of CD24 and CK7 identified CD24-positive cells as EVT (Fig. 4A) in male control placentas. Within the female control decidua we also see an abundance of CD24-positive cells stained in green (Fig. 4D). Again, Cytokeratin-7 (lower left corner, CK7, pink) was used as EVT specific marker. Single CD24 expression is shown in the upper right corner. Double expression of CD24 and CK7 identified CD24-positive cells as EVT (Fig. 4D) in female control placentas. CD24 is expressed on acute

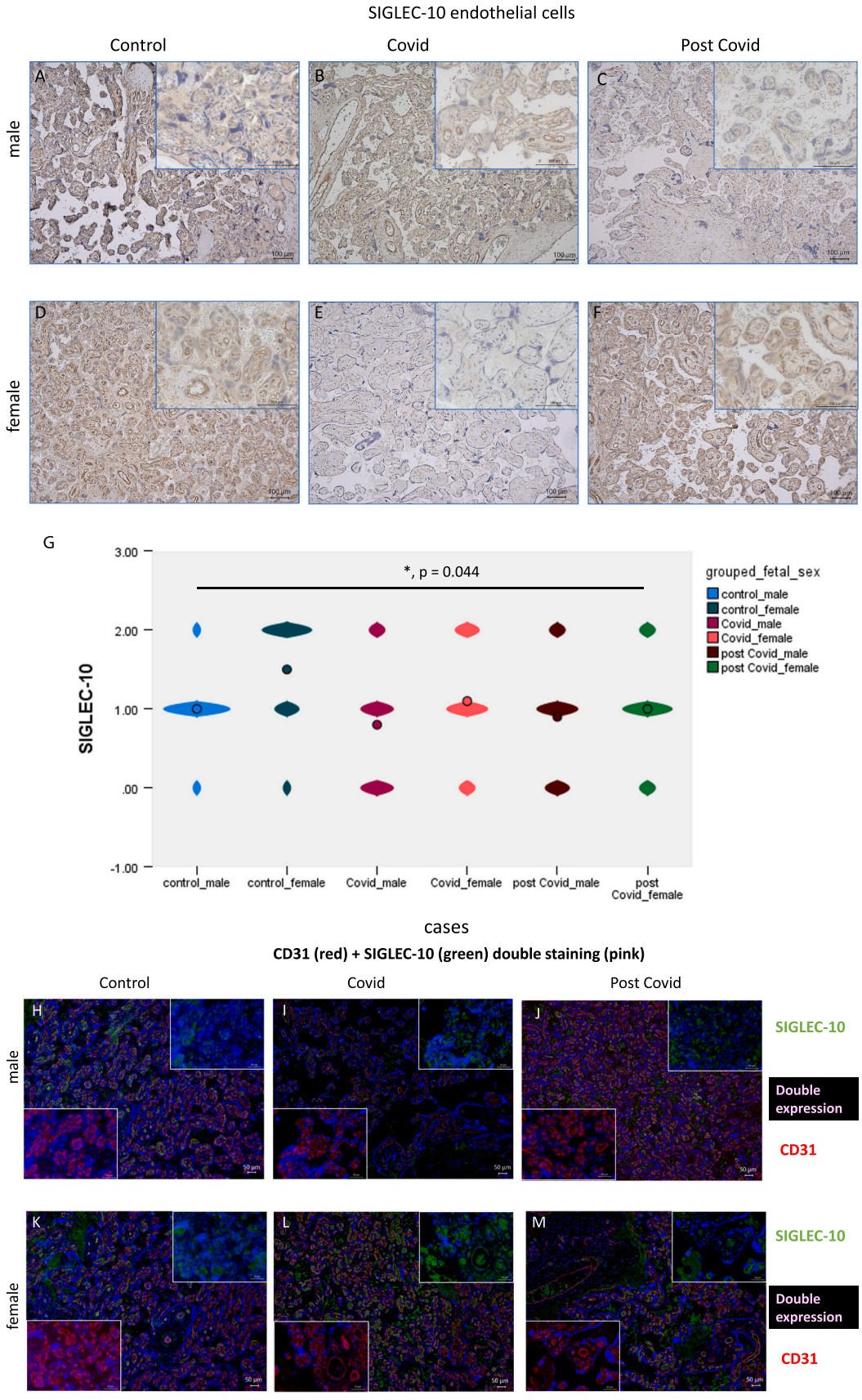

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Fig. 3. SIGLEC-10 is expressed in endothelial cells in moderate numbers within villous tissue in healthy control male placentas (A) as well as in female placentas (D), magnification 10x and insert 40x. Significantly reduced expression of CD24 was observed in acute covid-19 endothelial cells of male (B) and female placentas (E), and post covid19 endothelial cells of male (C) and female placentas (F) magnification 10x and insert 40x. A summary of the staining results is shown as violin plot in G. Significant differences are marked with an asterisk and the p-value is stated. Identity of endothelial cells as SIGLEC-10 expressing cells was analyzed by double immune fluorescence. SIGLEC-10 expression is shown (green fluorescence, upper right insert) together with CD31 (red fluorescence, lower left insert) together with the overlay in male (H) and female controls (K), in acute covid male (I) and female (L) placentas and in post covid male (J) and female (M) placentas.

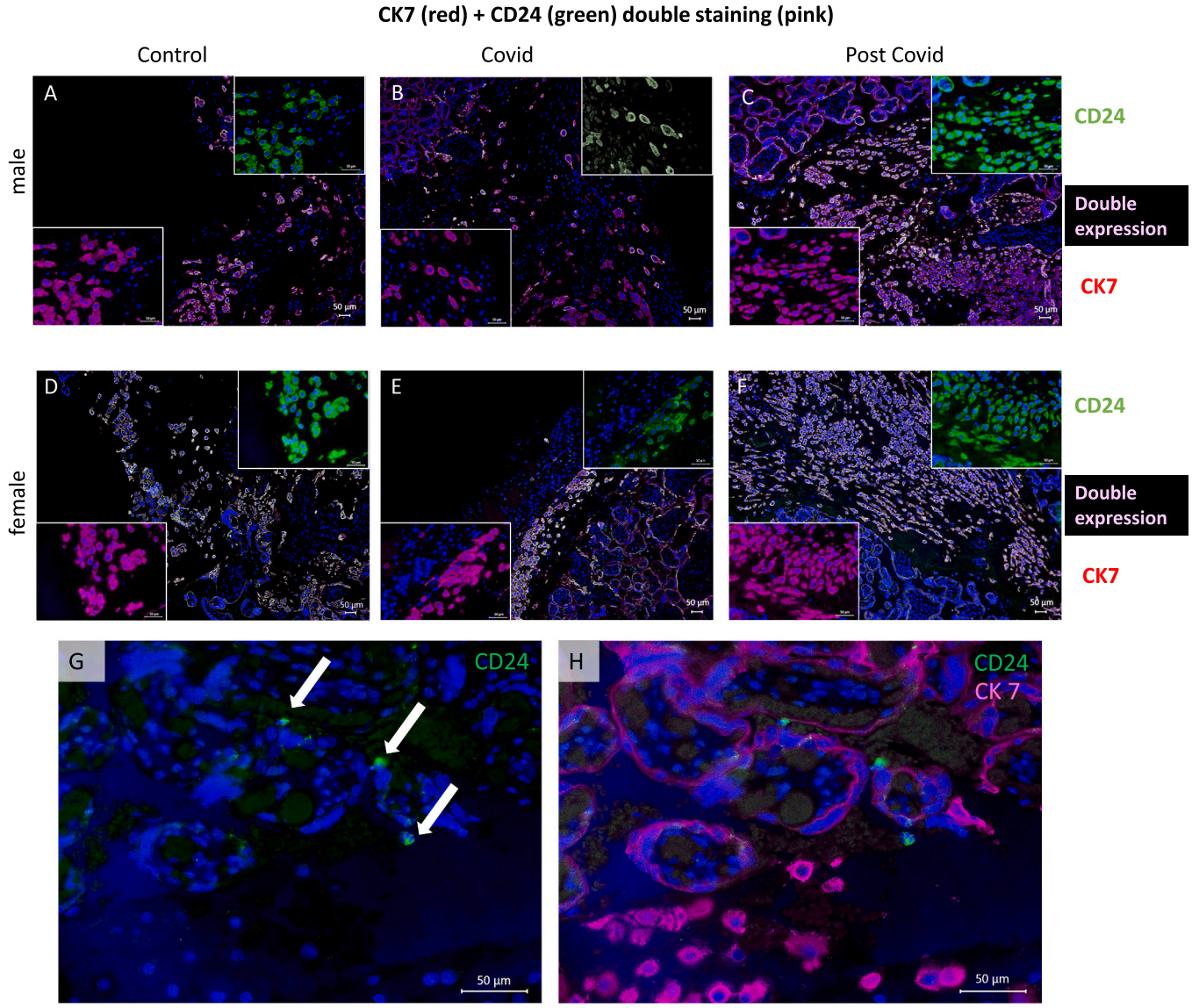

Fig. 4. Identity of extravillous trophoblast cells (EVTs) as CD24 expressing cells was analyzed by double immune fluorescence. CD24 expression is shown (green fluorescence, upper right insert) together with CK7 (red fluorescence, lower left insert) together with the overlay in male (A) and female controls (D), in acute covid male (B) and female (E) EVTs and in post covid male (C) and female (F) EVTs. Some cells in acute covid placentas express CD24 (green fluorescence, G) but do not express CK7 (pink fluorescence, H).

male (Fig. 4B) and female placentas (Fig. 4E). Staining explanation is the same as in controls. Also in post covid placentas, CD24 is expressed on male (Fig. 4C) as well as female extravillous trophoblast cells (Fig. 4F). The identification and color explanation is the same as in controls.

In acute covid-19 female placentas, only few cells express CD24 (Fig. 4G) marked with white arrows. Double immune fluorescence staining showed that these cells do not express CK7 (Fig. 4H) and therefore are not EVT in acute covid-19 placentas.

3.5. Identification of CD24 expressing cells in covid-19 decidua

In covid-19 placentas CD24 is expressed on some cells that are not EVTs as seen in Fig. 4H. Therefore, we performed double immune fluorescence staining for the identification of these cells within the decidua of covid-19 placentas. CD24 positive cells were stained in green (Fig. 5A). Double immunofluorescence with a CD3 antibody (stained in red) showed that CD24-positve cells are not of T-cell origin (Fig. 5B). Another covid-19 decidua was stained for CD24 (Fig. 5C in green) and double staining of this case with CD68 (red) showed that CD24-positive cells in the decidua are not macrophages (Fig. 5D). Staining with CD15

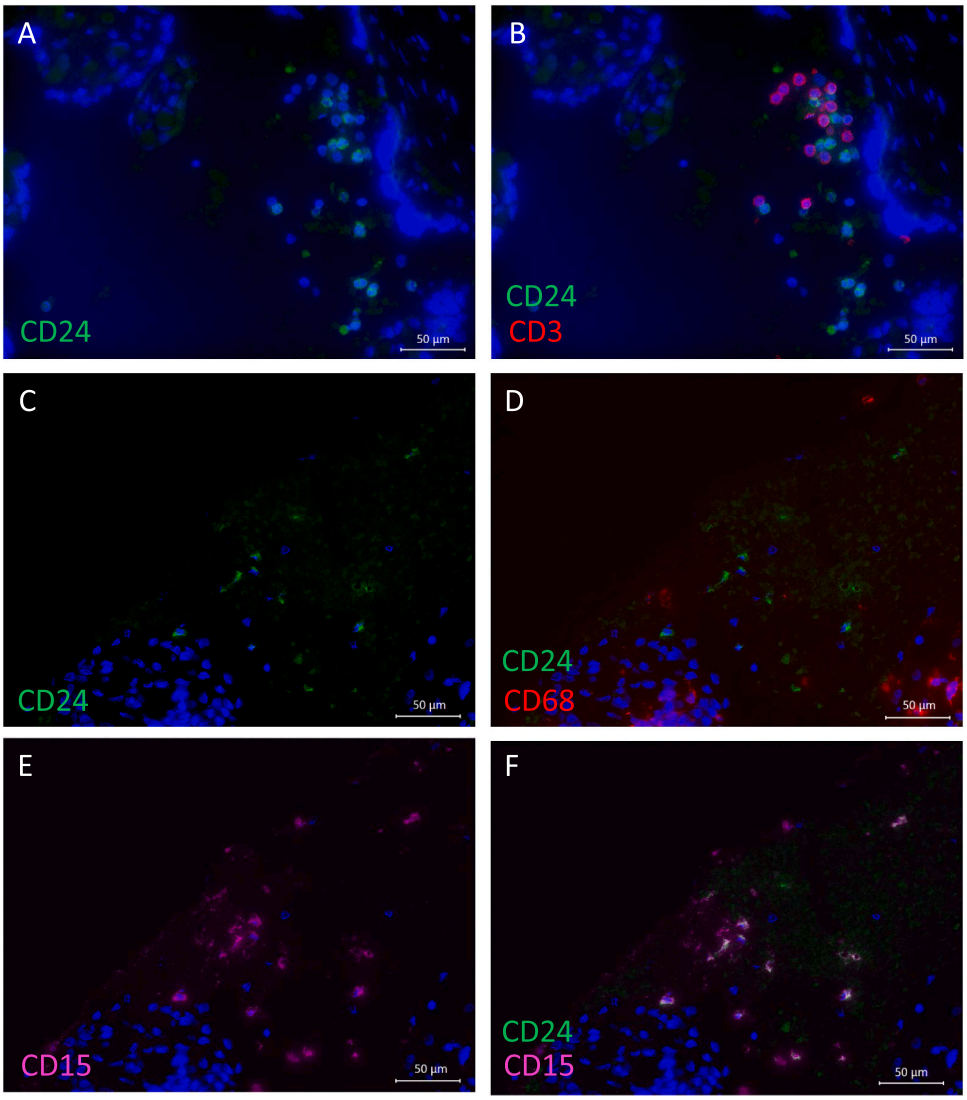

Fig. 5. CD24-positive cells (green fluorescence) of acute covid-19 placentas with female fetuses (A) are not positive for CD3 (T-cell marker, pink fluorescence (B), although both cell types seem to interact with each other (B), magnification 40x. CD24-positive cells (green fluorescence) of acute covid-19 placentas with female fetuses (C) are not positive for CD68 (macrophage marker, red fluorescence (D). CD15 (neutrophil marker, pink fluorescence; E) seems to match with CD24 (C) and double expression of both markers (F) proved that CD24-positve cells in female acute covid placentas are CD15 positive and therefore of neutrophile origin, magnification 40x.

(Fig. 5F, pink) showed a match with CD24 (Fig. 5E) and double expression of CD24 and CD15 proved the double expression of both markers in one cell and we could identify the CD24-positive cells within the covid-19 decidua as neutrophils as the also carry multi-lobed nuclei.

3.6. *Identification of CD24 expressing cells in villous placental tissue*

Within villous tissue of normal control third trimester placentas of female origin, Hofbauer cells were stained with CD163 (pink) and are present in a great abundance. These cells show an expression of CD24 (green) in the nucleus and are marked with white arrows (Fig. 6A). In female covid-19 villous tissue, the expression of CD24 is downregulated in CD163 positive Hofbauer cells (Fig. 6B) as no double expression of both CD24 and CD163 can be seen.

In covid-19 villous tissue, we also found CD24-positive cells stained in green (Fig. 6C). These cells (red staining) do not express CD68 (Fig. 6D). Staining with CD15 (Fig. 6E) in pink showed that this CD24-positve cell is a neutrophil granulocyte and double expression of both, CD24 and CD15 could proof this (Fig. 6F). A cell positive for both, CD24 and CD15 is marked with a white arrow (Fig. 6F).

4. Discussion

Within this study, we could show that the expression of the immune checkpoint molecule CD24 is reduced in acute covid-19 extravillous trophoblast cells as well as in fetal Hofbauer cells in placentas of female origin. In addition, CD24 is upregulated in post-covid-19 Hofbauer cells in placentas of male origin. In addition, double immunofluorescence staining of female covid-19 placentas showed an influx of CD24-positive neutrophils into the decidua. Few cases also showed influx of CD24-positive neutrophiles into fetal villous tissue of placentas with female origin.

The combination of neutrophils and CD24 in cases with viral and other infections is known already for some time (Chen et al., 2012; Nkwanyana et al., 2009; Velly et al., 2021; Wagner et al., 2008). Several recent studies support a link between lesion and inflammation, with lesion-associated molecular patterns (DAMPs) playing an important role in severe covid-19 pathology. In addition, in a recent study, the role of DAMPs and components of the DAMP signaling cascade, including SIGLECs and their corresponding ligands CD24 and CD52, in COVID-19 was analyzed (Parthasarathy et al., 2022). Recently it was shown that

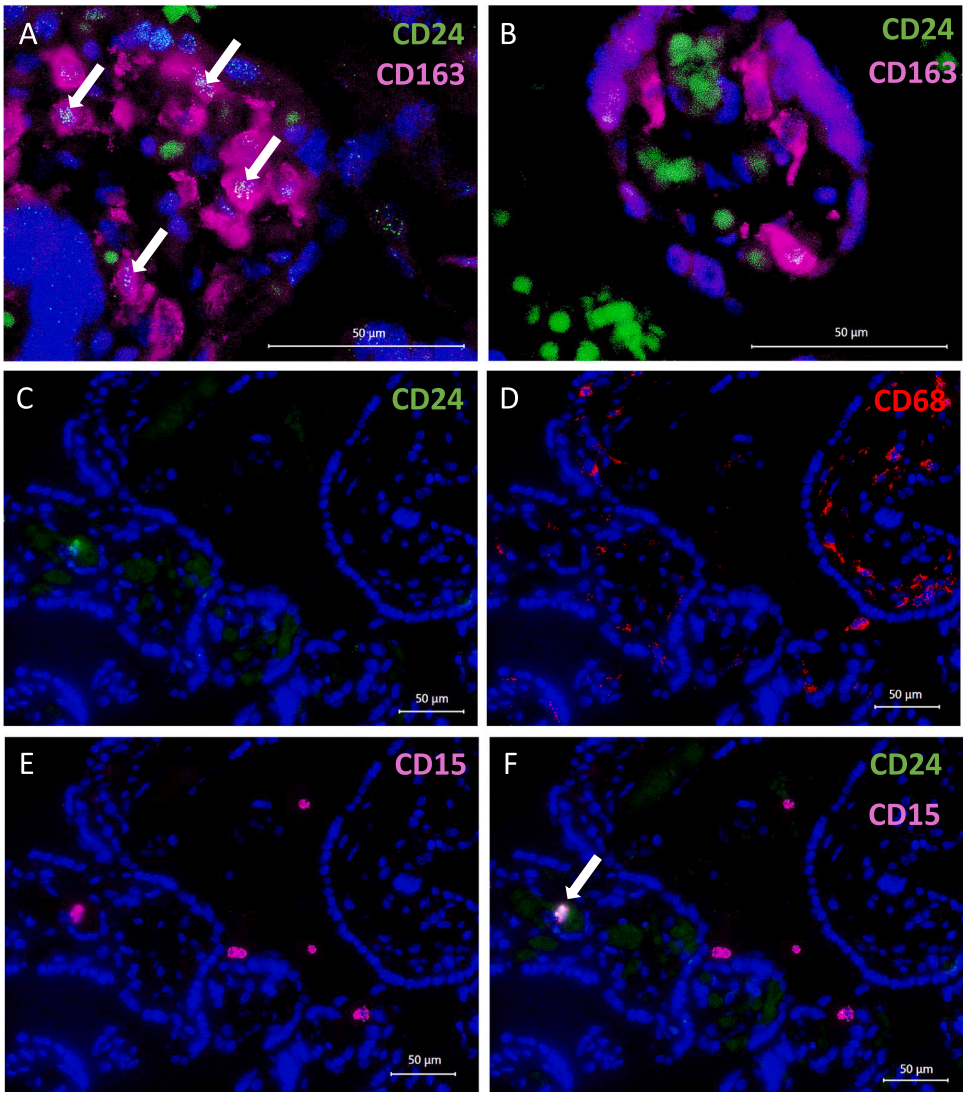

Fig. 6. Identity of Hofbauer cells as CD24-expressing cells in female healthy control placentas was proven by CD24 (green fluorescence) and CD163 (Hofbauer cell marker, pink fluorescence) double immunofluorescence staining (A). Hofbauer cells expressing CD24 are marked with white arrows. In acute covid 19 Hofbauer cells of female origin (pink fluorescence), CD24 (green fluorescence) is diminished (B). Almost no double expression of both markers is found. Within villous tissue of acute covid-19 placentas, few CD24-positive cells could be identified (green fluorescence, C). There is no double expression of CD24 and CD68 (macrophage marker, red fluorescence, D). Staining of CD15 (neutrophil marker, pink fluorescence, E) showed a match with CD24 and double expression of both markers (F) proved that CD24 positive cells within villous tissue are neutrophiles, all magnification 40x).

neutrophil derived extracellular vesicles (NDEVs) accumulate at the sites of infection or inflammation and their circulating levels are elevated in covid-19 patients (Guervilly et al., 2021). A very recent study of Bonifay et al. showed that CD15, CD24 and CD18 were the markers with the highest expression on NDEVs after covid-19 infection (Bonifay et al., 2022). The same group of authors proposed that the detection of this type of NDEVs could also be used on placenta samples but these studies were never performed. Expression of CD24 on placental samples was performed recently. Specifically in preeclampsia (Nagy et al., 2008) CD24 expression was shown, this was also proven by our research group (Sammar et al., 2021; Sammar et al., 2022). In first trimester pregnancy, co-localization of CD24 with its ligand SIGLEC-10 was observed in endometrial glands and in decidual cells in close vicinity to extracellular trophoblasts. This previous study was the first to demonstrate the early presence of CD24 in the placenta cytotrophoblast layers, placental bed and maternal uterine glands (Sammar et al., 2017). The presence of the CD24-Siglec-10 in these regions of fetal-maternal interactions suggests a possible role in mediating immune tolerance at the fetal-maternal interface (Sammar et al., 2017).

Our study showed differences in CD24 expression, specifically in Hofbauer cells in covid-19 female and in addition, in post-covid-19 male Hofbauer cells. Down regulation of CD24 in acute covid-19 Hofbauer cells could be explained as a response to acute viral infection. It is already known from other acute inflammatory diseases that down-regulation of CD24 plays a specific role in tissue protection as shown on autoimmune hepatitis (Zheng et al., 2018). On the other hand, the post-covid CD24-upregulation in male Hofbauer cells and its therefore upregulated immune modulation could be a hint for a persisting immune modulation specifically for the male post-covid offspring. Hofbauer cells originate from yolk sac macrophages and are a part of a first immune system formation of the fetus (Stremmel et al., 2018; Stremmel et al., 2018). Because these yolk sac macrophages not only migrate to the placenta but also to the fetus to form live long tissue specific macrophages like microglia and others, they could be responsible for long-lasting complications referred to as the post-COVID syndrome or long COVID, including fatigue or neurological sequelae (Knoll et al., 2021). There are also other studies, that found sex specific differences in covid-19 placentas. Bordt et al. characterized placental immune

responses in women who were infected with SARS-CoV-2 during pregnancy. The study revealed differential placental immune responses between male and female fetuses which were associated with decreased antibody transfer to male fetuses (Bordt et al., 2021; Bordt et al., 2021).

Another striking finding was, that we identified CD24-positive neutrophils in acute covid-19 placentas. We also checked the identity of the CD24-positive immune cells for immune cell populations other than neutrophils. For T-cell identity, we used CD3 and for macrophage identity, we used CD68. Neither CD24 was expressed on CD3 positive immune cells nor CD24 was expressed on CD68 positive immune cells in acute covid-19 placentas or specifically in the decidua of acute covid-19 placentas. Investigation of Redline et al. identified an early neutrophil-predominant intervillous infiltrate (Redline et al., 2022) on a covid-19 placenta. Although CD24 expression was never described before in an acute or post-covid-19 placenta, it is already known that upregulation of CD24 is combined with an aged neutrophil phenotype that inhibits inflammation by reducing chemotaxis, ROS production, and NADPH oxidase (Feng et al., 2023). This was already shown by earlier studies of Elghetany & Patel, which proposed that CD24 is expressed on neutrophilic granulocytes from the myelocytic stage onward and is usually not expressed on promyelocytes (Elghetany and Patel, 2002). In addition, we also found few CD24-positive neutrophils within the villous tissue on the fetal side of the acute covid-19 placenta. Usually, these cells were only identified on the maternal side. Therefore, this rare transfer of CD24-positive neutrophils could be a sign of micro chimerism, but this has to be confirmed in additional studies.

5. Conclusion

The data of our study showed a downregulation of CD24 in extravillous trophoblast cells and fetal Hofbauer cells in acute female covid-19 cases. In addition, in male cases we saw an upregulation of CD24 on fetal Hofbauer cells in a post-covid-19 situation. In addition, we identified CD24-positve neutrophils within the acute covid-19 placenta. Because available clinical data are consistent with a protective effect of CD24 in Covid-19 cases, these neutrophils could be a part of a mechanism of protection. Additional studies are required to confirm these beneficial effects.

CRediT authorship contribution statement

Christian Dannecker: Funding acquisition, Formal analysis, Data curation. **Peter Altevogt:** Resources, Project administration, Conceptualization. **Udo Jeschke:** Writing – original draft, Formal analysis, Conceptualization. **Marei Sammar:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Marina Seefried:** Writing – original draft, Investigation, Conceptualization. **Johanna Mittelberger:** Writing – review & editing, Resources, Formal analysis. **Manuela Franitza:** Validation, Project administration, Formal analysis. **Fabian Garrido:** Software, Resources, Data curation. **Carl Mathis Wild:** Validation, Supervision, Software. **Nina Ditsch:** Methodology, Formal analysis, Data curation. **Oleksii Protsepko:** Visualization, Resources, Methodology. **Christina Kuhn:** Resources, Methodology, Investigation.

Declaration of Competing Interest

N.D. reports funding from MSD, Novartis, Pfizer, Roche, AstraZeneca, TEVA, Mentor, and MCI Healthcare. C.D. is funded by Roche, AstraZeneca, TEVA, Mentor, and MCI Healthcare. All other authors declare no conflict of interest.

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