Induction and function of the mucosal immune system

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• The MRKAd5 HIV-1 Gag/Pol/Nef candidate vaccine advanced to a phase 2b test-of-concept trial known as STEP, conducted by Merck & Co., Inc., and the HIV Vaccine Trials Network (HVTN).

• The vaccine neither prevented infection nor had an impact on early plasma virus levels in those who received the vaccine compared with the placebo recipients (2007).

• Additional studies with mucosal and biopsy specimens will be required to explore whether activation of cells at the mucosal sites were different between vaccine and placebo recipients.
HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals

Most uninfected partners of couples discordant for HIV infection show evidence of HIV-specific secretory IgA at mucosal sites (vaginal wash)

Editorial: Sarah Rowland-Jones:
Dimers are a girl’s best friend
The mucosae are an enormous battlefield
Mucosal effector sites provide secretory IgA (SIgA) antibodies

Section through skin
Hornified layer
Epithelial cells

Airways and oral cavity
Mucus and cilia
Epithelial cells
Glands
Plasma cells

Gastrointestinal mucosa
Surface epithelium
Glands (crypts)
Plasma cells

Normal human colon

At the border of hell!
80% of all plasma cells are located in gut mucosa
An adult exports 3 g of SIgA to the gut lumen per day
Local formation and export of mucosal immunoglobulins

Stroma

Plasma cells
- IgA+J
- IgM+J

Dimeric IgA (pIgA)
- J chain

Gland
- Free SC

Lumen
- pIgR (mSC)
- SIgA
- SIgM
- IgA
- IgA-coated bacteria

Mucus
- Pentameric IgM
- IgG(±J)

Inductive site and effector site of gut immune system

Modified from: MacDonald & Monteleone
Science 2005; 307: 1920-25

- Germinal center
- B cell
- Homing
- Lamina propria
- pIgR
- Distant sites
- Normal effector site
- SIgA
- IgA, IgG
- Teff
- Treg
- pIgA
- Lymphocyte differentiation
- Barrier function
- IgA development
- IgA export
- Bug control
- Bacterial sensing by epithelium
- Antigen uptake
- Gut homing
- IgAlgG
- Peyer's patch (GALT)
- T and B cells
- M cells
- Intraepithelial lymphocyte
- Plasma cell
- IgA export
- Lymphocyte differentiation
- Antigen uptake
- Gut homing
- Normal effector site
- Lamina propria
- Regulatory T cells
Immunoglobulin A (IgA) is the major antibody class in humans.

Serum IgA (monomeric IgA)

Dimeric IgA (pIgA) with J chain (J)

Secretory IgA (SIgA) with bound SC (the cleaved ectodomain of pIgR)

SC aff.

Parotid gland

Merge

SC (free or bound) exhibits several anti-microbial properties so the sacrificial pIgR might have originated from the innate defence system to drive the ‘IgA pump’

Immunoglobulin A (IgA) is the major antibody class in humans.

Serum IgA
(monomeric IgA)

Dimeric IgA
(plIgA) with J chain (J)

Secretory IgA (SmIgA) with bound SC (the cleaved ectodomain of plgR)

Plasma cell

Epithelial cell

Systemic antibodies: Mainly IgG
Mucosal antibodies: Mainly secretory IgA (SIgA)
GALT structures are strategically situated in relation to the greatest concentration of commensal bacteria (indigenous microbiota)

- Peyer’s patches: distal ileum (nos. 100-250)
- Appendix: cecum
- Isolated lymphoid follicles (ILFs): distal large bowel (nos. ~ 30 000)
Maintenance of immune homeostasis in the human gut

Inductive site
Peyer's patch (GALT)

Effector site
Immune exclusion: SlgA/SlgM

- Productive stimulation
- Oral tolerance (suppression)

- CD4
- IgA
- IgM
- IgG
- IgE
- DTH
- CD4
- Mφ

Other secretory effector tissues

- Endothelial gatekeeper function

- Mesenteric lymph node
- Thoracic duct
- Peripheral blood
Inductive site (GALT)  Effector site (normal intestinal mucosa)

Maintenance of immune homeostasis in the human gut

Inductive site
Peyer's patch (GALT)

Effector site
Immune exclusion: SIgA/SIgM

Productive stimulation
Oral tolerance (suppression)

Endothelial gatekeeper function

Other secretory effector tissues
Mucosal leukocyte homing in the normal gut

Gut associated lymphoid tissue (GALT)

Endothelial gatekeeper function

Effector site: normal gut mucosa

MAdCAM-1

Peripheral blood

Peyer’s patch

Microvascular gut endothelium

Gut associated lymphoid tissue (GALT)

Endothelial gatekeeper function

Effector site: normal gut mucosa

MAdCAM-1
Human NALT anlagen: prenatal (19 wks). Nasal ILFs in 40% < 2 yrs (inducible?). Rodent NALT: postnatal organogenesis.

Tonsils and adenoids are well designed for antigen trapping

Inductive sites
- Tonsils & adenoids (NALT)
- TALT
- LALT

Human palatine tonsil

Human palatine tonsil

GC
Crypt

Reticular crypt epithelium (cytokeratin)

Scanning electron-microscopy (Owen, 1988)
Integration and compartmentalization in the mucosal immune system

- Tonsils & adenoids (83%)
- Blood circulation
- Cervical lymph nodes (78%)
- Mesenteric lymph nodes
- Inguinal lymph nodes
- Bone marrow
- Lacrimal gland (86%)
- Nasal & salivary glands (60%)
- Bone marrow (67%)
- Cervix mucosa (40%)
- Inguinal lymph nodes (60%)
- Peyer's patches (43%)
- Gut (9%)
- Duodenum
- Colon (28%)
- Colon (0%)
- Gut
Frequency of various tissue samples with $C_{\mu}$ deletion, reflecting dissemination of $s$lgD$^+$lgM$^-$ effector B cells from Waldeyer's ring (tonsils and adenoids) to systemic and mucosal immune compartments.

IgA def.: tonsils & adenoids: 
- Recurrent tonsillitis: n = 13
- Normal tonsils: n = 9
- Hyperplastic adenoids: n = 10

IgA def.: lacrimal & salivary glands:
- Lacrimal glands: n = 6
- Nasal mucosa: n = 26
- Salivary glands: n = 8
- Duodenum: n = 23

IgA def.: duodenum:
- Small bowel: n = 22

IgA def.: colon:
- Large bowel: n = 15

IgA def.: colon:
- Uterine endocervix: n = 5

Percentage of positive samples
Compartmentalization of the integrated mucosal immune system

**Inductive sites**
- Tonsils and adenoids (NALT)
- BALT
- Peyer's patches (GALT)
- Appendix and colonic-rectal isolated follicles (GALT)

**Effector sites**
- Lacrimal, nasal and salivary glands
- Bronchi
- Mammary glands
- Small bowel
- Large bowel
- Female genital tract

**Shared homing molecules (CCR10)**
- L-sel. (CD62L)
- CCR7
- α4β1
- CCR10

**Systemic homing**
- α4β7
- CCR9
- CCR10

**Johansen, Baekkevold, Carlsen, Farstad, Soler, Brandtzaeg, Blood 2005; 106:593-600**
The nature of secretory immunity in the female genital tract is obscure

- The most active SIgA system occurs in the gut, but secretory immunity also operates in the female genital tract; there are J chain-producing IgA\(^+\) plasma cells and plgR/SC expression in the cervical mucosa and fallopian tubes.

- The origin of these local IgA\(^+\) plasma cells remains partly undefined because organized inductive mucosa-associated lymphoid tissue (MALT) is generally absent from the genital tract.
Role of the endometrium in secretory immunity

- Like cervical glandular epithelium, the endometrium can also perform pIgR-mediated external translocation of dimeric IgA
- These dimers appear to be mainly derived from serum, partly under hormonal regulation
- Paracellular diffusion of IgG and monomeric IgA through epithelia is also an important part of humoral immunity in the female genital tract
There is considerable local production as well as leakage of IgG into the female genital tract

- Human cervical mucosa often contains a substantial number of IgG-producing plasma cells
- It is possible that these plasma cells are derived from NALT or perhaps from the draining cervical lymph nodes
- Intranasal vaccination also appears to be quite efficient for systemic immunization, providing high-affinity serum IgG antibodies
Class distribution and J-chain expression of IgA- and IgG-producing plasma cells in human cervical mucosa

Immunocyte class distribution (%)

J-chain expression (%)

Brandtzaeg and Johansen (unpublished)
IgA- and IgG-producing plasma cells with J-chain expression in cervical mucosa
IgA- and IgG-producing plasma cells with J-chain expression in duodenal mucosa

IgA + J chain

IgG + J chain
Components of humoral immunity in the human female genital tract

Adapted from: Bjercke and Brandtzaeg (1993), Brandtzaeg et al. (1993), Kutteh and Mestecky (1994) and Crowley-Norwick et al. (1995)
IgA- and IgG-producing cells in adenoid specimen
Tonsillar germinal centre (GC) and extrafollicular Ig-producing cells express J chain (% of total isotype)

<table>
<thead>
<tr>
<th>Isotype</th>
<th>Normal</th>
<th>Recurrent Tonsillitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgD</td>
<td>82%</td>
<td>50%</td>
</tr>
<tr>
<td>IgM</td>
<td>55%</td>
<td>63%</td>
</tr>
<tr>
<td>IgA</td>
<td>29%</td>
<td>2%</td>
</tr>
<tr>
<td>IgG</td>
<td>36%</td>
<td>45%</td>
</tr>
</tbody>
</table>

Tonsillar germinal centre contains IgG-producing plasmablasts which often show J-chain expression.
B-cell differentiation in germinal centres

Mantle zone

Lymphoid follicle

GC

IgA + J chain

Migration to distant secretory effector sites

Extra-follicular compartments

The nasal route is attractive for mucosal vaccine administration. But be aware of direct communication between the olfactory bulb and CNS.
Nasal anatomy and location of regional lymphoid tissue

Waldeyer’s ring
Organized lymphoid tissue with M cells

Adenoids
Tubal tonsil

Palatine tonsil
Lingual tonsil

Olfactory region
Ciliated mucosa

Skin

Nasal mucosa
Extremely rich in dendritic cells

Cervical IgA and IgG antibodies to group B streptococci serotype III in pregnant women according to colonization state

<table>
<thead>
<tr>
<th>Colonization state</th>
<th>Number of subjects</th>
<th>Median (range) of antibodies (kU/ml)</th>
<th>IgA</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix only positive</td>
<td>8</td>
<td>12.2 (3.0-227.7)*</td>
<td>6.8 (0.9-22.9)**</td>
<td></td>
</tr>
<tr>
<td>Rectum only positive</td>
<td>5</td>
<td>10.7 (1.8-19.1)</td>
<td>4.5 (2.8-8.1)**</td>
<td></td>
</tr>
<tr>
<td>Cervix and rectum positive</td>
<td>11</td>
<td>16.0 (8.0-52.9)**</td>
<td>13.6 (2.1-32.3)**</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>19</td>
<td>3.2 (0.2-17.7)</td>
<td>1.3 (0.2-3.6)</td>
<td></td>
</tr>
</tbody>
</table>

*aStatistical differences compared with non-colonized women (Mann-Whitney U test): *p<0.05; **p<0.01

Adapted from Hordnes et al. Vaccine 1996; 64:1643-52
Effects of parenteral immunization with albumin on concurrent penetration (2h) of human serum albumin (HSA) and transferrin (Trf) through rabbit sublingual mucosa

IgG antibodies may increase mucosal permeability for bystander molecules

Confirmed in vivo (mice) that this complication is caused by IgG but not SIgA antibodies
Lim & Rowley, 1982

Naïve animals

Immunized (IgG anti-HSA)

Brandtzaeg & Tolo Nature 1977; 266: 262-63
Conclusions

- Mucosal vaccination offers several distinct advantages for protection of the female genital tract (SIgA balancing IgG antibodies)
- Nasal spray might be more acceptable than an intravaginal or rectal vaccine
- A combined nasal/vaginal/rectal approach would probably be most effective but perhaps socially less acceptable
Acknowledgements

Laboratory for Immunohistochemistry and Immunopathology (LIIPAT) is part of Centre for Vaccinology and Immunotherapy (CEVI, 2001) and Centre of Excellence for Immune Regulation (CIR, 2007), funded by the Research Council of Norway, University of Oslo and Rikshospitalet University Hospital

Marie Curie Training Network: CROSS-TALK (EC, 2008)