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

Nina V. Palkola, Sari H. Pakkanen, Oskari Heikinheimo, Jussi M. Kantele, Anu Kantele

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 (α4β7, 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)/10⁶ 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 α4β7, 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 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...
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: α4β7 guides the cells to the intestine (Berlin et al., 1989), L-selectin to peripheral lymph nodes (PLN) (Campanini 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 α4β7 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 ASC and ISC by ELISPOT, as described earlier (Kantele et al., 1997; Kantele et al., 1996b). In brief, mAbs specific to L-selectin (Leu-8), α4β7 (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).

2.2. 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), α4β7 (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 × 10⁶ 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)/10⁶ 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 × (number of ASC in HR+ population)/(total of ASC in HR+ and HR− populations)

or ISC:

% HR+ ISC = 100 × (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
Table 1
Patient demographics, clinical data, number of pathogen-specific antibody-secreting cells (ASC), and proportions of Ig-isotypes.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Age</th>
<th>Infection</th>
<th>Pathogen</th>
<th>Daya</th>
<th>Fever °C</th>
<th>CRP mg/L</th>
<th>ASC/10⁶ PBMC</th>
<th>IgA ASC%</th>
<th>IgG ASC%</th>
<th>IgM ASC%</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
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<td>23</td>
<td>Salpingitis</td>
<td><em>Escherichia coli</em></td>
<td>7</td>
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<td>189</td>
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<td>6</td>
<td>12</td>
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<td>&gt; 200</td>
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<td>NA</td>
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<tr>
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<td><em>Streptococcus pyogenes</em></td>
<td>9</td>
<td>&gt; 39</td>
<td>300</td>
<td>20</td>
<td>16</td>
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<td>84</td>
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</table>

a number of days between onset of symptoms and blood sample.

b (IgA + IgG + IgM) ASC.

c ASC and all ISC.

c ASC and all ISC.

3.2. Isotype distributions of ASC and ISC

The geometric mean for IgA ASC was 140 (95%CI 28–866)/10⁶ PBMC, for IgG ASC 103 (22–405), and for IgM ASC 50 (13–181) (Fig. 3A). IgA ASC were detected in all patients; one patient had no IgG and another no IgM ASC. IgA ASC predominated in 7/10 and IgG in 3/10 patients with the highest responses and IgM in the two low-responders (Fig. 3A).

Circulating ISC were seen in all three isotypes for all subjects, implying that none of the volunteers had agammaglobulinemia (Fig. 3B and C). Fig. 3 presents the geometric means of IgA, IgG, and IgM ISC for infections at other mucosal sites (Kantele et al., 1988, 1994; Palkola et al., 2012, 2016). Detecting such cells also in upper FGT infections encourages their use as tools for evaluating immune responses in the FGT.
4.2. Timing of sampling

As plasmablasts only circulate transiently before homing into their target tissues, sampling timing is critical. We utilized our previous experience of acute mucosal infections in the urinary (Kantele et al., 2008) and respiratory tracts (Palkola et al., 2012, 2016), and the gut (Kantele et al., 1988), and the kinetics of plasmablasts in the circulation as reported in gastroenteritis (Kantele, 2012) and after oral (Kantele, 1990,1991; Kantele et al., 1988) and rectal vaccinations (Kantele et al., 1998). Antigen-specific plasmablasts enter the blood approximately on the third day after antigen encounter, peak in frequency on day seven, and disappear gradually by days 14–16. Prolonged antigenic stimulus may lengthen the detection period (Kantele, 1991, 2012). Accordingly, to catch the peak of the response, we only recruited patients with a history of symptoms lasting 7–10 days. The timing proved successful: pathogen-specific ASC were found in all patients.

4.3. Numbers of circulating plasmablasts (ASC and ISC)

The vigorous ASC response reveals the remarkable inductive capacity of the upper FGT, consistent with previous reports (Wira et al., 2015; Iwasaki, 2010). Indeed, the magnitude of the response exceeded that seen in infections of the upper (Palkola et al., 2016) and lower (Palkola et al., 2012) respiratory tracts, yet resembled that in infections at anatomically close sites i.e. gastroenteritis (Kantele et al., 1988) or upper urinary tract infection (UTI) (Kantele et al., 1994). As explanation, we have suggested polyclonal stimulation associated with severe natural infections (Kantele et al., 1988; Palkola et al., 2012). According with our findings, human memory B lymphocytes have been shown to differentiate into plasma cells in response to polyclonal stimuli, such as...
microbial products or bystander T cell help. Specific antibody-secreting cells have been found in such settings even in the absence of their cognate antigen (Bernasconi et al., 2002).

4.4. Immunoglobulin isotype distribution of pathogen-specific plasmablast response

While IgA predominated in the pathogen-specific plasmablast responses, also IgG ASC were abundant. Indeed, pathogen-specific IgA and IgG ASC have been shown to accumulate in the genital tract as a response to mucosal immunization or infection in animals (Bekri et al., 2017; Cuburu et al., 2009; Johansson et al., 1998) and in salpingitis in humans (Kutteh et al., 1990). Both isotypes can contribute to protection against sexually-transmitted pathogens (Bomsel et al., 2011; Li et al., 2011; Mascola et al., 2000; Shin and Iwasaki 2013). The high IgA ASC numbers accord not only with the previously reported up-stream increase of IgA levels in FGT secretions (Kozlowski et al., 2002; Quenzel et al., 1997), and predominance of IgA ASC in mucosae of the upper FGT (Kutteh et al., 1990), but also the active IgA transport (pIgR) operating on the epithelium of upper FGT (Iwasaki, 2010). Moreover, IgA is the general mucosal isotype, predominating among the circulating pathogen-specific ASC in gastroenteritis (Kantele et al., 1988; Kantele et al., 1996a; Pakkanen et al., 2010) and upper UTI (Kantele et al., 2008), and after oral vaccination (Kantele 1990; Kantele et al., 1986, Pakkanen et al., 2010). Like the high IgA ASC numbers in this study, high IgA antibody levels have been associated with FGT, and the mechanisms transporting IgG (nFcR) exist throughout the FGT (Li et al., 2011). Indeed, in human cervico-vaginal secretions, IgG levels even exceed those of IgA (Li et al., 2011; Kozlowski et al., 2002).

4.5. Homing profiles of plasmablasts

Homing molecules directing lymphocytes into the human FGT have been poorly described. Studies with rodents report recruitment of IgA cells into the uterus by the CCR10-CLL28 interaction (Cha et al., 2011; Cuburu et al., 2009). As for T lymphocytes, a unique memory-T-cell population co-expressing α4β7 and CLA has been detected in the endocervix of patients with Chlamydia infection (Kelly et al., 2009), and ligands such as E-selectin (ligand to CLA), ICAM-1, and VCAM-1 have been found inducible on human endometrium (Tabibzadeh et al., 1994). Moreover, α4β7-integrin-expressing T cells have been found in infected murine FGT (Davila et al., 2014). For the present study of circulating plasmablasts in human FGT infection, we chose to investigate α4β7, L-selectin, and CLA, since these markers have been extensively explored in infections of other mucosal sites (Kantele et al., 2008, Kantele et al., 1996a; Palkola et al., 2015, 2016; Pakkanen et al., 2010).

4.6. General characteristics of HR profile

α4β7 and L-selectin were both expressed on two thirds of the circulating pathogen-specific plasmablasts and CLA on only a small proportion. Such a HR profile implies that α4β7 and L-selectin contribute, but CLA less, in the dissemination of B cells from the upper FGT. Although we could not explore plasmablasts in the FGT tissues, this HR profile may, at least partly, represent that required for homing to the FGT, as migrating effector lymphocytes favour the initial activation site (Sigmundsdottir and Butcher, 2008). It should be highlighted that FGT lacks organized structures equivalent to intestinal Payer’s patches. Instead, draining lymph nodes (the common iliac, internal iliac, external iliac, and the inguinal femoral lymph nodes), may serve directly as activation sites (Iwasaki, 2010). The anatomically close rectal and urogenital mucosal sites at least partly share the same draining lymph nodes. It is thus of interest to compare the HR profile in FGT infection to that of infections in various human anatomical compartments, especially those of the intestinal and urinary tract.

4.6.1. Comparison with intestinal HR profile

The HR profile of circulating ASC in the upper FGT infection differed from the gut-seeking profile with respect to α4β7: although substantial, the proportion of α4β7-expressing cells (60%) did not reach that of gut-seeking lymphocytes (90–100%) reported after oral immunization (Kantele et al., 1997; Quiding-Järbrink et al., 1997) and in acute gastroenteritis (Kantele et al., 1996a; Kantele et al., 2008). The main ligand for α4β7, mucosal cell adhesion molecule (MadCAM-1) is highly abundant in the gut lamina propria (Briskin et al., 1997), whereas in FGT, MadCAM-1 expression has only been reported in tissue culture models of chlamydia infection (Kelly et al., 2001), not in non-infection settings (Johansson et al., 1999). L-selectin, on the other hand, may figure more importantly in infections of the FGT (67%) than the intestine (40%; Kantele et al., 2008; Kantele et al., 1996a). L-selectin-expressing plasmablasts dominate a systemic profile (proportion of L-selectin high, α4β7 moderate and CLA low) induced in PLN after parenteral immunization (Kantele et al., 1997, 2008; Quiding-Järbrink et al., 1997). The substantial proportion of L-selectin-expressing cells in FGT infections may reflect both the systemic part of the immune protection and a contribution of L-selectin in targeting effector B cells into the FGT. We are unaware of any studies exploring whether the ligand of L-selectin, peripheral lymph node addressin (PNAd), is abundant within the FGT.

4.6.2. Comparison with HR profile in respiratory tract

It is interesting to compare the HR profiles of the FGT and the respiratory tract, for a cross-talk has been reported between nasal and genital surfaces (Johansen et al., 2005; Kozlowski et al., 2002). Antigen-specific ASC and cell-mediated immune responses have been reported in murine/primate FGT after intranasal (Cha et al., 2011; Johansson et al., 1998) and sublingual (Cuburu et al., 2009) immunizations. Accordingly, in line with our data, a high to moderate proportion of both L-selectin- and α4β7-expressing ASC has been shown after intranasal cholera vaccination in humans (Quiding-Järbrink et al., 1997). In contrast, in respiratory tract infections (sinusitis, tonsillitis, pneumonia) the proportion of L-selectin-expressing cells appears higher (79–82%) and that of α4β7-expressing cells lower (15–44%) than in upper FGT infections (67% and 60%, respectively) (Palkola et al., 2015, 2016). The slight differences between the HR profiles reported for tonsillitis and sinusitis and after intranasal immunization may reflect differences in response induction, with intranasal immunization only stimulating a restricted area in the Waldeyer’s ring (resulting in high α4β7 proportions) and natural infections impacting more scattered induction sites.

4.6.3. Comparison with HR profile for UTI

A comparison with the urinary tract is of interest, as it may share draining lymph nodes with FGT. Indeed, the homing profile of pathogen-specific ASC in acute upper UTI (α4β7 61%, L-selectin 51%, CLA 13%) (Kantele et al., 2008) clearly resembles that found for FGT. The present HR profile (high to moderate proportion of α4β7 and L-selectin, and low of CLA-expressing cells) might thus represent one shared by the urogenital area.

4.7. Limitations

Three limitations should be mentioned: small number of subjects, limited coverage of various homing markers, and the fact that we did not assess the possible effect of hormonal status. Future studies should cover other potential receptors and the impact of hormonal status on HR expression.

5. Conclusions

This study is the first to show that pathogen-specific plasmablasts appear in the circulation in response to bacterial infection in the upper
FGT, the response predominated by IgA, followed by IgG and IgM plasmablasts. These circulating cells provide a tool for future investigations seeking to noninvasively explore immune responses elicited at this site. A homing profile resembling that reported previously for the upper urinary tract was revealed, suggesting a common urinogenital homing from these sites.

Author contributions

Conceived and designed the experiments: NVP, JMK, AK. Performed the experiments: NVP, OH, SHP. Analyzed the data: NVP, SHP, JMK, AK. Contributed reagents/materials/to collection of patient samples/analysis tools: OH, JMK, AK. Wrote the paper: NVP, OH, SHP, JMK, AK.

Conflicts of interest

All authors declare no conflicts of interest.

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