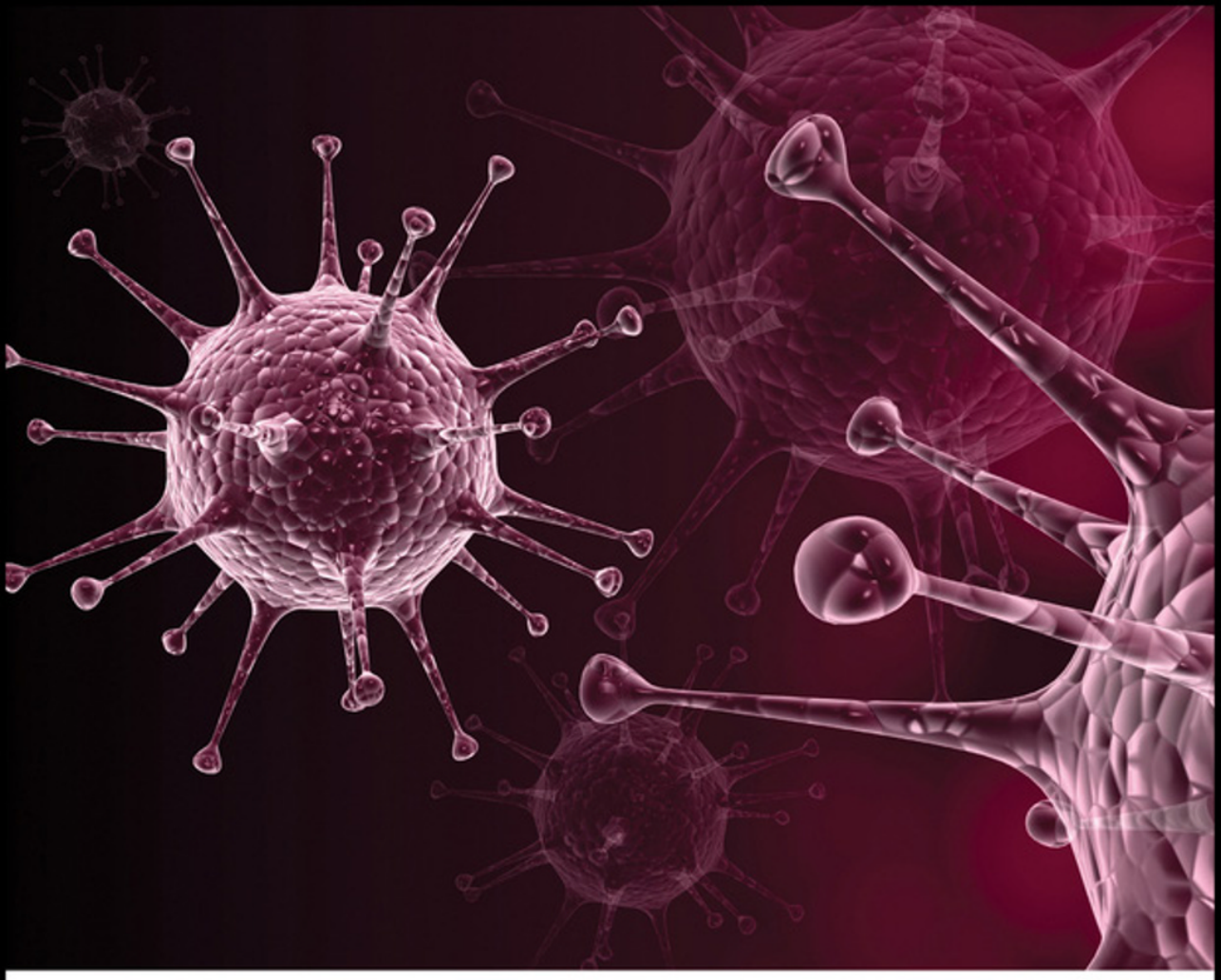

Diagnostics to Pathogenomics of Sexually Transmitted Infections

Edited by

Sunit K. Singh

WILEY Blackwell

Diagnostics to Pathogenomics of Sexually Transmitted Infections

Diagnostics to Pathogenomics of Sexually Transmitted Infections

Edited by Sunit K. Singh, PhD

Professor of Molecular Immunology
Head, Molecular Biology Unit
Professor Incharge-Centre of Experimental Medicine & Surgery
Faculty of Medicine
Institute of Medical Sciences (IMS)
Banaras Hindu University (BHU), Varanasi, India

WILEY Blackwell

This edition first published 2019
© 2019 John Wiley & Sons Ltd

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by law. Advice on how to obtain permission to reuse material from this title is available at http://www.wiley.com/go/permissions.

The right of Sunit K Singh to be identified as the author of the editorial material in this work has been asserted in accordance with law.

Registered Office
John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA

Editorial Office
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

Limit of Liability/Disclaimer of Warranty
While the publisher and authors have used their best efforts in preparing this work, they make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives, written sales materials or promotional statements for this work. The fact that an organization, website, or product is referred to in this work as a citation and/or potential source of further information does not mean that the publisher and authors endorse the information or services the organization, website, or product may provide or recommendations it may make. This work is sold with the understanding that the publisher is not engaged in rendering professional services. The advice and strategies contained herein may not be suitable for your situation. You should consult with a specialist where appropriate. Further, readers should be aware that websites listed in this work may have changed or disappeared between when this work was written and when it is read. Neither the publisher nor authors shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

Library of Congress Cataloging-in-Publication Data

Names: Singh, Sunit K., editor.
Title: Diagnostics to pathogenomics of sexually transmitted infections /edited by, Sunit K. Singh.
Description: Hoboken, NJ : Wiley Blackwell, 2018. | Includes bibliographical references and index. |
Identifiers: LCCN 2018024559 (print) | LCCN 2018024972 (ebook) | ISBN9781119380900 (Adobe PDF) | ISBN 9781119380955 (ePub) | ISBN 9781119380849 (hardcover)
Subjects: | MESH: Sexually Transmitted Diseases
Classification: LCC RC200 (ebook) | LCC RC200 (print) | NLM WC 140 | DDC616.95/1–dc23
LC record available at https://lccn.loc.gov/2018024559

Cover Design: Wiley
Cover Image: © mathagraphics/Shutterstock

Set in 10/12pt Warnock by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

Contents

About the Editor – Prof. Sunit K. Singh

Prof. Sunit K. Singh completed his bachelor's degree from GB Pant University of Agriculture and Technology, Pantnagar, India, and master's degree program from the CIFE, Mumbai, India. After receiving his master's degree, Prof. Singh joined the Department of Pediatric Rheumatology, Immunology, and Infectious Diseases, Children's Hospital, University of Wuerzburg, Wuerzburg, Germany, as a biologist. Prof. Singh completed his PhD degree from the University of Wuerzburg in the area of molecular infection biology.

Prof. Singh completed his postdoctoral trainings at the Department of Internal Medicine, Yale University, School of Medicine, New Haven, Connecticut, USA, and the Department of Neurology, University of California Davis Medical Center, Sacramento, California, USA, in the areas of vector-borne infectious diseases and neuroinflammation, respectively. He has also worked as a visiting scientist in several institutions of repute, such as Albert Einstein College of Medicine, New York, USA, Chonbuk National University, Republic of Korea, Institute of Parasitology, Ceske Budejovice, Czech Republic, and University of Geneva, Switzerland. He has an vast experience in the area of Infectious Diseases.

Prof. Singh has served as a scientist and led a research group in the area of molecular neurovirology and inflammation biology at the CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India. Currently, he is a professor of molecular immunology leading a research group in the area of human molecular cirology and immunology in the department of Molecular Biology, Faculty of Medicine, Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi, India. His main areas of research interest are molecular neurovirology and immunology.

There are several awards to his credit, including the Skinner Memorial Award, Travel Grant Award, NIH-Fogarty Fellowship, and Young Scientist Award. Prof. Singh has published many research papers in the areas of molecular virology and inflammation biology in various peer-reviewed journals. Prof. Singh has edited several books, including *Neuroviral infections-Vol-I & Vol-II*, *Viral Hemorrhagic Fevers, Human respiratory viral infections*, *Human Emerging & Re-emerging Infections-Vol-I & Vol-II*, *Viral Infections and Global Change* and *Neglected Tropical Diseases of South Asia*. Prof. Singh is associated with several international journals of repute as an editor and editorial board member.

Contributors

Kourosh Afshar
Division of Pediatric Urology
Department of Urologic Sciences
University of British Columbia
Vancouver, British Columbia, Canada

Charles W. Armitage
Institute of Health and Biomedical Innovation & School of Biomedical Sciences
Queensland University of Technology
Brisbane, Australia

Margaret E. Bauer
Department of Microbiology and Immunology
Indiana University School of Medicine
Indianapolis, IN, USA

Kenneth W. Beagley
Institute of Health and Biomedical Innovation & School of Biomedical Sciences
Queensland University of Technology
Brisbane, Australia

Gert Bellen
Femicare vzw
Tienen, Belgium

Anuradha Bishnoi
Department of Dermatology and Venereology
Postgraduate Institute of Medical Education & Research
Chandigarh, India

Alison J. Carey
Institute of Health and Biomedical Innovation & School of Biomedical Sciences
Queensland University of Technology
Brisbane, Australia

Nuno Cerca
Centre of Biological Engineering
LIBRO - Laboratory of Research in Biofilms Rosário Oliveira
University of Minho
Braga, Portugal

Sarita Martins De Carvalho Bezerra
Ceder-Hospital Santo Amaro
Recife, Pernambuco, Brazil

Gilbert G.G. Donders
Femicare vzw
Tienen, Belgium
and

Department of OB/Gyn
Antwerp University
Antwerp, Belgium
and
Regional Hospital Hart
Tienen, Belgium

Giorgia Giuffrida
Dermatology Clinic
University of Catania
Catania, Italy

Sivtrigaile Grinceviciene
Femicare vzw
Tienen, Belgium
and
Department of Biothermodynamics and Drug Design
Institute of Biotechnology
Vilnius University
Vilnius, Lithuania

Piotr B. Heczko
Department of Bacteriology
Microbial Ecology and Parasitology
Jagiellonian University Medical College
Cracow, Poland

Danica K. Hickey
Institute of Health and Biomedical Innovation & School of Biomedical Sciences
Queensland University of Technology
Brisbane, Australia

Diane M. Janowicz
Department of Medicine
Indiana University School of Medicine
Indianapolis, IN, USA

Ayse Serap Karadag
Department of Dermatology
Medical Faculty Goztepe, Training and Research Hospital
Istanbul Medeniyet University
Istanbul, Turkey

Behnam Kazemi
Division of Pediatric Urology,
Department of Urologic Sciences
University of British Columbia
Vancouver, British Columbia, Canada

Piotr Kochan
Department of Microbiology
Jagiellonian University Medical College
Cracow, Poland

Francesco Lacarrubba
Dermatology Clinic
University of Catania
Catania, Italy

Suncanica Ljubin-Sternak
Clinical Microbiology Department
Teaching Institute of Public Health "Dr Andrija Stampar", & Medical Microbiology Department
School of Medicine
University of Zagreb
Zagreb, Croatia

Marcio Martins Lobo Jardim
Ceder-Hospital Santo Amaro
Recife, Pernambuco, Brazil

Andrew E. MacNeily
Division of Pediatric Urology
Department of Urologic Sciences
University of British Columbia
Vancouver, British Columbia
Canada

Rahul Mahajan
Department of Dermatology and Venereology
Postgraduate Institute of Medical Education & Research
Chandigarh, India

Jiri Mestecky
Department of Microbiology
University of Alabama at Birmingham
Birmingham, Al, USA
and

Institute of Immunology and Microbiology
First School of Medicine
Charles University
Prague, Czech Republic

Giuseppe Micali
Dermatology Clinic
University of Catania
Catania, Italy

Lenka Mikalová
Department of Biology
Faculty of Medicine
Masaryk University
Brno, Czech Republic

María Teresa Pérez-Gracia
Área de Microbiología
Departamento de Farmacia
Instituto de Ciencias Biomédicas
Facultad de Ciencias de la Salud
Universidad CEU-Cardenal Herrera
Valencia, Spain

Filip Rob
Dermatovenereology Department
Second Medical Faculty of Charles University
Na Bulovce Hospital
Prague, Czech Republic

Aliona Rosca
Centre of Biological Engineering, LIBRO - Laboratory of Research in Biofilms Rosário Oliveira
University of Minho
Braga, Portugal

Katerina S. Ruban
Femicare vzw
Tienen, Belgium

Michael W. Russell
Department of Microbiology and Immunology
University of Buffalo
Buffalo, NY, USA

Andreas Sauerbrei
Section Experimental Virology
Institute of Medical Microbiology
Jena University Hospital, Friedrich-Schiller University of Jena
Jena, Germany

Santosh Kumar Singh
Molecular Biology Unit
Faculty of Medicine
Institute of Medical Sciences
Banaras Hindu University
Varanasi, Uttar Pradesh, India

Sunit K. Singh
Molecular Biology Unit
Faculty of Medicine
Institute of Medical Sciences
Banaras Hindu University
Varanasi, Uttar Pradesh, India

David Šmajs
Department of Biology
Faculty of Medicine
Masaryk University
Brno, Czech Republic

Magdalena Strus
Department of Bacteriology
Microbial Ecology and Parasitology
Jagiellonian University Medical College
Cracow, Poland

Beatriz Suay-García
Área de Microbiología
Departamento de Farmacia
Instituto de Ciencias Biomédicas
Facultad de Ciencias de la Salud
Universidad CEU-Cardenal Herrera
Valencia, Spain.

Juliana Uchiyama
Ceder-Hospital Santo Amaro
Recife, Pernambuco, Brazil

Tugba Kevser Uzuncakmak
Department of Dermatology
Medical Faculty Goztepe
Training and Research Hospital
Istanbul Medeniyet University
Istanbul, Turkey

Barbara Van Der Pol
School of Medicine
University of Alabama
Birmingham, AL, USA

Preface

Sexually transmitted diseases (STDs) constitute a significant part of the total disease burden globally. These diseases exert a high emotional toll due to the social stigma connected to afflicted individuals, as well as an economic burden on individuals and on the healthcare system. Both affect the community's social and economic development adversely. STDs affect men and women of all backgrounds, irrespective of their economic status. These can be acquired and transmitted through unsafe sexual practices and by various pathogens, including bacteria, fungi, viruses, and parasites. The nucleic acid-based molecular assays enable rapid and accurate identification of infections. These untreated infections lead to complications such as infertility and cervical cancer.

The spread of STDs decreases with the use of contraceptive tools and continuation of treatment. Some STDs are associated with poor pregnancy outcome and high morbidities and mortalities in neonates. In the developing world, the incidence and prevalence of STDs are both very high. Early detection and treatment of STDs reduces the spread of infection and may avoid serious complications. Significant advances in the diagnosis and management of STDs have resulted in prevention, diagnosis, treatment, and better patient care.

This book provides an overview of sexually transmitted diseases. It includes the most common viral, bacterial, and protozoan infections that compromise the sexual health and well-being of any society. There is a need to strengthen the public health systems for controlling the sufferings associated with the sexually transmitted diseases by utilizing need-based affordable and sustainable control measures.

There is need for incremental advancement in efforts to control, eliminate, or eradicate STDs. More efficient and proactive healthcare systems with easy access to affordable medicines are required for proper management of STDs globally.

Dr. Sunit K. Singh, PhD
Professor of Molecular Immunology,
Head, Molecular Biology Unit
Professor Incharge-Centre for Experimental Medicine and Surgery
Faculty of Medicine,
Institute of Medical Sciences (IMS)
Banaras Hindu University (BHU), Varanasi, India

1

Mucosal Immunity in Sexually Transmitted Infections

Jiri Mestecky[1,2] *and Michael W. Russell*[3]

[1] *Department of Microbiology, University of Alabama at Birmingham, Birmingham, Al, USA*
[2] *Institute of Immunology and Microbiology, First School of Medicine, Charles University, Prague, Czech Republic*
[3] *Department of Microbiology and Immunology, University of Buffalo, Buffalo, NY, USA*

1.1 Introduction

Quantitative evaluation of the cells involved in the immune system, such as lymphocytes, plasma cells, macrophages, dendritic cells, and epithelial cells, together with their products, including antibodies, cytokines, and humoral factors of innate immunity, convincingly revealed that the immune system associated with the mucosae is greater than its systemic counterpart (Russell et al. 2015a). This fact should not be surprising, as the development of the entire immune system during evolution and continuously in everyday life is driven by stimulation with commensal microbiota, antigens present in food and inhaled air, as well as pathogens throughout the enormous surface area of mucosal sites, which far exceeds the skin surface.

The mucosal immune system comprises anatomically remote and physiologically distinct compartments that provide protection at various mucosal sites. Although the genital tract shares some common features with other mucosae, including the presence of humoral factors and cells of innate immunity, and the origin of cells involved in antibody production and T cell-mediated immunity, there are also many distinct features characteristic of the genital tract (Russell and Mestecky 2002, 2010; Mestecky et al. 2005). The spectrum of antigens including commensal or pathogenic microorganisms, and sperm is different from those at other mucosal sites. Furthermore, the primary physiological role of the genital tract is reproduction, which involves the acceptance of allogeneic sperm and semi-allogeneic offspring. This distinct physiological role influences the immune system of the genital tract with respect to the induction or suppression of immune responses, which must be considered in the development and application of vaccines against infectious agents of sexually transmitted diseases.

Diagnostics to Pathogenomics of Sexually Transmitted Infections, First Edition.
Edited by Sunit K. Singh.
© 2019 John Wiley & Sons Ltd. Published 2019 by John Wiley & Sons Ltd.

1.2 Innate Immunity in the Genital Tract

Like other mucosal tracts, the genital tract is rich in cellular and humoral components of innate immunity, but the contributions of these disparate factors to defense against sexually transmitted infections (STI) is not well understood. Typically, more information is available for the female than for the male tract. Distinction must be made at the outset between humoral antimicrobial defense factors, usually proteins of diverse nature and mode of action, and nonspecific factors such as pattern recognition receptors and cytokines that orchestrate the inflammatory and adaptive immune responses, and that recruit, activate, and induce both cellular and molecular defense mechanisms.

1.2.1 Humoral Defense Factors in Female Secretions

Secretions of the male and female genital tracts contain an array of innate antimicrobial defense factors similar to those found in other, often better studied secretions, such as milk, saliva, and intestinal and respiratory secretions. These include lactoferrin, lysozyme, peroxidase, defensins, and other proteins secreted by epithelial cells (Hajishengallis and Russell 2015; Ouellette 2015) (Table 1.1). While many of these are constitutively produced, some are upregulated or induced by cytokines, such as IL-17 and IL-22 generated by Th17 cells or by innate lymphoid cells, especially those designated as ILC3. However, there is relatively little information on the role these factors play in defense of the genital tract against STI pathogens. On the other hand, it may be argued that the presence of these factors sets the minimum requirements for the colonization of mucosal surfaces, as organisms that cannot adapt to the conditions created by

Table 1.1 Some Innate Defense Factors Found in the Human Genital Tract.

Factor	Female	Male
Lactoferrin	1 μg ml^{-1} (vaginal fluid)	1.2 mg ml^{-1} (semen)
	0.1 mg ml^{-1} (cervical mucus plug)	Identified by IHC[a] in urethral epithelial cells
Lysozyme	13 μg ml^{-1} (vaginal fluid)	Identified by IHC in glands of Littré and intra-epithelial cells
	1 mg ml^{-1} (cervical mucus plug)	
Peroxidase	Identified in vaginal fluid	
Defensins	HD-5 and HBD-1 found in cervicovaginal secretions, endocervical and endometrial cells	HD-5 present in urethral secretions as proHD-5, activated by proteases
SLPI[b]	Produced in glandular epithelium	Identified by IHC in urethral epithelial cells
MBL[c]	Found in cervicovaginal lavage	1–25 ng ml^{-1} (semen)

[a]Immunohistochemical staining.
[b]Secretory leukocyte protease inhibitor.
[c]Mannose-binding lectin.

these factors would be unable to establish themselves as either commensals or pathogens.

In addition, female genital secretions contain abundant mucus, which can form a physical plug at the cervix, and at ovulation under the influence of estrogen, this liquefies to facilitate passage of sperm. The vaginal environment is normally acidic, maintained largely by the dominant presence of *Lactobacillus* sp., and an increase in pH is associated with dysbiosis that can result in bacterial vaginosis (Russell et al. 2005).

Lactoferrin is a non-heme iron-binding protein (M_r ~80 000) related to serum transferrin, but found in most external secretions (reviewed in Hajishengallis and Russell 2015). In the presence of bicarbonate ion, it binds Fe^{3+} with extremely high affinity even at acidic pH down to pH 3. This effectively keeps the secretions in a free-iron-depleted state, which means that both commensal and pathogenic bacteria colonizing mucosal surfaces must develop alternative mechanisms for obtaining this essential element. Bacteria also use iron-sensing mechanisms to detect when they are located within animal systems, and respond by activating a wide variety of genes involved not only in iron acquisition but also in adapting to the *in vivo* environment. Approximately half of all gonococcal isolates express lactoferrin-binding proteins, LbpA and LbpB, through which they can extract iron from human lactoferrin (Anderson et al. 2003). However, strains that lack LbpA and LbpB are fully virulent, whereas the corresponding transferrin-binding proteins, TbpA and TbpB, are proven virulence factors (Cornelissen et al. 1998). Lactoferrin has also been shown to have anti-viral activity, including against HIV, herpesvirus, and hepatitis B virus (van der Strate et al. 2001).

It has been difficult to establish conclusively that lactoferrin (or transferrin) exerts anti-bacterial effects through iron deprivation: as noted above, bacteria that colonize mucosal surfaces have other means of obtaining iron from their environment. Instead, it appears that the cationic nature of lactoferrin (pI ~9) and its ability to release by proteolysis basic "lactoferricin" peptides from its N-terminus may be responsible for observed antibacterial effects.

Lysozyme is a small (M_r ~14 000) cationic (pI 10.5) protein with muramidase activity that hydrolyses bacterial peptidoglycan (reviewed in Hajishengallis and Russell 2015), and is found in genital secretions and other body fluids (Table 1.1). However, most commensal and pathogenic bacteria are resistant to lysis by lysozyme due to modifications of peptidoglycan structure and its close association with other cell wall structural materials that impede access. Other nonenzymic modes of antibacterial action have been described, including bactericidal activity due to its cationic nature.

Peroxidase activity has been described in vaginal fluid as in other secretions (Table 1.1). Secretory peroxidases utilize H_2O_2 to catalyze the oxidation of halides and pseudohalides to toxic products, but (unlike myeloperoxidase found in phagocytes) they cannot oxidize chloride to hypochlorite (reviewed in Hajishengallis and Russell 2015). Instead, the preferred substrate appears to be thiocyanate (SCN^-), which is found in secretions as a detoxification product of cyanide, and is oxidized to hypothiocyanite ($OSCN^-$). Both this anion and its conjugate acid, HOSCN, inhibit the growth and metabolism of many bacterial species including streptococci and lactobacilli, which often generate the required H_2O_2.

Defensins are small cationic proteins (Mr < 5000) containing three characteristic pairs of cysteine disulfide bonds, the arrangement of which defines the α- and β-defensin families (Ouellette 2015). The α-defensin HD-5 and β-defensin HBD-1 have both been identified in cervical mucus (Quayle 2002). Defensins likely act by permeabilizing bacterial membranes, creating pores by insertion into the lipid bilayers. Low levels of defensins in vaginal secretions have been associated with bacterial vaginosis (Martin and Ferris 2015).

1.2.2 Innate Defense Factors in the Male Tract

The presence of innate defense factors in the male reproductive tract has been much less well studied. However, several mucins are expressed, and lactoferrin, lysozyme, α-defensin HD-5, and secretory leukocyte protease inhibitor (SLPI) have been identified immunohistochemically in urethral epithelial cells and the glands of Littré (Table 1.1) (Anderson and Pudney 2015). HD-5 occurs mainly in an inactive precursor form in urethral secretions, where it is activated by proteases possibly derived from neutrophils during inflammation (Porter et al. 2005). HD-5 is bactericidal for *Neisseria gonorrhoeae* and Mannose-binding lectin, which initiates complement activation through the lectin pathway, has been found in human semen at very low concentrations (1–25 ng ml^{-1}) and it binds to *N. gonorrheae* in a strain-variable manner probably dependent on the lipooligosaccharide (LOS) structure (Wing et al. 2009).

1.3 Immunoglobulins in Secretions of the Genital Tract

In contrast to external secretions of lacrimal, salivary, and lactating mammary glands and the gastrointestinal tract, in which secretory immunoglobulin A (S-IgA) represents the dominant Ig isotype, both male and female human genital tract secretions contain slightly more immunoglobulin G (IgG) than IgA (Kutteh et al. 1996; Baker et al. 2015) (Table 1.2). Furthermore, in females the levels and Ig distribution display marked hormonally dependent differences during the menstrual cycle (Hocini and Barra 1995; Kutteh et al. 1996; Rodgriques Garcia et al. 2015; Crowley-Nowick et al. 1997a; Wira et al. 2005, 2015). Consequently, evaluation of humoral immune responses should take into the account the timing of collection of such fluids to provide comparable results (Mestecky et al. 2011). Irrespective of the phase of the menstrual cycle, IgG appears as the dominant isotype (Kutteh et al. 1996). Variations in Ig levels are dependent on the expression of epithelial cell receptors involved in the transcellular transport of Igs of various isotypes (Menge and Mestecky 1993; Baker et al. 2015).

1.3.1 Female Genital Tract Secretions

Although the reported total levels of Igs in female genital tract secretions are slightly underestimated due to dilution with collection fluids (Jackson et al. 2015), the dominance of IgG is generally accepted irrespective of the assays used for Ig measurement. However, there are marked differences in levels of total Ig of all major isotypes during the menstrual cycle (Kutteh et al. 1996). The highest

Table 1.2 Levels, Properties, and Biological Activities of Ig in the Genital Tract.

	IgG	IgG1	IgG2	IgG3	IgG4	IgA	IgA1	IgA2	IgM
Cervico-vaginal secretions[a]	1–285	18.3 (75%)	5.9 (24%)	0.1 (0.3%)	0.3 (0.7%)	3–133	50%	50%	5–118
Preejaculate	0.1–6.4					0.2–17.3			trace
Semen	4.7–142.3					0.03–96.0			
Intestinal Fluid	4					166			8
Specificity for:									
proteins	++	++	+/−	++	++	++	++	+/−	+
polysaccharides	++	+	+++	+/−	+/−	++	+	++	+
Complement activation	++	++	+++	+++	−	−	−	−	++
Receptors for epithelial transport into external secretions[b]									
FcRn	+	+	+	+/−	+	−	−	−	−
pIgR	−	−	−	−	−	+	+	+	+
Cleavage by bacterial IgA1 proteases	_					+	+	−	−
Number of Ag-binding sites per molecule	2	2	2	2	2	2 monomer 4 dimer S-IgA 8 tetramer			10

(Based on refs: Brown and Mestecky 1988; Raux et al. 2000; Vidarsson et al. 2014; Jackson et al. 2015).
[a]Enormous variation is due to differences in collection procedures and sample processing. Ig levels are also strongly dependent on stage of the menstrual cycle.
[b]Receptors for Fc regions of IgG, IgA, and IgM are also expressed on other cell types in the systemic and mucosal tissues.

levels are present shortly before ovulation (days –4 to –1) and the lowest levels at the time of ovulation. This may be partially due to increased production of mucus by the uterine endocervix and therefore dilution of Ig content. Decreased levels of Igs and innate immune factors at the time of ovulation may result in compromised protection, termed the *window of vulnerability* (Wira and Fahey 2008; Rodriguez Garcia et al. 2015). Low levels of Igs are present in vaginal fluids before and after ovulation due to the formation of the mucous plug at the uterine opening. The distribution of IgG subclasses in cervicovaginal secretions resembles that of plasma (Raux et al. 2000). Functional differences among IgG subclasses are relevant to the associated protective mechanisms, including the specificity of antibodies for certain types of antigens, ability to activate complement, and reactivity with IgG Fc receptors expressed on various types of cells, which influences their distribution in body tissues and fluids (Hocini and Barra 1995; Vidarsson et al. 2014; Baker et al. 2015) (Table 1.2). For example, antibodies of the IgG1, 2, and 3 subclasses specific for HIV-derived antigens differ in their level and association with protection: although IgG1 antibodies are dominant, the levels of IgG2 and IgG3 are of importance for their HIV reactivity (Arnold et al. 2007). IgG is also the dominant Ig isotype present in male genital tract secretions (Moldoveanu et al. 2005).

IgA is present in female genital tract secretions at levels that are lower than those of IgG but that follow the same pattern of changes over the menstrual cycle. In humans, IgA occurs in IgA1 and IgA2 subclasses that display differences in protein structure and glycosylation patterns of their heavy chains (Woof and Mestecky 2015). Furthermore, IgA1 and IgA2 are differentially distributed in various body fluids, and they exhibit some diverse effector functions and specificities for certain types of antigens (Woof and Mestecky 2015). Heavy chains of IgA1 contain a unique hinge region (HR) between the Cα1 and Cα2 constant region domains. The HR contains a duplicated 8 amino acid insertion of repeated proline, serine, and threonine residues with a variable number of *O*-linked glycans. Importantly, the HR of human and hominoid primate IgA1 is the principal substrate of bacterial IgA1 proteases, which cleave IgA1 into Fab and Fc fragments, thereby interfering with the Fc-mediated effector functions of IgA1 (Kilian and Russell 2015). Genital pathogens *N. gonorrheae* and *Ureaplasma urealyticum* are among the diverse group of organisms that secrete IgA1 proteases. While all gonococcal isolates constitutively produce IgA1 protease, its significance in gonococcal infection remains unclear (Cooper et al. 1984; Hedges et al. 1998).

Antibodies specific for particular types of antigens exhibit characteristic IgA subclass associations. Antibodies to proteins, glycoproteins, viruses, and sperm are present mostly in the IgA1 subclass, whereas those specific for lipopolysaccharides, lipoteichoic acid, and polysaccharides occur predominantly in the IgA2 subclass (Brown and Mestecky 1988; Woof and Mestecky 2015). Interestingly, sperm immobilized by agglutination with IgA1 antibodies can regain their mobility after treatment with bacterial IgA1 proteases (Kutteh et al. 1995a). In serum, ~85% of IgA is present in the IgA1 subclass. In contrast, different external secretions display distinctive IgA subclass distributions (Woof and Mestecky 2015). Tears, saliva, nasal, and small intestinal secretions contain mainly IgA1, whereas

in secretions of the large intestine and milk, IgA2 is present at slightly higher levels than IgA1. In secretions of the female genital tract, IgA2 is also higher than IgA1 but in semen IgA1 predominates (Kutteh et al. 1996; Moldoveanu et al. 2005). The IgA subclass distribution in secretions reflects the proportion of IgA1- and IgA2- producing cells in the respective tissues (see below) (Pakkanen et al. 2010). In contrast to exclusively monomeric (m) IgG or polymeric (p) immunoglobulin M (IgM), both m and p forms of IgA exist and are characteristically distributed in various body fluids (Moldoveanu et al. 2005; Woof and Mestecky 2015). While in serum IgA occurs almost exclusively as mIgA, in external secretions such as milk or saliva, approximately 90% or more is present as S-IgA, which consists of pIgA (mainly dimers and tetramers) associated with a small polypeptide called joining (J) chain and secretory component (SC) acquired during epithelial transport (see below). In both female and male genital tract secretions, IgA occurs in three molecular forms: mIgA, pIgA, and S-IgA. The proportions of the individual forms are quite variable and reflect contributions of IgA from the circulation as well as local production.

1.3.2 Origin of Igs in Human Genital Tract Secretions

Immunochemical and immunohistochemical investigations of the properties of Igs in female and male genital tract secretions and mucosal tissues have revealed that they are of circulatory as well as local origin (Kutteh et al. 1996; Moldoveanu et al. 2005). Indirect evidence for the circulatory origin of IgG in semen was provided by systemic immunization studies, which indicated that plasma-derived specific antibodies are found in semen of systemically immunized males (Moldoveanu et al. 2005; Underdown and Strober 2015). The parallel kinetics and Ig properties of antibody responses in serum and semen from volunteers immunized systemically with several vaccines indicated the circulatory origin of seminal antibodies. Interestingly, intranasal immunization with live attenuated influenza virus vaccine resulted in the induction of IgA antibodies in semen. Thus, both systemic and mucosal tissues contribute to the pool of antibodies in male genital tract secretions (Moldoveanu et al. 2005). In secretions of the female genital tract, the relative contribution of Igs from the circulation or local production is strongly dependent on the timing of fluid collection during the menstrual cycle (Kutteh et al. 1996; Crowley-Nowick et al. 1997b; Wira et al. 2005, 2015). The most important organ involved in the transport of circulating or locally produced antibodies into genital secretions is the uterus (Crowley-Nowick et al. 1995; Kutteh et al. 1995b). Uterine epithelial cells express polymeric Ig receptor (pIgR) for pIgA and IgM, and neonatal Fc receptor (FcRn) for IgG (Baker et al. 2015). Hysterectomy results in a highly significant decrease in IgA and a less pronounced depression of IgG (Jalanti and Isliker 1977), probably due to partially preserved transport of IgG mediated by vaginal epithelial cells, which express FcRn but not pIgR. The structural and functional differences between FcRn and pIgR reflect their physiological involvement in protection (Baker et al. 2015). FcRn expressed on placental cells is involved in the selective transport of IgG from maternal into the fetal circulation. In some species, but not humans, FcRn expressed on inestinal epithelial cells is responsible for the selective uptake

of milk IgG into the newborn circulation (Baker et al. 2015). FcRn is a bidirectional, recyclable receptor that, depending on pH, binds IgG at the basolateral surface and releases it at the apical surface, or vice versa, binds and internalizes IgG at the apical surface and releases it into the circulation. In the genital tract, FcRn is also involved in the transport of IgG into genital tract secretions. Importantly IgG from the genital tract may be taken up, depending on intravaginal pH: recent results suggest that IgG complexed to HIV may be taken up by epithelial cells of genital and intestinal origin and thereby enhance HIV infection (Gupta et al. 2013).

In sharp contrast, pIgR represents a unidirectional and sacrificial receptor involved in transepithelial transport of pIgA and IgM (Baker et al. 2015). It is a heavily glycosylated protein that displays Ig domain-like structure and is expressed on the basolateral surfaces of epithelial cells. IgA or IgM in their polymeric forms and containing J chain is bound to pIgR through covalent and noncovalent interaction and transcytosed through the epithelial cells. At the apical surface, pIgA (or IgM) is released with the bound extracellular part of pIgR, called SC, which stabilizes the structure of S-IgA, enhances resistance to proteolysis, and contributes through its glycan moiety to the protective activity (see below). Thus, pIgR (unlike FcRn) is not recycled and the large *N*-terminal segment of pIgR, SC, remains associated with pIgA or IgM. The expression of pIgR on epithelial cells is regulated by several cytokines (e.g. IFNɣ, IL-4, IL-17) and in the genital tract also by hormones such as estrogens (Menge and Mestecky 1993; Baker et al. 2015).

1.3.3 Functions of Genital Tract Antibodies

The protective function of mucosal antibodies has been amply documented in many studies performed in humans as well as in animals (Mestecky et al. 2010; Russell et al. 2015b). Mucosal antibodies induced as a consequence of infection and by active or passive immunization confer protection against various microbial pathogens. Recent results, however, indicate that antibodies, especially those of the IgA isotype, significantly contribute to the maintenance of commensal mucosal microbiota through specific antibody and glycan-dependent binding, with the formation of biofilms at mucosal niches (for review see Mestecky and Russell 2009a). Thus, mucosal antibodies play an essential role in the regulation of commensal as well as pathogenic microbiota to maintain desired homeostasis at mucosal surfaces. Commensal bacteria present in the oral cavity or intestinal tract have been found to be coated with IgA *in vivo* (Mestecky and Russell 2009a); it seems likely that this also occurs in the female genital tract with physiological impact in the maintenance of the vaginal commensal microbiota but this has not been documented.

The protective effect of genital tract antibodies against bacterial infections is not well-understood. One likely reason for this is the lack of demonstrable states of protective immunity against most STIs, as discussed below, and in the absence of such a state mechanisms of protective immunity remains speculative. It is often assumed that immunity to *N. gonorrhoeae* will involve complement-mediated bacteriolysis, which is undoubtedly important for immunity to the

related *N. meningitidis*, as well as opsonophagocytosis by neutrophils, which are typically abundant in the exudate induced in symptomatic gonococcal infection. Both complement-mediated bacteriolysis and phagocytosis by neutrophils have been demonstrated *in vitro* using IgG antibodies generated by immunizing experimental animals, or IgG derived from human sera (Russell et al. 2015c). However, it has also been shown that *N. gonorrheae* possesses multiple mechanisms for resisting complement, including the sialylation of its LOS, the ability to bind complement-regulatory proteins C4-binding protein and factor h, and the induction of antibodies to reduction-modifiable protein (Rmp) that block lysis mediated by antibodies against porin or LOS (Lewis et al. 2010). In addition, IgA antibodies have been shown to inhibit IgG antibody-mediated bacteriolysis of meningococci, a property that extends to the Fab fragments generated by IgA1 proteases that are produced by all strains of *N. gonorrheae* (Russell et al. 1989; Jarvis and Griffiss 1991). The availability of a complete functional (lytic) complement system in genital tract secretions is also an overlooked factor. While C3, the most abundant component, is readily detected (and is exploited by *N. gonorrheae* for one mechanism of attachment to C3-receptor-bearing epithelial cells (Edwards and Apicella 2004), this does not necessarily mean that a complete lytic system is present as other essential components occur at much lower concentrations and are readily inactivated by proteolysis. The levels of complement in the human female tract fluctuate markedly during the menstrual cycle, being highest at menses with the influx of blood. It has also become clear that *N. gonorrheae* can survive within neutrophils by mechanisms that involve inhibition of both oxygen-dependent and oxygen-independent intracellular killing (Criss and Seifert 2012). IgA or even IgG antibodies can be expected to inhibit attachment to and invasion of epithelial cells (Russell et al. 2015a), but the extent to which this mechanism operates against STI pathogens is unknown at present.

In the case of *C. trachomatis*, the picture is complicated by its obligatory biphasic life-cycle, in which extracellular metabolically inactive "elementary bodies" can invade epithelial cells, whereas the intracellular replicating "reticulate bodies" are noninvasive. Thus, inhibition of initial infection is likely to require neutralizing antibodies against the elementary bodies, but the intracellular replicating forms are shielded from these and immunity appears to depend on IFNɣ-driven, $CD4^+$ T cell-mediated mechanisms (Rank and Whittum-Hudson 2010). In murine models, protection against repeat infection may require antibody production arising from previous infection, whereas immunity to primary infection depends more on cellular mechanisms with IFNɣ playing a major role (Morrison et al. 2000, 2011). Thus, mechanisms of protective immunity depend on the stage of infection. However, inflammatory immune responses especially involving $CD8^+$ T cells and the generation of TNFɣ appear to be responsible for the tissue damage caused by chlamydial infection (Murthy et al. 2011).

The importance of antibodies in the female genital tract in protection against viral infection has been demonstrated in several studies (Mestecky et al. 2010; Russell et al. 2015c). For example, passive immunization with SIV-specific antibodies of IgG and IgA isotypes protected rhesus macaques against intravaginal challenge with SIV (for review, see Xu et al. 2015). Therefore,

active immunization with HIV-derived antigens is a highly desirable goal of ongoing studies to prevent HIV infection by the most frequent route through an antibody-dependent mechanism (McElrath 2015). The protective effect of antibodies, mostly of the IgG isotype, has been demonstrated in the prevention of infection with human papilloma virus (HPV). Systemic immunization with available HPV vaccines induces specific antibodies in the circulation as well as in genital tract secretions, derived from the circulatory pool (Russell et al. 2015c).

However, antibodies in the female genital tract can also be detrimental to reproduction. Sera and genital secretions of infertile women may contain antisperm antibodies of IgG and IgA isotypes that effectively inhibit sperm mobility and thus interfere with egg fertilization (Bronson and Fleit 2015). On the other hand, systemic immunization with selected sperm antigens has been extensively explored as a means of control of fertility and reproduction.

1.4 Cells of the Mucosal Immune System of the Genital Tract

1.4.1 Epithelial Cells

In the female genital tract, stratified squamous epithelial cells cover the surfaces of vagina and ectocervix, while in the upper genital tract – endocervix, endometrium, and Fallopian tubes – a single layer of columnar epithelial cells is present. These phenotypically distinct types of cells exhibit different immunological functions. In addition to a mechanical barrier, epithelial cells are the source of humoral factors of innate immunity (see above) and, due to the expression of receptors specific for the Fc regions of all major Ig isotypes, are involved in their transepithelial transport (Baker et al. 2015) (see above).

1.4.2 Immunoglobulin-Producing Cells

The numbers and phenotypes of Ig-producing cells have been evaluated by immunohistochemical methods on tissue sections of lower and upper genital tract or by ELISPOT on cells dissociated from the cervix of hysterectomized women (Kutteh et al. 1988; Crowley-Nowick et al. 1995). The highest numbers of such cells were found in the uterine endocervix and ectocervix, followed by the Fallopian tubes and vagina; ovaries and endometrium were devoid of Ig-producing cells. The isotype distribution of these cells differed with respect to the dominance of IgG or IgA: by immunofluorescence IgA^+ cells were dominant, but by ELISPOT more IgG- than IgA-secreting cells were detected. This difference may be partially due to the source of tissues, isolation of cells for ELISPOT, and the counting of spots formed not only by plasma cells but also by epithelial cells that had internalized IgG. Regardless, the distribution of Ig isotypes in genital tissues is markedly different from other mucosal tissues such as the intestine, in which ~90% of Ig-producing cells are IgA-positive (Brandtzaeg 2015). However, similar to other mucosal tissues, the majority of IgA cells is positive for intracellular

J chain, suggesting their production of pIgA. Because the plasma of healthy individuals contains only small quantities of pIgA, it is likely that S-IgA or pIgA present in cervicovaginal fluid is of local rather than circulatory origin.

The distribution of IgA1- or IgA2-producing cells in the cervix is reminiscent of the large intestine but remarkably different from other mucosal tissues (Woof and Mestecky 2015). The relative proportions of IgA1- or IgA2-producing cells in most mucosal tissues favor IgA1, while in the large intestine and uterine cervix roughly equal numbers of IgA1- and IgA2-positive cells are present (Crago et al. 1984).

In the human male genital tract tissues, Ig-producing cells are present in the penile urethra in glands of Littré with a predominance of IgA (Anderson and Pudney 2015). These cells are also positive for J chain and are localized in the vicinity of pIgR-positive columnar epithelial cells (Pudney and Anderson 1995). Thus, the complementary cellular distribution required for the assembly of S-IgA is present in the penile urethra. Indeed, immunochemical analyses of preejaculate revealed the dominance of IgA in this fluid in contrast to semen (Moldoveanu et al. 2005).

Studies of the origins of Igs and the most effective immunization routes for inducing immune responses in genital secretions have revealed that B and T cells come from remote inductive sites, enter the circulation, and then lodge in mucosal tissues through interaction of lymphocyte homing receptors (integrins) with addressins expressed on endothelial cells of post-capillary venules, where terminal differentiation into effector cells occurs (Mikhak et al. 2015). In the genital tract, the homing receptor $\alpha4\beta1$ is dominant rather than $\alpha4\beta7$ (which is typical of cells that home to the intestinal tract), and it interacts with VCAM-1 and ICAM-1 ligands. Importantly, intranasal or sublingual inductive lymphoepithelial tissues may be the main source of such lymphocytes, thereby explaining the preferential elicitation of humoral responses by these routes of immunization (see above).

1.4.3 T Cells and Other Cell Types

Phenotypic and functional studies of T cell populations in genital tissues of individuals with STI other than HIV have not been extensively addressed, mainly due to difficulty in obtaining relevant tissues and low yields of lymphocytes. This problem can be at least partially overcome by using menstrual blood (Sabbaj et al. 2011; Moylan et al. 2017) as a rich source of lymphocytes with phenotypic profiles that are distinct from cells obtained from peripheral blood.

T cells of $CD4^+$ and $CD8^+$ subsets are present in the female genital tract in the cervix and endometrium as isolated cells, intraepithelial lymphocytes and lymphoid follicles (Crowley-Nowick et al. 1995; Rodriguez Garcia et al. 2015), and they display T-helper (Th), immunoregulatory (Treg) or cytotoxic functional profiles. Th1, Th2, and Th17 are involved in regulation of local immune responses. Cytotoxic T lymphocytes and natural killer (NK) cells are present in the endocervix and endometrium, and participate in local defense mechanisms as demonstrated in SIV-infected rhesus monkeys or HIV-infected women (for review see Xu et al. 2015). Cytotoxic activity has also been demonstrated in $CD8^+$ cells, but the patterns of activity and dependence on hormonal state vary

and appear to be suppressed in the secretory phase when fertilization and implantation take place (White et al. 1997). Aggregates of lymphoid cells in the endometrium fluctuate during the menstrual cycle and are maximum during the secretory phase. These consist of $CD19^+$ B cells surrounded by $CD8^+$ T cells and an outer sheath of $CD14^+$ macrophages (Yeaman et al. 1997). However, their function remains unclear. Transient aggregates of dendritic cells (DC) and $CD4^+$ T cells have been observed in the vaginas of HSV-infected mice (Gillgrass et al. 2005).

Other cell types present in the female genital tract tissues include macrophages, DC and NK cells with characteristic phenotypic properties and functional activities (Russell and Mestecky 2010; Lambrecht et al. 2015; Smythies et al. 2015). Studies of these cell populations in patients with STI are limited (Russell et al. 2015c). Four main populations of antigen-presenting cells (APC) have been identified in human vaginal mucosa: Langerhans cells and $CD14^-$ DC, which polarize toward Th2 responses, and $CD14^+$ DC and macrophages, which polarize toward Th1 (Duluc et al. 2013). DC have also been described in the uterine stroma and within the cervical epithelium (Hussain et al. 1992; Pudney et al. 2005), and functional APC activity has been demonstrated in uterine, cervical, and vaginal tissues (Fahey et al. 1999; Wallace et al. 2001). APC activity appears to vary with tissue location and hormonal status: in rats, estradiol has been shown to enhance APC activity by uterine epithelial cells but to suppress it in uterine stroma and vaginal (Wira et al. 2015). The suppression of APC function by estradiol is mediated by TGF-β (Wira et al. 2002). Monocytes and macrophages are relatively few, and neutrophils are the most abundant phagocytes occurring in the fallopian tubes, especially during the inactive phase of the menstrual cycle. NK cells ($CD56^{hi}$ and $CD16^{lo}$) are frequent in the endometrium, and have an important role in regulating the response to the implanted fetus (Shivhare et al. 2015).

The abundance of TGFβ in genital tract tissues is consistent with a regulatory environment: indeed $Foxp3^+$ Treg cells are induced in the presence of high levels of TGFβ. However, the additional presence of IL-6, IL-21, or IL-1 drives the differentiation of Th17 cells (Korn et al. 2009). $CD3^+/TCR\alpha\beta^+$ cells lacking both CD8 and CD4 have been described with regulatory activity in the mouse genital tract (Johansson and Lycke 2003). $Foxp3^+$ Treg cells and IL-10-dependent type 1 regulatory T cells are induced in mice infected with *N. gonorrheae* (Imarai et al. 2008; Liu et al. 2014). Gonococcal infection also induces the production of IL-17 but not IFNγ or IL-4 in mice (Liu et al. 2012). The role of Th17 and regulatory T cells in STI merits further investigation.

1.5 Induction of Immune Responses in the Genital Tract

The primary immunological role of the female genital tract is to accept allogeneic sperm and foster the implantation and growth of a semi-allogenic fetus without inducing a deleterious immune response. Furthermore, the immune system of mucosal tissues, including the genital tract, facilitates the survival of commensal microbiota with a concomitant capability to respond to mucosal pathogens (Aymeric and Sansonetti 2015). This goal is achieved by the parallel

induction of mucosal tolerance toward commensals and the fetus, and active immune responses to harmful microorganisms (Czerkinsky et al. 1999; Russell and Mestecky 2002, 2010). However, the human female genital tract differs from other mucosal compartments in lacking so-called inductive sites that are present in the intestinal and respiratory tracts, such as intestinal Peyer's patches (PP), which have the ability to internalize and process antigens. This is accomplished by unique epithelial microfold (M) cells that take up and deliver antigens to underlying dendritic and lymphoid cells for the induction of humoral and cellular immune responses (Brandtzaeg 2015; Williams and Owen 2015). These mucosal inductive sites are the source of B and T cells that populate anatomically remote mucosal tissues and glands (e.g. salivary, lacrimal, and lactating mammary glands), where terminal differentiation takes place resulting in the production and secretion of antibodies mainly of the S-IgA isotype, and effector T cells with cytotoxic and regulatory functions (for review see Boyaka et al. 2005; Mikhak et al. 2015).

Ample attempts have been made in animal models as well as in humans to induce, by various immunization routes, pathogen- or sperm-specific antibodies to prevent infection or induce infertility in the female genital tract (Kutteh et al. 1993; Russell and Mestecky 2002, 2010). Furthermore, in many studies, local immune responses to agents of STI have been evaluated (Russell et al. 2015c). In humans, vaginal or intrauterine immunization with soluble antigens such as ferritin, bovine serum albumin, or inactivated polio virus vaccine did not stimulate vigorous local humoral responses, although oral or intramuscular immunization induced antibody responses of all major isotypes in serum and IgG responses in cervico-vaginal secretions (Ogra and Ogra 1973; Vaerman and Ferin 1974, for reviews see Kutteh et al., 1993; Russell and Mestecky 2010). Furthermore, intravaginal immunization with a live recombinant canarypox virus containing HIV genes failed to induce immune responses to HIV-derived antigens as well as to the canarypox vector (Wright et al. 2004). However, intravaginal immunization within the exceptionally potent antigen and adjuvant, cholera toxin B subunit (CTB) stimulated local responses (Wassen et al. 1996; Kozlowski et al. 1997; Johansson et al. 1998, 2001; Kozlowski 2002). Repeated oral or intravaginal immunization with CTB in a gel induced local specific antibody responses in most women, with better response induced by intravaginal vaccination (Wassen et al. 1996). Alternative immunization routes, including rectal, oral, intranasal, or sublingual antigen application, have been explored (Forrest et al. 1990; Czerkinsky et al. 1999, 2011). Such approaches exploit the common mucosal immune system whereby antigen exposure at an inductive site generates corresponding immune responses at remote mucosal effector sites, including the genital tract (McDermott and Bienenstock 1979; Mestecky 1987). Repeated rectal immunization of women with inactivated influenza virus vaccine induced specific IgA antibodies in vaginal secretions and IgG antibodies in cervical secretions six months later, suggesting that this route may be effective for genital antibody responses (Crowley-Nowick et al. 1997a, 1997b). The effectiveness of rectal or oral immunization with a bacterial antigen for the induction of humoral responses in secretions of the genital and intestinal tracts, and in saliva was extensively addressed in subsequent studies using the live attenuated *Salmonella*

typhi Ty21a vaccine (Kantele et al. 1998; Kutteh et al. 2001: Pakkanen et al. 2010). In addition to antibody responses, the phenotype of antibody-secreting cells in peripheral blood was determined with respect to the expression of systemic and mucosal homing receptors. Oral immunization induced pronounced humoral responses in vaginal secretions and saliva, while rectal immunization was more effective in the induction of antibodies in saliva, tears, and rectal secretions; no differences were noted with respect to the intestinal tract and serum responses. The number of specific antibody-secreting cells was comparable in both groups of volunteers: almost all cells expressed dominant α4β7, the intestinal homing receptor, and a minority of cells expressed L-selectin, the peripheral lymph node receptor. Interestingly, the combination of initial oral immunization with a rectal boost significantly increased vaginal and cervical fluid antibodies dominated by IgA, compared to women immunized only orally or rectally (Kutteh et al. 2001). Antibody responses to another attenuated strain of *S. typhi* administered by oral or rectal routes demonstrated preferential S-IgA responses by the oral route for the vaginal and cervical secretions in a limited number of volunteers (Nardelli-Haefliger et al. 1996). Intranasal or sublingual immunization of experimental animals with a variety of antigens has been also explored in many studies for the induction of humoral immune responses in the female genital tract (for review see Russell et al. 1996; Wu and Russell 1997; Czerkinsky et al. 2011). Microbial antigens given by these immunization routes induced female genital tract responses manifested by the presence of IgA and IgG antibodies. However, analogous studies performed in humans are rather limited. Repeated intranasal immunization with different doses of CTB elicited prolonged IgA and IgG responses in vaginal secretions and sera only when higher doses of CTB were used (Bergquist et al. 1997).

1.5.1 Induction of Humoral Immune Responses in Human Male Genital Tract Secretions

In contrast to abundant studies of secretions of the human and animal female genital tract, analyses of immune responses in males immunized by mucosal or systemic routes are rather limited (Moldoveanu et al. 2005). Immune responses to orally (*S. typhi* Ty21a) or systemically (influenza virus, pneumococcal polysaccharide, diphtheria, and tetanus toxoids) administered vaccines were compared in a large study involving 82 healthy volunteers. Oral immunization with *S. typhi* Ty21a vaccine (see above) induced moderate IgA, IgG, and IgM responses in seminal plasma, nasal fluid, saliva and in serum. Interestingly, levels of specific antibodies in seminal plasma paralleled those in rectal lavage fluid with respect to the peak of humoral response. Systemic immunization with influenza virus induced IgG and IgA antibodies in both seminal plasma and serum, which remained detectable, although at lower levels, for 6 months after immunization. After systemic immunization with pneumococcal polysaccharide, or diphtheria or tetanus toxoid vaccines, comparable IgG responses in sera and seminal plasma were induced, suggesting the systemic origin of antibodies in seminal plasma. Intranasal immunization with the live attenuated influenza vaccine induced limited antibody responses in seminal plasma (Moldoveanu et al. 2005).

1.5.2 Immune Responses in the Genital Tract after Infections

1.5.2.1 Gonorrhea

It is well-known that gonorrhea can be acquired repeatedly with little or no evidence for the development of protective immunity arising from prior episodes of infection. It is generally assumed that this is because *N. gonorrhoeae* has the capacity to vary the expression and epitope specificity of most of its major surface antigens to an extraordinary extent (Jerse et al. 2014). In addition, it possesses several mechanisms to interfere with complement activation (Lewis et al. 2010). Thus, conventional thinking is that while anti-gonococcal antibodies are induced, *N. gonorrhoeae* evades their effects through extensive antigenic variation and the inhibition of complement-mediated lysis. However, antibodies reactive with *N. gonorrhoeae* can be demonstrated in most samples of human serum regardless of infection, probably induced by nasopharyngeal exposure to *N. meningitidis* and other commensal *Neisseria* species. Quantitative studies revealed that proven cases of gonococcal infection were associated with only weakly elevated serum or local secretory antibodies, even against the homologous isolate of *N. gonorrhoeae*, and that these responses were not sustained (Hedges et al. 1999). Subsequent studies in vaginally infected mice have shown that *N. gonorrhoeae* suppresses Th1- and Th2-driven adaptive immune responses by mechanisms dependent on TGFβ, IL-10, and the generation of type 1 regulatory T cells (Liu et al. 2014), while concomitantly inducing Th17-driven innate responses (Feinen et al. 2010). This situation can be counter-manipulated by neutralizing TGFβ and IL-10, or by the local application of microencapsulated IL-12, to generate antibody and Th1-driven cellular responses, establish immune memory, and afford resistance to challenge infection (Liu et al. 2013). Mice have also been successfully immunized by intravaginal administration of gonococcal outer membrane vesicles (which contain most of the surface antigens) together with microencapsulated IL-12. Resistance to challenge depended on both B cells and IFNɣ, but the cellular and molecular mechanisms of defense have not yet been determined (Liu et al. 2017). It has been suggested that gonorrhea might ultimately be self-limiting, implying that eventually the human immune system develops responses capable of elminating the infection, but it is totally unethical to perform studies involving the withholding of treatment that would be necessary to investigate this.

1.5.2.2 Chlamydia

In contrast, in genital infection with *C. trachomatis*, it appears that partial protective immunity can be induced by prior infection (Geisler 2010). Again, antigenic variation especially in the major outer membrane protein is an important factor, and in the absence of a defined state of protective immunity no clear consensus exists over the determinants or correlates of protection. However, repeated exposure and the ensuing host responses are held responsible for the inflammatory tissue damage that results from untreated chlamydial infection. It has been proposed that prompt treatment of chlamydial infection forestalls the development of adaptive immune responses by limiting the duration of exposure to chlamydial antigens (Brunham and Rekart 2008). If correct,

this hypothesis implies that *C. trachomatis*, like *N. gonorrhoeae*, has the ability to suppress or at least delay the onset of immune responses that might be effective against it, and that eventually the host immune system might break through and mount protective responses. IL-10 induced by *Chlamydia* has been found to modulate antigen-presentation by dendritic cells and drive them into a regulatory response mode, and in its absence, *Chlamydia* is more rapidly cleared (Omosun et al. 2015).

1.5.2.3 Human Immunodeficiency Virus (HIV)

Current epidemiological data indicate that almost all HIV infections are transmitted heterosexually through the genital and intestinal tracts (Mestecky 2007; Mestecky et al. 2009, 2014). Although the virus spreads promptly from the genital tract to cause systemic infection, there are individuals, usually sex workers, who despite frequent HIV exposure remain uninfected and sero-negative (highly exposed persistently sero-negative individuals). In a search for the mechanisms of this apparent resistance to HIV infection, secretions of the genital tract have been evaluated for the presence of local antibodies that might play a protective role. Indeed, numerous studies (for reviews see Hirbod and Broliden 2007; Mestecky 2007) have reported the presence of HIV-specific antibodies of the IgA isotype. In sharp contrast, other studies (reviewed in Mestecky 2007) failed to confirm these results. In a large blindly performed study (Mestecky et al. 2011) of sera and vaginal secretions of HIV-infected or sero-negative African sex workers, six US- and Europe-based laboratories independently evaluated these fluids using well established assays, including ELISA with a broad spectrum of HIV-derived antigens, Western blot, and virus neutralization analyses. Although dominant IgG and IgA HIV-specific antibodies were detected with remarkable concordance in sera and vaginal secretions of HIV-infected women, no local IgA antibodies were detected in highly exposed seronegative sex workers. Therefore, the ability of sexually encountered HIV to induce local IgA responses remains controversial. In HIV-infected individuals, the majority of antibodies in sera as well as in external secretions, in which S-IgA is the dominant Ig isotype (e.g. intestinal fluid), HIV-specific antibodies were always predominantly IgG (Wright et al. 2002; Mestecky et al. 2004). Mechanisms involved in limited IgA responses to HIV have been explored (Xu et al. 2009). Apparently, HIV-infected macrophages and dendritic cells suppress the differentiation of B cells to IgA- or IgG2-producing plasma cells through the introduction of HIV negative factor (nef) into these B cells.

1.5.2.4 Human Papilloma Virus

HPVs are double-stranded DNA viruses, which infect squamous epithelial cells, including those of the genital tract, with remarkable species specificity and tissue tropism. Of more than 220 genotypes, HPV types 16, 18 and to a lesser degree 31, 33, and 35 are of importance in the development of premalignant (dysplasia) and malignant cervical lesions (for review see Chow et al. 2010). Importantly, only a small percentage of HPV-infected women develop cervical cancer. HPV infection induces cellular and humoral immune responses in plasma and genital tract secretions. The levels of HPV-specific antibodies are

higher in women with cervical cancer but the relative proportions of IgG vs. IgA antibodies display a characteristic pattern and kinetics (Nguyen et al. 2005; Russell et al. 2015c;). Humoral responses to HPV are induced with delayed kinetics (Hagensee et al. 2000) and IgA antibodies appear earlier than IgG. Furthermore, a comparative study (Nguyen et al. 2005) of HPV16-specific antibodies indicated that, in women with cervical cancer, IgG responses in vaginal washes were higher than in women with cervical dysplasia or those undergoing hysterectomy for other reasons. Interestingly, lower IgA responses were detected in cervical cancer and dysplastic patients than in those with hysterectomy. Apparently and by analogy with HIV infection (see above), HPV induces low IgA-associated responses in women with cervical cancer or dysplasia. Due to inherent difficulties in obtaining a sufficient number of T cells from cervical tissue, most studies of cell-mediated immunity have been performed using lymphocytes from peripheral blood (Evans et al. 1997). T cells with cytotoxic activity have been detected in draining lymph nodes.

Systemic immunization with two currently available HPV vaccines induces specific antibodies of the IgG isotype in plasma as well as in genital tract secretions (Kwak et al. 2011; Petaja et al. 2011; Wang et al. 2016). Although local responses in the cervix, manifested by the presence of HPV-specific antibody-secreting cells, have not been demonstrated, based on other studies of the origin of IgG in genital tract after systemic immunization (Underdown and Strober 2015), it is highly probable that such antibodies are of circulatory origin and are selectively transported in the genital secretion by an epithelial FcRn-dependent mechanism. Sublingual mucosal immunization was less effective in the induction of such antibodies (Huo et al. 2012).

1.6 Concluding Remarks

STIs continue to present serious problems due to their high morbidity, economic impact, and the difficulties encountered in their prevention. The immunological uniqueness of the genital tract compared to other compartments of the mucosal and circulating immune system must be considered in the evaluation of humoral and cellular immune responses, whether these are induced by infection or by immunization. In addition, the selection of relevant STI-derived antigens capable of inducing protective responses, as well as choice of novel adjuvants or immunoregulatory cytokines for co-administration, appropriate immunization routes and antigen-delivery systems, are all factors that need to be considered for the development of future vaccines.

References

Anderson, J.E., Hobbs, M.M., Biswas, G.D. et al. (2003). Opposing selective forces for expression of the gonococcal lactoferrin receptor. *Mol. Microbiol.* 48: 1325–1337.

Anderson, D. and Pudney, J. (2015). Human male genital tract immunity. In: *Mucosal Immunology*, 4e (ed. J. Mestecky, W. Strober, M.W. Russell, et al.) Chapter 109, 2125–2140. Amsterdam: Elsevier/Academic Press.

Arnold, J.N., Wormald, M.R., Sim, R.B. et al. (2007). The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu. Rev. Immunol.* 25: 21–50.

Aymeric, L. and Sansonetti, P. (2015). Discriminating pathogens from commensals at mucosal surfaces. In: *Mucosal Immunology*, 4e (ed. J. Mestecky, W. Strober, M.W. Russell, et al.) Chapter 50, 429–454. Amsterdam: Elsevier/Academic Press.

Baker, K., Blumberg, R.S., and Kaetzel, C.S. (2015). Immunoglobulin transport and immunoglobulin receptors. In: *Mucosal Immunology*, 4e (ed. J. Mestecky, W. Strober, M.W. Russell, et al.) Chapter 19, 349–408. Amsterdam: Elsevier/Academic Press.

Bergquist, C., Johansson, E.-L., Lagergard, T. et al. (1997). Intranasal vaccination of humans with recombinant cholera toxin B subunit induces systemic and local antibody responses in the upper respiratory tract and the vagina. *Infect. Immun.* 65: 2676–2684.

Boyaka, P.N., McGhee, J.R., Czerkinsky et al. (2005). Mucosal vaccines: an overview. In: *Mucosal Immunology*, 3e, vol. 47 (ed. J. Mestecky, M.E. Lamm, W. Strober, et al.) Chapter, 855–874. Amsterdam: Elsevier/Academic Press.

Brandtzaeg, P. (2015). The mucosal B cell system. In: *Mucosal Immunology*, 4e (ed. J. Mestecky, W. Strober, M.W. Russell, et al.) Chapter 21, 429–454. Amsterdam: Elsevier/Academic Press.

Bronson, R. and Fleit, H.B. (2015). Immunologically mediated male and female reproductive failure. In: *Mucosal Immunology*, 4e (ed. J. Mestecky, W. Strober, M.W. Russell, et al.) Chapter 111, 2157–2182. Amsterdam.: Elsevier/Academic Press.

Brown, T.A. and Mestecky, J. (1988). Subclass distribution of IgA antibodies to microbial and viral antigens. In: *Mucosal Immunity and Infections at Mucosal Surfaces* (ed. W. Strober, M.E. Lamm, J.R. McGhee, et al.) Chapter 33, 340–345. New York: Oxford University Press.

Brunham, R.C. and Rekart, M.L. (2008). The arrested immunity hypothesis and the epidemiology of chlamydia control. *Sex. Transm. Dis.* 35: 53–54.

Chow, L.T., Broker, T.R., Steinberg, B.M. et al. (2010). The natural history of human papillomavirus infections of the mucosal epithelia. *APMIS* 118: 422–449.

Cooper, M.D., McGee, Z.A., Mulks, M.H. et al. (1984). Attachment to and invasion of human fallopian tube mucosa by an IgA1 protease-deficient mutant of *Neisseria gonorrhoeae* and its wild-type parent. *J. Infect. Dis.* 150: 737–744.

Cornelissen, C.N., Kelley, M., Hobbs, M.M. et al. (1998). The transferrin receptor expressed by gonococcal strain FA1090 is required for the experimental infection of human male volunteers. *Mol. Microbiol.* 27: 611–616.

Crago, S.S., Kutteh, W.H., Moro, I. et al. (1984). Distribution of IgA1-, IgA2-, and J chain-containing cells in human tissues. *J. Immunol.* 132: 16–18.

Criss, A.K. and Seifert, H.S. (2012). A bacterial siren song: intimate interactions between *Neisseria* and neutrophils. *Nat. Rev. Microbiol.* 10: 178–190.

Crowley-Nowick, P.A., Bell, M., Edwards, R.P. et al. (1995). Normal uterine cervix: characterization of isolated lymphocyte phenotypes and immunoglobulin secretion. *Amer. J. Reprod. Immunol.* 34: 241–247.

Crowley-Nowick, P.A., Bell, M.D., Brockwell, R. et al. (1997a). Rectal immunization for induction of specific antibody in the genital tract of women. *J. Clin. Immunol.* 17: 370–379.

Crowley-Nowick, P.A., Edwards, R.P., Moldoveanu, Z. et al. (1997b). Menstrual cycle: effects on vaccine-induced antibodies in genital tract secretions. In: *Mucosal Solutions. Advances in Mucosal Immunology*, vol. 1 (ed. A.J. Husband, K.W. Beagley, R.L. Clancy, et al.), 393–401. Australia: The University of Sydney.

Czerkinsky, C., Anjuere, F., McGhee, J.R. et al. (1999). Mucosal immunity and tolerance: relevance to vaccine development. *Immunol. Rev.* 170: 197–222.

Czerkinsky, C., Cuburu, N., Kweon, M.N. et al. (2011). Sublingual vaccination. *Hum. Vacc.* 7: 110–114.

Duluc, D., Gannevat, J., Anguiano, E. et al. (2013). Functional diversity of human vaginal APC subsets in directing T-cell responses. *Mucosal Immunol.* 6: 626–638.

Edwards, J.L. and Apicella, M.A. (2004). The molecular mechanisms used by *Neisseria gonorrhoeae* to initiate infection differ between men and women. *Clin. Microbiol. Rev.* 17: 965–981.

Evans, E.M., Man, S., Evans, A.S. et al. (1997). Infiltration of cervical cancer tissue with human papillomavirus-specific cytotoxic T-lymphocytes. *Cancer Res.* 57: 2943–2950.

Fahey, J.V., Prabhala, R.H., Guyre, P.M. et al. (1999). Antigen-presenting cells in the human female reproductive tract: analysis of antigen presentation in pre- and post-menopausal women. *Am. J. Reprod. Immunol.* 42: 49–57.

Feinen, B., Jerse, A.E., Gaffen, S.L. et al. (2010). Critical role of Th17 responses in a murine model of *Neisseria gonorrhoeae* genital infection. *Mucosal Immunol.* 3: 312–321.

Forrest, B.D., Sharman, D.J.C., and LaBrooy, J.T. (1990). Specific immune response in humans following rectal deliver of live typhoid vaccine. *Vaccine* 8: 209–212.

Geisler, W.M. (2010). Duration of untreated, uncomplicated *Chlamydia trachomatis* genital infection and factors associated with chlamydia resolution: a review of human studies. *J. Infect. Dis.* 201 (Suppl 2): S104–S113.

Gillgrass, A.E., Tang, V.A., Towarnicki, K.M. et al. (2005). Protection against genital herpes infection in mice immunized under different hormonal conditions correlates with induction of vagina-associated lymphoid tissue. *J. Virol.* 79: 3117–3126.

Gupta, S., Gasch, J.S., Becerra, J.C. et al. (2013). The neonatal Fc receptor (FcRn) enhances human immunodeficiency virus type 1 (HIV-1) transcytosis across epithelial cells. *PLoS Pathog.* 9: 1–13.

Hagensee, M.E., Koutsky, L.A., Lee, S.K. et al. (2000). Detection of cervical antibodies to huma papillomavirus type 16 (HPV-16) capsid antigens in relation to detection of HPV-16DNA and cervical lesions. *J. Infect. Dis.* 181: 1234–1239.

Hajishengallis, G. and Russell, M.W. (2015). Innate humoral defense factors. In: *Mucosal Immunology*, 4e (ed. J. Mestecky, W. Strober, M.W. Russell, et al.) Chapter 15, 251–270. Amsterdam: Elsevier/Academic Press.

Hedges, S.R., Mayo, M.S., Kallman, L. et al. (1998). Evaluation of immunoglobulin A1 (IgA1) protease and IgA1 protease-inhibitory activity in human female genital infection with *Neisseria gonorrhoeae*. *Infect. Immun.* 66: 5826–5832.

Hedges, S.R., Mayo, M.S. et al. (1999). Limited local and systemic antibody responses to *Neisseria gonorrhoeae* during uncomplicated genital infections. *Infect. Immun.* 67: 3937–3946.

Hirbod, T. and Broliden, K. (2007). Mucosal immune responses in the genital tract of HIV-1-exposed uninfected women. *J. Int. Med.* 262: 44–58.

Hocini, H. and Barra, A. (1995). Systemic and secretory humoral immunity in the normal human vaginal tract. *Scand. J. Immunol.* 42: 269–274.

Huo, Z., Bissett, S.L., Giemza, R. et al. (2012). Systemic and mucosal immune responses to sublingual or intramuscular human papilloma virus antigens in healthy female volunteers. *PLoS One* 7: e33736.

Hussain, L.A., Kelly, C.G., Fellowes, R. et al. (1992). Expression and gene transcript of fc receptors for IgG, HLA class II antigens and Langerhans cells in human cervico-vaginal epithelium. *Clin. Exp. Immunol.* 90: 530–538.

Imarai, M., Candia, E., Rodriguez-Tirado, C. et al. (2008). Regulatory T cells are locally induced during intravaginal infection of mice with *Neisseria gonorrhoeae*. *Infect. Immun.* 76: 5456–5465.

Jackson, S., Moldoveanu, Z., and Mestecky, J. (2015). Appendix I: collection and processing of human mucosal secretions. In: *Mucosal Immunology*, 4e (ed. J. Mestecky, W. Strober, M.W. Russell, et al.), 2345–2354. Amsterdam: Elsevier/Academic Press.

Jalanti, R. and Isliker, H. (1977). Immunoglobulins in human cervico-vaginal secretions. *Int. Arch. Allergy Appl. Immunol.* 53 (5): 402–408.

Jarvis, G.A. and Griffiss, J.M. (1991). Human IgA1 blockade of IgG-initiated lysis of *Neisseria meningitidis* is a function of antigen-binding fragment binding to the polysaccharide capsule. *J. Immunol.* 147: 1962–1967.

Jerse, A.E., Bash, M.C., and Russell, M.W. (2014). Vaccines against gonorrhea: current status and future challenges. *Vaccine* 32: 1579–1587.

Johansson, E.-L., Ras, C., Fredriksson, M. et al. (1998). Antibodies and antibody-secreting cells in the female genital tract after vaginal or intranasal immunization with cholera toxin B subunit or conjugates. *Infect. Immun.* 66: 514–520.

Johansson, E.-L., Wassen, L., Holmgren, J. et al. (2001). Nasal and vaginal vaccinations have differential effects on antibody responses in vaginal and cervical secretions in humans. *Infect. Immun.* 69: 7481–7486.

Johansson, M. and Lycke, N. (2003). A unique population of extrathymically derived $\alpha\beta TCR^{+}CD4^{-}CD8^{-}$ T cells with regulatory functions dominates the mouse female genital tract. *J. Immunol.* 170: 1659–1666.

Kantele, A., Hakkinen, M., Moldoveanu, Z. et al. (1998). Differences in immune responses induced by oral and rectal immunizations with *Salmonella typhi* Ty21a: evidence for compartmentalization within the common mucosal immune system in humans. *Infect. Immun.* 66: 5630, PMCID: PMC108711–5635.

Kilian, M. and Russell, M.W. (2015). Microbial evasion of IgA functions. In: *Mucosal Immunology*, 4e (ed. J. Mestecky, W. Strober, M.W. Russell, et al.) Chapter 22, 455–469. Amsterdam.: Elsevier/Academic Press.

Korn, T., Bettelli, E., Oukka, M., and Kuchroo, V.K. (2009). IL-17 and Th17 cells. *Annu. Rev. Immunol.* 27: 485–517.

Kozlowski, P.A., Cu-Uvin, S., Neutra, M.R., and Flanigan, T.P. (1997). Comparison of the oral, rectal, and vaginal immunization routes for induction of antibodies in rectal and genital tract secretions of women. *Infect. Immun.* 65: 1387–1394.