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Short communication

Ly49 knockdown in mice results in aberrant uterine crypt formation and impaired blastocyst implantation

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ABSTRACT

Genetic knockdown (KD) of the mouse Ly49 receptor family is reported to result in infertility despite the presence of zona-enclosed blastocysts in the uterus. Ly49 receptors regulate leukocyte functions particularly Natural Killer (NK) cell functions and are analogous to human killer immunoglobulin-like receptors (KIRs). Histological analyses of gd3.5–4.5 B6.Ly49^{KD} uteri identified hatched but retarded blastocysts with pyknotic nuclei, aberrant endometrial crypt formation and impaired uterine lumen closure accompanied by a lack of primary decidualization These data support peri-implantation roles for leukocytes expressing the Ly49 receptor repertoire and may give insight into KIR-based regulation of human infertility.

1. Introduction

Implantation involves bidirectional interactions between the blastocyst and the temporarily receptive uterus during the window of implantation [1–3]. In mice, blastocyst attachment occurs late on gestational day (gd)3.5, followed by implantation, localized to crypts at the antimesometrial (AM) pole [2,4], and lumen closure at gd4.5 [1,2,5]. Aberrant crypt formation and impaired blastocyst implantation are associated with pregnancy failure [2].

In humans, a family of activating and inhibitory killer immunoglobulin-like receptors (KIRs) is associated with pregnancy success. Uterine Natural Killer (uNK) cells constitutively express these receptors [6]. Certain haplotype combinations of maternal KIR-fetal HLA that lack leukocyte activation potential are associated with infertility, recurrent miscarriage and other gestational complications [7,8]. UNK cells are present in non-pregnant human and mouse endometrium [9] but numbers expand tremendously in mesometrial decidua basalis after implantation [10]. UNK cells are associated with decidual integrity, angiogenesis and uterine lumen closure [11,12]. These actions are promoted by cytokine and angiokine production [13,14].

The Ly49 gene family [15], analogous to human KIRs, regulates mouse NK cell activity. We previously investigated pregnancies in C57BL/6 (B6) mice with knockdown (KD) of the entire Ly49 gene family (B6.Ly49^{KD}) [16]. Failure to establish pregnancy after mating occurred in approximately 70% of syngeneically-mated B6.Ly49^{KD} females, although zona-enclosed blastocysts of normal appearance were flushed at gd3.5 [16], suggesting peri-implantation embryonic losses. Our study objective was to establish the basis for B6.Ly49^{KD} female infertility.

2. Materials and methods

2.1. Animals

C57BL/6 (B6) mice were purchased from Charles River Laboratories; B6-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ alymphoid (NK-/uNK-,T-,B-) mice were purchased from Taconic Biosciences. Both strains were maintained at Queen's University. B6.Ly49^{KD} mice [17] were bred and maintained at the University of Ottawa. Mice over 6 weeks of age were mated syngeneically with copulation plugs considered gd0.5. Mice were euthanized by cervical dislocation. Breeding and animal manipulations were conducted in accordance with the Canadian Council on Animal Care guidelines and were approved by the Animal Care Committees of both universities.

Abbreviations: AM, antimesometrial; DC, dendritic cell; gd, gestation day; KIR, killer immunoglobulin like receptor; uNK, uterine natural killer (cell); WM-IHC, whole mount immunohistochemistry.

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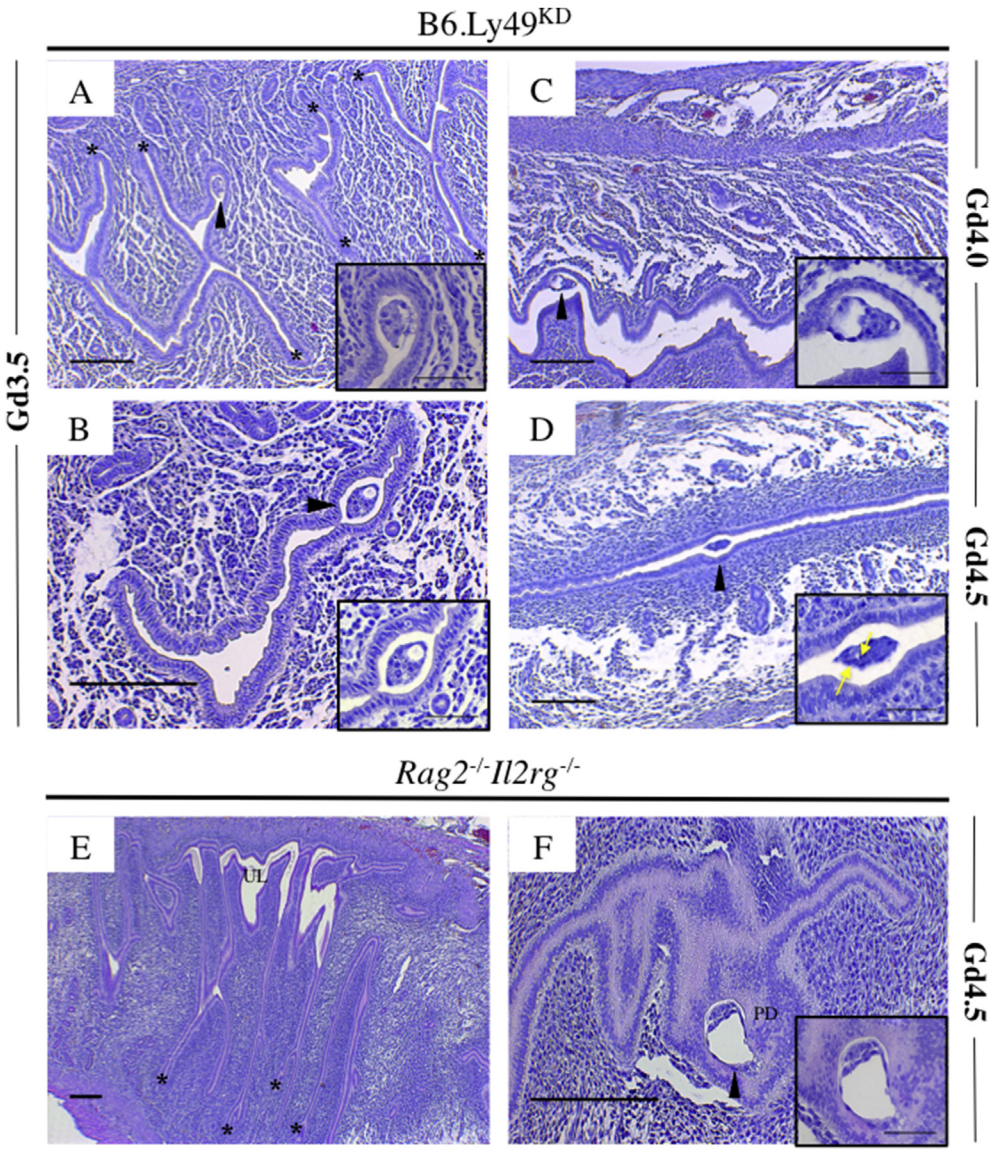

Fig. 1. Photomicrographs of longitudinal sections of H&E stained mouse uteri oriented with the mesometrial pole to the top of each panel. Hatched blastocysts (indicated by arrowheads) in B6.Ly49^{KD} uteri are shown at lower and higher power (insets) at gd3.5 (A–B), gd4.0 (C) and gd4.5 (D). Deeply invaginated crypts, typical of the window of implantation (2) are seen in B6.Ly49^{KD} only on gd3.5 (A) and extend anomalously towards the mesometrial as well as the AM pole. The larger, darkly stained, hyperchromatic cells present in B6.Ly49^{KD} blastocyst inner cell mass and trophoblast are suggestive of pyknosis and oncosis. Failure to induce invasion or primary decidualization is seen at gd4.0 (D) when the uterine lumen is abnormally dilated and at gd4.5 (E) when the uterine lumen has narrowed, indicating the window of implantation has passed. The small hyperchromatic spots (yellow arrows) in the retarded, dying gd4.5 blastocyst, are fragments of ruptured cell nuclei indicative of the process of karyorrhexis and blastomere necrosis. The uterus of a gd4.5 alymphoid Rag2$^{-/-}$IL2rg$^{-/-}$ mouse is shown at lower (E) and higher (F) power. Deep implantation crypts extend only towards the AM side of the uterine lumen (E) and blastocyst implantation (F; inset: higher magnification) is accompanied by initiation of decidualization. Both features are characteristic of gd4.5 implantation sites in normal mice (2). UL, uterine lumen; PD, primary decidualization. Scale bars: 200 μm, insets: 100 μm.

2.2. Histology

For paraffin sections, uteri were harvested from B6.Ly49^{KD} and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice at gd3.5 (n = 2 B6.Ly49^{KD}), gd4.0 (n = 2 B6.Ly49^{KD}) and gd4.5 (n = 3 B6.Ly49^{KD}, and n = 2 *Rag2*$^{-/-}$*Il2rg*$^{-/-}$). Samples were immersion fixed in 4% paraformaldehyde (PFA) for 24 h and paraffin-embedded. Six μm thick longitudinal sections were cut, stained with hematoxylin and eosin (H&E).

For whole mount immunohistochemistry (WM-IHC) B6 virgin, gd3.5 and gd4.5 uteri (n = 2/gd) were studied. Freshly dissected uteri were dissected, sectioned longitudinally, and incubated for 1 h in PBS-1%BSA-0.1% sodium azide (PBA) with 10 μg/mL of blocking antibody to the IgG Fc receptor (anti-CD16/CD32; supernatant of hybridoma 2.4G2, ATTC, Manassas, VA) and 2 μg/mL of fluorescently conjugated anti-CD45 APC (BioLegend, Cedarlane, Burlington ON), and anti-Ly49C/I PE (BD Pharmingen, Mississauga, ON) primary antibodies. Both the live, WM-IHC stained explants and the H&E-stained paraffin tissue sections were examined using a Zeiss M1 Imager microscope (Zeiss; Toronto, ON, Canada) equipped with Axiovision 4.8 software.

3. Results/discussion

At gd3.5, expanded B6.Ly49^{KD}, zona-free blastocysts were located within the uterine crypts at the AM pole of the uterus (Fig. 1A, B). Darkly stained pyknotic nuclei were randomly present