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Histamine Regulates Placental Cytokine Expression – In vivo Study on HDC Knockout Mice

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Abstract

Successful pregnancy is closely related to polarization toward a Th2 type immune response. As histamine is known to initiate Th2 dominance during inflammatory processes we raised the question whether histamine has any effect on the actual tuning of proper cytokine balance for the proceeding of the gestation. Histamine has multiple functions in the process of pregnancy, different studies have shown the direct and/or indirect presence of histamine action in the placenta as well. As HDC is the unique histamine producing enzyme in eukaryotes, we used HDC (so endogenous histamine)-deficient knockout mice as reliable model for studying histamine-related processes in vivo. We examined the placental histamine content and the expression of histamine receptors and Th1/Th2/Th3 type cytokines in the placenta. We showed for the first time the influence of histamine on the orchestrated regulation of placental cytokine expression. In the absence of local histamine the cytokine balance is shifted toward Th1 types at the maternal–placental interface, threatening pregnancy. We also measured splenic lymphocyte subpopulation ratios in pregnant and non-pregnant mice and found that in pregnancy they are independent of the presence of histamine.

Keywords: Histamine; Placental cytokine expression; Histamine receptors; Lymphocyte subsets

1. Introduction

Maternal immunosuppression is indispensable for successful pregnancy. The Th1/Th2 paradigm has been documented for long – that is lower ratio of Th1 and higher ratio of Th2 cytokines contribute to the maintenance of pregnancy [1,2]. Lately, a large number of studies have colored the picture of this paradigm, implying that several adaptations could operate in parallel to create and maintain maternal immunosuppression [3]. Besides, the state of pregnancy rather activates the innate immune system, meanwhile suppresses the adaptive one [4].

In eukaryotes, histamine is synthesized by a unique enzyme, L-histidine decarboxylase (HDC) [5]. Although mast cells are the main source of histamine, many other cell types express HDC and synthesize histamine themselves. Histamine acts through four types of receptors (H1R–H4R), their expression is tissue-specific [6,7]. The role of histamine in female reproductive processes has been described from several aspects [8–10]. Histamine has multiple functions in the process of pregnancy due to its vasoactive, differentiating and growth-promoting characteristics. Histamine is produced either by mast cells and/or uteral epithelial cells [11–13]. Pre- and post-implantation events are accompanied by high histidine decarboxylase (HDC) enzyme activity. HDC expression is much higher in the placenta than in any other organ and the presence of H1 and H2 receptors has also been shown in it [14–16]. All these findings suggest an important, still not yet clarified role of histamine in placental functions.

As HDC is the unique histamine producing enzyme in eukaryotes, the recently developed HDC (so endogenous histamine)-deficient knockout mice serve as a reliable model for

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studying histamine-related processes in vivo. Earlier we faced problems with the birth rate of our HDC knockout mice, their litter size was smaller than the one of wild types and their resorption rate was higher [17]. Considering that Th2 dominance is crucial during pregnancy – despite the necessity of the presence of some Th1 cytokines at different times of the gestational period – and that on the other hand during inflammatory processes histamine also initiates Th2 dominance [18,19], we raised the question whether histamine has any effect on the actual tuning of proper cytokine balance for the proceeding of the gestation. Since different studies have shown the direct and/or indirect action of histamine in the placenta, we focused on placental histamine receptor and cytokine expressions in HDC knockout (KO) and wild type (WT) pregnant mice. We intended to get a clearer picture about the placental contribution in the overall establishment of the pregnancy maintaining cytokine balance and its potential dependency on histamine. Moreover, healthy pregnancy is based on the balanced function of immune cells. Functional or numerical changes of these cells can result in reproductive failures. Therefore, in addition to the events at the feto-maternal interface, we measured lymphocyte subpopulation ratios at the systemic level too. The increasing prevalence of allergic diseases and the subsequent anti-histamine medication in the age of reproduction – even during pregnancy – underline the significance of this study.

2. Materials and methods

2.1. Animals

The experiments were conducted in accordance with accepted standards of animal care.

Histidine decarboxylase (HDC) gene targeted mice were generated as described [20]. Briefly, using isogenic mouse genomic DNA obtained by amplification of 129Sv-derived E14 ES cell DNA we designed the HDC targeting construct to replace a ~2.4 kb fragment extending from the *Spe*I site in intron 5 to the *Pst*I site in exon 9 with a PGK-*neo*^{r} cassette. The cloned fragments were linearized and introduced into the ES cell line R1 selected by G418 and gancyclovir. Out of six LA-PCR positive clones aggregated with CD1 morulae three of them were confirmed to be homologous recombinants by Southern blotting. Wild type and HDC knockout mice have been bred in our transgenic facility.

Normal rodent diet (histamine content >50 μmol/g) was provided ad libitum (Charles River, Hungary). Blood and spleen samples were taken from six non-pregnant 3–5-month-old female HDC knockout (KO) and wild type (WT) CD1 mice from multiple litters and from pregnant mice 14–15 days post coitum (dpc, counting 1 dpc as the day the plug was found) from multiple litters. Samples were collected with terminal ether anesthesia. Placentae were removed, bled out, snap frozen in liquid nitrogen, and then stored at −80 °C until use. Blood was taken from vena cava inferior. For cytokine expression measurements we took the placentae of four to five pregnant mice, two placentae from the same mother (8–10 placentae all together) in both experimental groups (wild type and HDC knockout).

2.2. Fluorimetric determination of histamine after HPLC

Organs and serum taken from the animals were weighted. The samples were stabilized and deproteinated in 0.5 M perchloric acid. After centrifugation, the supernatant was directly used for HPLC-separation by a LICHROSIL IC CA cation exchange column (flow 1 ml/min, mobile phase 0.75 g oxalic acid and 0.5 g tartaric acid/ml), post-column derivatisation with OPT (boric buffer, pH 9) and measured fluorimetrically (Ex 340 nm, Em 455 nm). The values were calculated by Borwin 1.5 software (Jasco, Gotha; Germany) linear regression.

2.3. Total cytoplasmic RNA preparation

Briefly, the following steps were carried out: homogenization of the samples by TRI reagent (Sigma), extraction with chloroform, precipitation with isopropanol, wash with alcohol. For H1 and H2 receptor studies RNA was treated by RNase-free DNase (Promega).

2.4. RT-PCR

Reverse transcription of an aliquot of total cytoplasmic RNA (1 μg) to cDNA was catalyzed by multi-reverse transcriptase (Perkin–Elmer) in the presence of 1 mM of each dNTPs, RNase inhibitor, random hexamers, 10× PCR buffer and $MgCl_2$ (Promega) to a total volume of 40 μl. The RT-mix was incubated at 42 °C for 10 min for reverse transcription, followed by 95 °C for 10 min to inactivate the reverse transcriptase. The cDNAs were stored at −20 °C until use.

2.5. Quantitative real-time PCR

The assays were performed based on Assays-on-Demand Gene Expression Products Protocol, Applied Biosystems.

Briefly, target cDNA samples synthesized from the RNA sample were amplified by AmpliTaq Gold DNA polymerase in the TaqMan Universal PCR Master Mix, using sequence-specific primers and TaqMan MGB probe. QRT-PCR was carried out using an ABI Prism 7000 Sequence Detection System (Applied Biosystems) and the associated ABI 2.0 software was used to analyze data and determine the threshold count regarding relative quantification of gene expression experiments referring to Sequence Detection System User Bulletin #2 (P/N 4303859). The primers and probes were designed using the Assays-on-Demand gene expression product list of Applied Biosystem.

Results are expressed as relative expression referred to the expression of housekeeping gene HGPRT. By using HGPRT endogenous control as an active reference we could normalize quantitation of our mRNA targets for differences in the amount of total RNA added to each reaction. Each reaction was carried out in duplicates and the average of 6–10 WT and KO placentae are shown, respectively. The measurement was repeated twice.

2.6. Cytofluorimetry (FACS analysis)

Spleen cell suspensions were stained by appropriate amount of fluorescently labeled monoclonal antibodies (usually 1 μl/10^6 cells from BD Pharmingen anti-mouse monoclonal antibodies). T cells were defined by the cell surface expression of CD3, Th cells by CD3/CD4 double positivity and Tc cells by the coexpression of CD3 and CD8. Splenic B and NK cells were characterized by their CD19 or NK1.1 expression. 10^5 leukocytes were acquired from each sample within 2 h after labeling, using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). The analysis was performed using the CellQuest Pro software (Becton Dickinson, San Jose, CA).

2.7. Statistical analysis

Student's t test was used to compare WT and HDC KO cytokine expressions and lymphocyte ratios. Results are presented as mean ± sem. $p \leq 0.05$ values were considered as statistically significant.

3. Results

3.1. Histamine concentrations at the feto-maternal interface

To clarify the importance of the presence of histamine at the feto-maternal interface, we measured the histamine concentration of the placenta, of the anatomically adjacent part of the uterus and of the serum by HPLC. In wild type (WT)