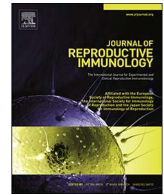
Journal of Reproductive Immunology 126 (2018) 69–75

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri



Circulating pathogen-specific plasmablasts in female patients with upper genital tract infection

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ARTICLE INFO

Keywords:
Lymphocyte homing
Adhesion molecule
Homing receptor
Pathogen-specific antibody-secreting cell
Plasmablast
Upper female genital tract
ELISPOT

ABSTRACT

Mucosal antibodies constitute the first line of adaptive immune defence against invaders in the female genital tract (FGT), yet the sequence of events leading to their production is surprisingly poorly characterized. We explored the induction of pathogen-specific antibody-secreting cells (ASC) as a response to an acute infection in the upper FGT.

We recruited 12 patients undergoing surgery due to an upper FGT infection (7/12 blood culture positive, 5/12 negative) and six healthy controls. Pathogens were sampled during surgery and PBMC collected in the acute phase of the disease (days 7–10). We searched by ELISPOT circulating pathogen-specific ASC and explored their frequency, immunoglobulin isotype distribution, and expressions of homing receptors ($\alpha_4\beta_7$, L-selectin, and CLA).

All patients had circulating ASC specific to the infective bacteria; the geometric mean was 434 (95%CI 155–1234) ASC (IgA + IgG + IgM)/10^6 PBMC. IgA ASC predominated in 7/12, IgG ASC in 3/12, and IgM ASC in 2/12 cases. Of all the pathogen-specific ASC, 60% expressed $\alpha_4\beta_7$, 67% L-selectin, and 9% CLA.

This study is the first to show induction of pathogen-specific ASC in the peripheral blood in bacterial infection in the human FGT. Our findings reveal that such FGT-originating pathogen-specific ASC are predominated by IgA ASC and exhibit a homing receptor profile resembling that of ASC in acute urinary tract infection. The data thus suggest a characteristic profile shared by the urogenital tract.

1. Introduction

The female genital tract (FGT) is uniquely challenged to provide protection against pathogens without compromising reproduction. The FGT comprises upper (Fallopian tubes, uterus, endocervix) and lower (ectocervix, vagina) parts, each harbouring a characteristic immune defence influenced by hormonal changes (Wira et al., 2015). Mucosal antibodies act as key players providing the first specific line of defence against a variety of pathogens (Woof and Mestecky, 2005). Nonetheless, the induction of specific antibody response in the human FGT is insufficiently characterized as yet.

An antigen encountered at mucosal sites activates its cognate naïve B lymphocytes which enter the lymphatics and return, via blood circulation, to mucosal sites (Brandtzaeg and Johansen, 2005; Sigmundsdottir and Butcher, 2008). Consistent with this migration, effector B cells, plasmablasts, can be found in the human circulation after mucosal antigen encounter: circulating antigen-specific antibody-secreting cells (ASC) have been reported after oral (Czerkinsky et al., 1987; Kantele, 1990; Kantele et al., 1986), rectal (Kantele et al., 1998; Kutteh et al., 2001), or intranasal vaccinations (Quiding-Järbrink et al., 1997), and in various mucosal infections such as gastroenteritis (Kantele et al., 1988, 2008; Kantele et al., 1996a; Pakkanen et al., 2010), pyelonephritis and cystitis (Kantele et al., 1994, 2008), and tonsillitis, sinusitis, and pneumonia (Palkola et al., 2012, 2016). Thus far, circulating plasmablasts have not been explored in acute bacterial infection in the genital tract.

Homing of lymphocytes from circulation into tissues is a selective process where tissue-specific homing receptors (HR) and chemokine

Abbreviations: FGT, female genital tract; ASC, pathogen-specific antibody-secreting cell; CLA, cutaneous lymphocyte antigen; HR, homing receptor; CCR, chemokine receptor; PLN, peripheral lymph node; ISC, immunoglobulin-secreting cell

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https://doi.org/10.1016/j.jri.2018.02.005
Received 13 October 2017; Received in revised form 12 January 2018; Accepted 21 February 2018
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receptors (CCR) on the circulating cells recognize their respective ligands in the target tissue (Brandtzaeg and Johansen, 2005; Sigmundsdottir and Butcher, 2008). Tissue-specific HR have been identified: $\alpha_{4\beta7}$ guides the cells to the intestine (Berlin et al., 1993), L-selectin to peripheral lymph nodes (PLN) (Camerini et al., 1989), and cutaneous lymphocyte antigen (CLA) to cutaneous sites (Berg et al., 1991). Lymphocytes typically home back to sites where the antigen was initially encountered (Sigmundsdottir and Butcher, 2008). Accordingly, after intestinal antigen encounter, a high proportion of plasmablasts express $\alpha_{4\beta7}$ and lower proportions L-selectin (Kantele et al., 1997; Kantele et al., 1996a; Quiding-Järbrink et al., 1997) or CLA (Kantele et al., 2003), while antigen encounter at other mucosal sites elicits a different homing profile (Kantele et al., 2008; Palkola et al., 2015, 2016; Quiding-Järbrink et al., 1997). Likewise, in mice, the set of receptors operating in the homing process differs between the various mucosal sites (e.g. nasal *vs.* intestinal) (Csencsits et al., 1999). There is, however, a lack of data on HR expressions associated with antigen encounter in the FGT.

We sought to identify circulating FGT-originating plasmablasts in patients with acute bacterial infection in the upper FGT. We explored ASC specific to bacteria isolated from the site of infection and characterized the response in terms of magnitude, isotype distribution, and HR expressions.

2. Methods

2.1. Study design

We investigated circulating plasmablasts (pathogen-specific ASC and all immunoglobulin-secreting cells, ISC) and their homing potentials in patients with bacterial upper FGT infection and healthy controls (Fig. 1).

PBMC were analyzed for ASC and ISC by ELISPOT. Immunomagnetic cell sorting was combined with ELISPOT to determine their expression of HR. Samples for bacterial cultures were obtained during surgery and the bacteria applied as an antigen to detect pathogen-specific ASC.

The Ethics Committee of Helsinki University Hospital approved the study protocol (411/E5/02). Written informed consent was obtained from volunteers.

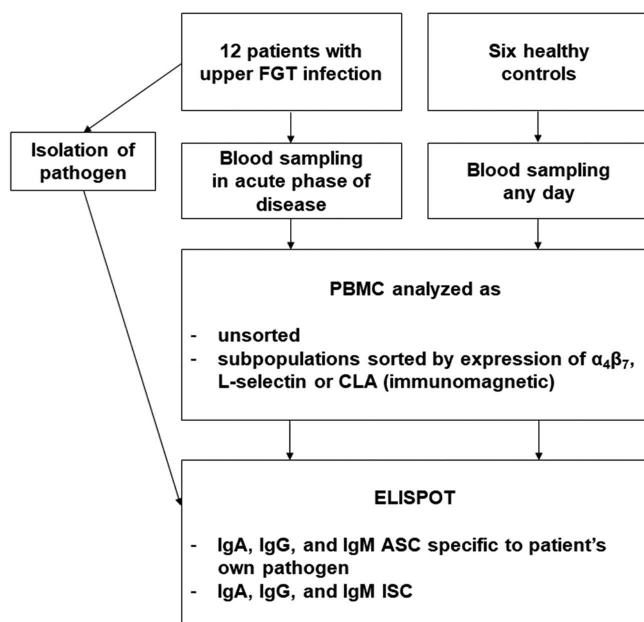

Fig. 1. Flow diagram of the study (ASC – pathogen-specific antibody-secreting cell, ISC – immunoglobulin-secreting cell).

2.2. Study subjects

A total of 12 patients (aged 18–51) with acute bacterial upper FGT infection were recruited at Helsinki University Hospital (Table 1). Blood culture proved positive for 7/12 (invasive) and negative for 5/12 (non-invasive) patients. Six healthy volunteers (35–47 years) served as controls.

2.3. Sorting of PBMC by homing receptor expression

PBMC were sorted into HR^+ and HR^- cell populations by immunomagnetic cell sorting as described earlier (Kantele et al., 1997; Kantele et al., 1996b). In brief, mAbs specific to L-selectin (Leu-8), $\alpha_4\beta_7$ (ACT-1), or CLA (HECA-452) were incubated with PBMC. After washings, magnetic Dynal M-450 beads (coated with sheep anti-mouse IgG; Oslo) were added to separate HR^+ from HR^- cells. The efficiency of the separation has been reported previously (Kantele et al., 1997).

2.4. Enumeration of ASC and ISC

Unsorted PBMC and subpopulations HR^+ and HR^- were analyzed for pathogen-specific ASC and ISC by ELISPOT, as described earlier (Kantele, 1990). Briefly, for ASC, each patients' own bacterial isolate or panel of these isolates (healthy controls) was used to coat microtiter plate wells. The isolates were applied as suspensions of formalin-killed whole bacteria (8×10^6 bacteria/mL PBS; 50 µL/well for three hours at 37 °C or overnight at 20 °C). For controls, coating suspensions of six patients were tested, each in their individual wells. The concentration of the coating antigen was thus the same for patients and controls. For ISC, human IgA, IgM, (Dako, Glostrup, Denmark), or IgG (Sigma, Immuno Chemicals, St. Louis, MO) –specific antisera were used for coating. After washings and blocking, aliquots of unsorted PBMC or HR^+ and HR^- subpopulations were allowed to secrete antibodies in the wells. Next, alkaline phosphatase-conjugated anti-human IgA, IgG (Sigma) or IgM (Southern Biotech, Birmingham, AL) were added, followed by the substrate (5-bromo-4-chloro-3-indolyl phosphate, Sigma) in melted agarose; spots were enumerated under a light microscope.

2.5. Statistical analysis

The numbers of pathogen-specific ASC and all ISC were given as geometric means of ASC/ISC (IgA + IgG + IgM)/10^6 PBMC with 95% confidence intervals (95%CI) as counted using bootstrapping in four groups: all patients, blood-culture positive and negative patients, and healthy controls.

HR expressions were determined as percentages of ASC:

$\% HR^+ASC = 100 \times$ (number of ASC in HR^+ population)/(total of ASC in HR^+ and HR^- populations)

or ISC:

$\% HR^+ISC = 100 \times$ (number of ISC in HR^+ population)/(total of ISC in HR^+ and HR^- populations).

The proportion of ASC or ISC expressing the various HR were given as arithmetic means with SD. To obtain reliable statistics in the HR analyses, we only included those with ≥ 20 identified spots.

Independent-samples Mann-Whitney *U* test and related-samples Wilcoxon Signed Rank test were applied for comparisons (SPSS 24.01; SPSS Inc). $P < 0.05$ was considered significant.

3. Results

3.1. Number of ASC and ISC

Pathogen-specific ASC (IgA + IgG + IgM) were found in all patients

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