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Short communication

The CD200 tolerance-signaling molecule and its receptor, CD200R1, are expressed in human placental villus trophoblast and in peri-implant decidua by 5 weeks' gestation

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ABSTRACT

CD200 expression in murine trophoblast and decidua prevents semi-allogeneic and LPS-induced abortions by binding to CD200 receptor-bearing cells to suppress NK activity, induces IDO in macrophages, and promotes the generation of regulatory T cell subsets. CD200 and its receptor CD200R1 reported in 7–9 weeks' gestation human villus trophoblasts are reduced in spontaneous abortion syncytiotrophoblasts. By specific antibody staining, we find that both CD200 and CD200R1 are expressed even earlier, by 5 weeks' gestation, by villus trophoblasts and by decidual cells. Expression of CD200 was validated using two independent antibodies. CD200–CD200R1 signaling may be required for human pregnancy success.

1. Introduction

The IgG superfamily glycoprotein CD200 suppresses macrophage/microglial activation, promotes IDO production by macrophages and dendritic cells, suppresses NK and mast cells, induces γδT suppressor cells, and promotes Treg cells by altering dendritic cell maturation (reviewed in Clark and Gorczynski, 2013). CD200$^{-/-}$ mice develop severe autoimmunity and inflammation (Nathan and Muller, 2001). In the CBAxDBA/2 mouse model of spontaneous abortions triggered by TNF-α + IFN-γ, pregnancy loss is prevented by the expression of CD200 in trophoblast and decidua (Clark et al., 2001). LPS-induced abortion in B6 mice is blocked by up-regulating CD200 expression (Yu et al., 2008). CD200 has recently been described in human placental trophoblasts at parturition and in first-trimester (7–9 weeks) gestation placental villus trophoblasts, and in spontaneous abortion villi expression in syncytiotrophoblasts is significantly reduced (Wang et al., 2014). CD200 lacks an intracellular tail able to activate CD200-expressing cells and must bind to CD200 receptor (CD200R)-expressing cells to act (Clark and Gorczynski, 2013). There are at least two CD200R types expressed in murine pregnancy placenta/decidua and one CD200R type in humans (CD200R1) (Wright et al., 2003; Gorczynski et al., 2004). Until recently, expression of CD200R was

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thought to be restricted to lymphomyeloid cell subsets, but Wang et al. (2014) recently described CD200R on 7- to 9-week human placental villus trophoblasts. We also described CD200R expression on term placental trophoblasts and on a high proportion of early human breast cancers (Clark et al., 2014, 2015).

In this paper, we examined the expression of CD200 and CD200R1 in the placental and decidual tissue of a 5-week gestation pregnancy, which is earlier than any previous reports.

2. Materials and methods

Archived human pregnancy tissues from elective terminations and archived human tonsil tissue was studied with the approval of the Hamilton Integrated Research Ethics Board. From terminations at 5, 7, and 9 weeks, we selected for the current report a specimen at a gestational age of 5 weeks (Carnegie stage).

Immunohistochemistry was performed on 4-μg tissue sections on positively charged slides using a potent rabbit anti-CD200Fc serum from which anti-Fc activity had been absorbed (RB846) kindly provided by Dr. R. Gorczynski (Toronto General Research Institute) along with pre-immune rabbit serum as previously described (Clark et al., 2014, 2015). Dako ENVISION followed by AEC was used to detect rabbit IgG binding, and hematoxylin was used as the counterstain. CD200 staining was validated using an antigen-affinity purified rabbit anti-CD200 (anti-KLH-CD200, Antibodies Online, ABIN761396), and CD200R1 was stained using an antigen-affinity-purified rabbit anti-CD200R1 (anti-KLH-CD200R1, ABIN1715098). Rabbit monoclonal anti-Ki-67 (Thermoscientific Cat. RM106, USA) was used to detect proliferating cells. Antigen retrieval was performed in EDTA pH8.0 buffer, except for Ki-67, where pH 6.0 citrate was used. Stained slides were scanned using Imagescope and digital photographs were taken at 400×.

3. Results

Human tonsil stained for CD200 primarily in the follicular areas populated by B cells (B), which are known to be strongly $CD200^+$, in contrast to the area populated primarily by T cells (T). RB846 gave stronger staining (1/1000 dilution) that ABIN anti-CD200 (1/250, 4 μg IgG/ml) and pre-immune control rabbit serum (1/1000, ≈8–15 μg/ml IgG) was negative. ABIN anti-CD200R1 (1/250, 4 μg/ml IgG)

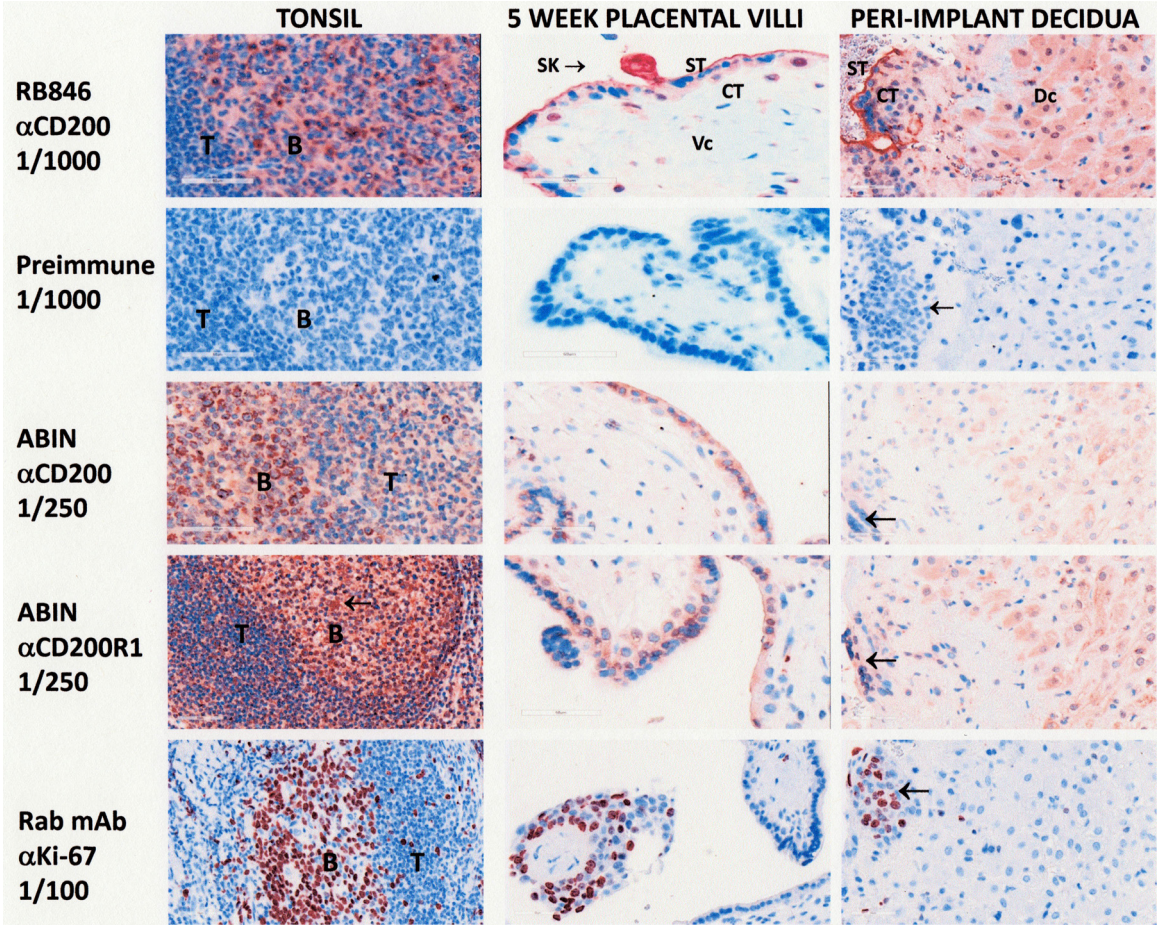

Fig. 1. Immunohistochemical staining of tonsil and placental villi and peri-implant decidua of 5 weeks' gestation. T = T cell region, B = B cell follicle, ST = syncytiotrophoblasts, CT = cytotrophoblasts, SK = syncytiotrophoblast knot, Vc = villus core, Dc = decidual cells. Magnification 400×. Scale bar 60 μm.