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Original article

Characterization of estrogen-responsive epithelial cell lines and their infectivity by genital *Chlamydia trachomatis*

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Abstract

Chlamydial attachment and infectivity in vitro and ascending disease and sequelae in vivo have been reported to be enhanced/modulated by estrogen.

Endometrial carcinoma cell lines Ishikawa and HEC-1B and the breast cancer lines MCF-7 and HCC-1806 were examined for *Chlamydia trachomatis* E infectivity. Estrogen receptor (ER) presence was confirmed by Western blot and qRT-PCR analyses. FACS analysis was used to determine the percent of plasma membrane-localized ERs (mERs), and their activity was tested by estrogen binding and competitive estrogen antagonists assays. Chlamydiae grew in all cell lines with HEC (90%) >> MCF-7 (57%) > Ishikawa (51%) >> HCC-1806 (20%). The cell line ER isoform composition was re-defined as: ERα + ERβ + for MCF-7, HCC-1806 and Ishikawa; and ERβ only for HEC-1B. HeLa cells were also tested and found to express ERβ, but not ERα. A small percentage of both ERs were surface-exposed and functionally active. The endometrium-predominant ERβ isoform was found in all cell lines, including those most representative of the common sites of *C. trachomatis* infection. Thus, the role of chlamydial attachment/infectivity will now be analyzed in ERβ + and—isogenic HEC-1B cells.

Keywords: *Chlamydia trachomatis*; Chlamydia; Hormone modulation; Estrogen; Cell lines; Estrogen receptors

1. Introduction

Chlamydia trachomatis serovars D-K are the most common cause of bacterially-acquired sexually transmitted diseases (STDs) [1,2]. According to the World Health Organization, 340 million new cases of STDs occurred worldwide in 1999, of which 91 million were due to *C. trachomatis* [3,4]. In the USA, ~ 4 million new cases of chlamydial STDs are reported annually. The highest incidence of chlamydial infections is in females between 15 and 19 years of age [5]. Unfortunately, greater than 85% of women with chlamydial cervicitis are asymptomatic and are at risk for ascending infection. Indeed, Jones et al. [6] reported that *C. trachomatis* was isolated from the endometrium of 41% of women with asymptomatic cervicitis. Approximately half of the 1 million diagnosed cases of pelvic inflammatory disease (PID) per year are due to *C. trachomatis*. The total cost of PID and its sequelae, including tubal factor infertility and ectopic pregnancy, is estimated to be in the range of $10 billion annually [7]. It has recently become obvious that a substantial portion of PID cases does not present with classic signs and symptoms of acute PID and go unrecognized, leading to a condition termed "subclinical PID" [8]. New data suggest that 25% of these women are chlamydia-positive [9]. Thus, the consequences for chlamydia-positive women in their reproductive years can be devastating.

A key feature influencing reproductive physiology and the uterus, in particular, is the female hormones, estrogen and progesterone. Estrogen predominates in the first 10 days after menstruation and peaks at ovulation (~14–15 days). During this proliferative phase, growth of the endometrial glands increases and epithelial cells migrate out of the glands to re-seed the denuded endometrial surface. Progesterone peaks

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during the 10 days after ovulation, the secretory phase, for release of mucous, and if implantation does not occur, the progesterone and estrogen levels drop to orchestrate menstruation [10].

Moses et al [11], using the *C. psittaci* guinea pig inclusion conjunctivitis strain (GPIC), and Bose and Goswami [12], using the *C. trachomatis* biovar Lymphogranuloma venereum, were among the first investigators to show that estrogen enhances the attachment and infectivity of chlamydial elementary bodies (EBs) for HeLa cells in vitro.

A study by Sweet et al. [13] revealed a significant prediction for development of salpingitis as a complication of chlamydial infection if chlamydial infection occurred in the early, estrogen-dominant phases of the cycle. These latter clinical findings of the enhancing effects of estrogen on chlamydial infection in the upper genital tract have been validated in animal models in vivo [14,15]. For example, Rank et al. [15] found that when the arrival of chlamydiae in the upper genital tract coincided with high estradiol levels, a significantly greater percentage of animals developed chronic inflammation, fibrosis and tubal dilation of the oviducts.

In previous studies in our own laboratory attempting to characterize chlamydial infection parameters in primary human genital epithelial cells, endometrial gland epithelial cells (HEGEC) were obtained from discarded hysterectomy tissues and cultured ex vivo [16]. Analyses performed on a series of tissues obtained throughout the menstrual cycle clearly showed that estrogen-dominant HEGEC were dramatically more susceptible (> 90%) to serovar E attachment and infectivity than were progesterone-dominant epithelial cells; as the progesterone concentration increased in the latter 10 days of the cycle prior to menstruation, chlamydial attachment and infectivity decreased from 50% to 35% and, finally, 20% [17]. These unwitting observations supported earlier in vitro and in vivo findings that chlamydial infection might be modulated by female hormones. Davis et al. [18] showed that infectious EB of *C. trachomatis* serovar E, bound to the apical surfaces of the polarized human endometrial epithelial cell line HEC-1B, were associated with protein disulfide isomerase (PDI), a protein chaperone associated with the estrogen receptor (ER) complex [19]. Finally, further confirmation of the above findings was obtained by Guseva et al. [20] in a swine model. Both luminal and glandular epithelial cells from the uterus and uterine horns cultured ex vivo from mature female swine were more susceptible to *C. suis* S45 infection when the epithelial cells were obtained in the estrogen-dominant phase versus the progesterone-dominant phase.

In contradiction to the purported role of estrogen in enhancing chlamydial infection, serial clinical studies, performed to determine if there was a connection between a woman's menstrual cycle and chlamydial infection, showed a significant increase in cervical epithelial cell sensitivity to *C. trachomatis* infection in the late stages of the cycle [21–23]. While this stage is associated with high levels of both estrogen and progesterone, there is a predominance of progesterone in the genital tissues as well as in the bloodstream.

While the uterus is the major target of estrogen action, there are heterogeneous cell types in the uterus that respond differently to estrogen. The cellular action of steroid hormones is mediated through their ability to bind to receptors, which are basically ligand-activated transcription factors. In mammals, ERs are now known to exist in at least two types—the classical ERα and the newly discovered ERβ [24,25], and in three locations—the eukaryotic nucleus, cytoplasm and the plasma membrane. In the plasma membrane, a sub-population of ERs appears to concentrate mainly in discrete domains, known as caveolae. In caveolae, cross-talk with signaling molecules facilitates estrogen/ER cell biological action [26]. Higher concentrations of estrogen lead to increased numbers of ERs in uterine epithelial cells, and in humans, the maximum number of ERs occurs during the early proliferative phase of the menstrual cycle [27].

Since there are differences in the distribution of ERα and ERβ in human organs, tissues of different origins and their derived cell lines have been used to study the functions and actions of these receptors; human endometrial epithelial cells are richer in ERβ whereas breast carcinoma cells are richer in ERα. Thus, the purpose of this study was to examine well-studied epithelial cell lines of different origins and reportedly different hormone sensitivities in vitro in an attempt to begin to dissect the documented effect of estrogen on chlamydial infectivity in vitro.

2. Materials and methods

2.1. Cell lines

The epithelial cell lines obtained from the American Type Culture Collection (ATCC, USA) were: (i) ductal breast carcinoma MCF-7 (HTB-22, ATCC), designated as ER positive, and (ii) an ER-negative breast cancer cell line subclone, HCC-1806 (CRL-2335, ATCC); and (iii) the transformed endometrial line HEC-1B (HTB-113, ATCC). The Ishikawa cell line, originated also from endometrial carcinoma and characterized as ER positive, was kindly provided by Dr. Alison Quayle and S.J. Greene (Louisana State University Health Science Center, New Orleans, LA, USA). All cultures were routinely monitored and shown to be free of mycoplasma contamination. Stock cultures of the epithelial cell lines were propagated in flasks containing Minimum Essential Medium (MEM, Gibco, Grand Island, NY, USA) supplemented with 10% FBS. For estradiol binding assays, the epithelial cells were grown in phenol red-free MEM supplemented with 10% charcoal–dextran stripped FBS to reduce exogenous estrogen.

2.2. Chlamydia strain

A human urogenital isolate of *C. trachomatis* serovar E/UW-5/CX was originally obtained from S.P. Wang and C.-C. Kuo (University of Washington, Seattle, WA, USA) and