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Original article

CD1d-restricted NKT cells modulate placental and uterine leukocyte populations during chlamydial infection in mice

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Abstract

Invariant CD1d-restricted natural killer T cells play an important immunoregulatory role and can influence a broad spectrum of immunological responses including against bacterial infections. They are present at the fetal–maternal interface and although it has been reported that experimental systemic iNKT cell activation can induce mouse abortion, their role during pregnancy remain poorly understood. In the present work, using a physiological *Chlamydia muridarum* infection model, we have shown that, in vaginally infected pregnant mice, *C. muridarum* is cleared similarly in C57BL/6 wild type (WT) and CD1d$^{-/-}$ mice. We have also shown that infected- as well as uninfected-CD1d$^{-/-}$ mice have the same litter size as WT counterparts. Thus, CD1d-restricted cells are required neither for the resolution of chlamydial infection of the lower-genital tract, nor for the maintenance of reproductive capacity. However, unexpected differences in T cell populations were observed in uninfected pregnant females, as CD1d$^{-/-}$ placentas contained significantly higher percentages of CD4^{+} and CD8^{+} T cells than WT counterparts. However, infection triggered a significant decrease in the percentages of CD4^{+} T cells in CD1d$^{-/-}$ mice. In infected WT pregnant mice, the numbers of uterine CD4^{+} and CD8^{+} T cells, monocytes and granulocytes were greatly increased, changes not observed in infected CD1d$^{-/-}$ mice. An increase in the percentage of CD8^{+} T cells seems independent of CD1d-restricted cells as it occurred in both WT and CD1d$^{-/-}$ mice. Thus, in the steady state, the lack of CD1d-restricted NKT cells affects leukocyte populations only in the placenta. In *Chlamydia*-infected pregnant mice, the immune response against *Chlamydia* is dampened in the uterus. Our results suggest that CD1d-restricted NKT cells play a role in the recruitment or homeostasis of leukocyte populations at the maternal–fetal interface in the presence or absence of *Chlamydia* infection.

Keywords: *Chlamydia* infection; NKT cells; Mouse models; Leukocyte populations; Pregnancy

1. Introduction

Chlamydia trachomatis genital-tract infections lead to infertility or unsuccessful pregnancy in many animal species. Most of the damage due to *Chlamydiae* appears to be unrelated to the presence of the pathogen itself, but is due instead to the inflammation and fibrosis following infection.

In murine pregnancy models, the innate immune system, rather than the adaptive immune system, is responsible for

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infertility and spontaneous abortions [1,2]. The innate immune system provides rapid defense mechanisms based on recognition of pathogens by macrophages, neutrophils, natural killer (NK) and NKT cells, and secretion of inflammatory cytokines [3], which directly or indirectly lead to damage of Fallopian tubes or the placenta, leading to the demise of the fetus.

With the goal of better understanding the response of the maternal innate immune system to infection, we focused this study on the behavior of placental and uterine leukocytes, and especially CD1d-restricted NKT cells, during *Chlamydia* infection. CD1d-restricted NKT cells can be classified into type 1 NKT cells using an invariant Vα14-Jα18 TCR α chain (CD1d-restricted NKT cells) and type 2 NKT cells with diverse Vα TCR chains [4]. CD1d-restricted NKT cells, which can be activated by α-galactosylceramide (α-GalCer), have been more extensively studied than type 2 NKT cells [5]. However, little is known about the presence or function of uterine NKT cells and, particularly, CD1d-restricted NKT cells during pregnancy.

The activation of iNKT cells by α-GalCer, the ligand of the Vα14-Jα18 TCR chain of CD1d-restricted NKT cells, induced pregnancy loss, while the injection of α-GalCer into CD1d-deficient mice lacking CD1d-restricted NKT cells had no effect on pregnancy [6]. α-GalCer induces CD1d-restricted NKT cell activation and abortion in WT mice through a mechanism involving TNFα, IFNγ and perforin [6,7]. Proinflammatory cytokines such as TNFα, IL-1β and IFNγ appear to be critical mediators in the induction of pregnancy loss [8].

In general, the role of CD1d-restricted NKT cells during bacterial infections is not clear. CD1d-restricted NKT cells played a protective role in host defense against infection by some bacterial pathogens such as *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa* [9,10], but the presence of these cells was detrimental in the case of *Salmonella* and *Listeria* infections [11,12]. In the case of chlamydial infections, CD1d-restricted NKT cells conferred protection against *C. trachomatis*-induced arthritis [13], or *C. muridarum*-induced genital tract infection [14]; but Bilenki et al. [15] have shown, conversely, that CD1d-restricted NKT cells promote *C. muridarum* lung infection. Moreover, using another chlamydial species, *Chlamydophila pneumoniae*, Joyee et al. [16] reported that CD1d-restricted NKT cells play a crucial role in mediating the protective immune responses against lung infection. Hence, the role of CD1d-restricted NKT cells during chlamydial infection is still not fully understood. In the experiments by Bilenki et al. [15], *C. muridarum* was inoculated intranasally. We therefore sought to determine whether CD1d-restricted NKT cells may play the same role after intravaginal infection by *C. muridarum*, since the infection pathway, the defense mechanisms of each mucosa and the tissue distribution of CD1d-restricted NKT cells are most likely critical factors in the response to chlamydial infection.

To study the involvement of CD1d-restricted NKT cells in pregnancy and chlamydial infection, we compared 4 groups of pregnant mice: C57BL/6 (B6) WT and CD1d-deficient ($CD1d^{-/-}$) pregnant mice, infected or not with *C. muridarum*. We first verified that the course of infection was not affected in non-pregnant B6 mice by CD1d-deficiency or CD1d-restricted NKT cell activation by α-GalCer. Subsequently, we characterized the uterine and placental leukocytes in WT and $CD1d^{-/-}$ pregnant females to determine whether CD1d-restricted NKT cells had any effect on other leukocyte populations during infection with *C. muridarum*.

Our results indicate that although CD1d-restricted NKT cells are not required for the resolution of chlamydial infection of the lower-genital tract, they do play a role in the recruitment or homeostasis of several other uterine and placental leukocyte populations present at the fetal–maternal interface of vaginally-infected mice.

2. Materials and methods

2.1. Animals

C57BL/6JRj (B6) wild type (WT) mice were obtained from the breeding center CERJ Janvier (Le Genest Saint Isle, France) and $CD1d1^{-/-}$ mice (CD1d1 homozygous knock-out mice), back-crossed onto the C57BL/6J genetic background, were obtained from Dr. Van Kaer [17]. Colonies were raised and housed in specific pathogen-free conditions, following institutional guidelines.

2.1.1. Infection of nonpregnant animals with C. muridarum

We used five nonpregnant WT B6 and five nonpregnant $CD1d^{-/-}$ seven week-old mice per experiment. The mice were injected subcutaneously with progesterone (2.5 mg of Depo-Provera) 7 days before vaginal infection with 10^6 infectious forming units (IFU) of *C. muridarum* in a final volume of 20 μl.

2.1.2. Injection of nonpregnant mice infected with C. muridarum with α-galactosylceramide (α-GalCer)

We injected 10 nonpregnant C57BL/6 mice with progesterone 7 days before infection with 10^6 IFU of *C. muridarum*. Five of these mice were injected 4 times i.p. with 2.5 μg of α-GalCer (a gift from Dr. M. Bonin [18]), on days −2, 0, +3 and +7 after vaginal infection. The other 5 control mice were injected with excipient at the same times.

2.1.3. Infection of pregnant mice with C. muridarum

B6 WT and $CD1d^{-/-}$ pregnant mice were infected vaginally with 10^6 IFU of *C. muridarum*, 5.5 days *post coitum* (dpc) and sacrificed at 14.5 dpc. Uninfected B6 WT and $CD1d^{-/-}$ pregnant mice were used as controls and sacrificed at 14.5 dpc.

2.2. Quantification of infection

The course of infection was monitored by collecting cervico-vaginal swabs for 3 weeks after infection, and quantifying the *Chlamydiae* present on the swabs. We used sterile DACRON polyester tipped applicators from Puritan (Hardwood Products Company LP Guilford, Maine, USA) for the vaginal swabs. Bacterial DNA was extracted from the swabs