The information summarized in the various comprehensive presentations in this workshop represented a diverse spectrum of historical, evolutionary, and functional aspects of mammalian lactation and the process of breastfeeding.

This workshop was dedicated to Prof. Lars A. Hanson (MD, PhD) for his outstanding contributions to the understanding of the biology of milk and the dissemination of knowledge on breastfeeding to advance current practices of breastfeeding in the contemporary human society worldwide. The dedication ceremony was followed by scientific presentations in session I of the workshop with the keynote addressed by Olav T. Oftedal.

Oftedal provided an elegant perspective of the evolution of lactation in different mammalian species. Based on studies on synapsids (ancestral to mammals, which appear to have diverged from sauropsids [ancestral to crocodiles, lizards, and birds]), he proposed that lactation may have first evolved as a source of moisture and antimicrobial compounds for parchment-shelled eggs, followed by the evolution of some skin secretions, which eventually became milk. It was suggested that among basal animals (monotremes), each mammary gland develops as a triad in association with a hair follicle and sebaceous gland as a mammmopilo-sebaceous unit (MPSU).

In other mammalian species, such as marsupials, there is a similar triad, but the hair follicles are shed during development. In the diverse group of eutherian mammals, some show no association with the mammary hair, while others, such as the horse, develop as MPSU with mammary hair and sebaceous glands present in the mammary gland.
The MPSU also bears significant resemblance to apocrine glands (APSU), suggesting that mammary glands may have also evolved from an APSU-type structure. Recent studies have suggested that most constituents of mammalian milk are unique and found only in mammary secretions. He proposed that if a milk protein occurs in the milk of monotremes, marsupials, and eutherians, the major mammalian taxa, then the protein must have evolved before the groups diverged and are inherited from the ancestral taxa. These observations have provided unique and new insights into the genetic origin and functions of specific mammary constituents in the products of lactation. Oftedal briefly alluded to the 4 primary types of caseins, members of the secretory calcium-binding phosphoproteins (SCPPs), as an evolutionary challenge because of their diversity and the large size of the micelles in milk. These proteins have an ancient history in the evolution of mineralized tissues. Based on related SCPP genes, caseins may have evolved as protolacteal secretion that delivered calcium to eggs. Finally, his presentation discussed briefly the evolution of the milk fat globule membrane, lactose, and other neutral and acidic oligosaccharides.

The next presentation provided a brief historical overview of the immunology of milk and mammalian lactation. This presentation served as an introduction to the subsequent specific topics discussed in this workshop. Mother’s milk has been considered a complete food for the infant from times immemorial, and it has been associated with unique healing powers and beneficial effects. These include cure for insomnia, loss of appetite, ascites, piles, skin disorders, sexual dysfunction, muscle weakness, contraception, and prevention of cancer and infections. Breastfeeding developed a spiritual and religious importance in the Middle Ages in Europe, as evidenced by the deep faith and respect for Nursing Madonna, Virgin Mary, and the breastfed Jesus. The modern history of breast milk immunology can be traced to a publication by Paul Ehrlich as early as 1892 and subsequent demonstration of specific maternal antibody transport to the colostrum and milk. The immunologic composition of human milk and its biologic linkage to mucosa-associated lymphoid tissue was initially recognized by Gugler and Von Muralt, and by Lars Hanson. These elegant studies were followed by the identification of secretory IgA in human external secretions by Chodirkar and Tomasi, and Bienenstock and Tomasi, and in the human milk by Hanson and Johansson. Subsequent studies by Beer and Bellingham, Ogra et al., Mohr, and Okamoto and others identified several cellular and soluble immunologic factors in the human milk and their transport to the suckling neonate via the process of breastfeeding. It is now known that human colostrum and milk contains a wealth of immunologically active products derived from the innate and adoptive immunologic, microbiologic, dietary, and other maternal experiences in the maternal mucosal surfaces, es-
especially the gut, and the maternal circulation. This historical review was dedicated to the memory of Dr. S.S. Ogra, the principal investigator of most milk-related research carried out in her laboratory in the early 1970s and 1980s in the School of Medicine at the University at Buffalo. This presentation briefly reviewed lactation performance and the presence and function of diverse soluble elements detected in mammalian colostrum and milk to date. These included: secretory IgA and other immunoglobulin isotypes, antisecretory factor, soluble CD14, and soluble Toll-like receptors, as well as several cytokines and lymphokines. It also introduced the role of colostrum- and milk-associated cellular components, such as leukocytes, macrophages, epithelial cells, stem cells, and T lymphocytes, and cell-mediated immune responses. This overview also summarized earlier studies on the transfer of tuberculin-specific maternal cellular immunity to the neonate via breastfeeding and more recent investigations on the transfer of maternal cellular immunity and engulfment of maternal DNA via the transfer of leukocytes and stem cells. Finally, the risks and benefits of the colostrum and milk to the neonate and the developing infant were briefly considered here. Detailed discussion of the issues identified here follows in subsequent presentations in this session and sessions II and III of this workshop.

Jiri Mestecky reviewed in some detail the evidence for the existence of mucosa-associated lymphoid tissue and common mucosal immune sites for effective immunization in the mucosal system, and the importance of mammary glands as an integral component of the common mucosal immune system. He discussed recent studies on the structure, biologic activities, and the spectrum of antibodies of the IgA isotype specific for microbial, dietary, and other environmental antigens and macromolecules in the colostrum and milk. He concluded his presentation by identifying possible directions for future investigations in the immunobiology of the mammary gland and lactation. These include the routes for the most effective induction of IgA responses in milk, the identification of phenotypes of B lymphocytes that express homing receptors for the mammary gland, and the determination of effective timing for maternal immunization to provide optimal levels of protective immune reactivity in the colostrum and the milk for the neonate.

Helena Tlaskalová-Hogenová, Miloslav Kverka, and Jiří Hrdý introduced the wide spectrum of immunomodulatory components present in human milk and colostrum, including those of innate and adaptive immunity, and factors influencing the composition and colonization of newborn gut microbiota. They discussed the nature of autoantibodies and the spectrum of newly detected cytokines and lymphokines in human milk. The presentation was completed with an overview of different cellular components of and cytokine gene expression on
colostral cells in healthy and allergic mothers. Studies carried out to date have identified over 35 cytokines in the colostrum and milk, and some of them have been identified for the first time in human milk. Their possible functions include the development of intestinal lymphoid tissue, functional development of the gut structure, angiogenesis, central and enteric nervous system development, and establishment of immunologic homeostasis in the mammary gland as well as in the breastfeeding neonate.

Valerie Verhasselt discussed the influence of breastfeeding on the development of immunologic health in the breastfed neonate and infant. She began with the examination of the unique specificities of the neonatal immune system, including unique limitations to the development of immune responses after postnatal exposure to environmental antigens. She reviewed the role of TGF-β, vitamin A, several environmental allergens, and specific antibodies in the context of early life and long-term allergic disease susceptibility. Based on controlled epidemiological data and several experimental studies, she proposed that early-life oral exposure to allergens does not induce tolerance but may prime for allergic responses. It has been suggested that nonbreastfed infants are exposed to only few allergens, but in very high concentrations, such as β-lactoglobulins. On the other hand, breastfed infants are exposed to a wide variety of allergens in the maternal milk and colostrum but in extremely low concentrations. Additionally, breast milk provides the infant with significant amounts of TGF-β, vitamin A, and other cofactors which affect the integrity of the barrier of gut epithelium and regulate antigen transfer and presentation to the mucosa-associated lymphoid tissue. As a result, breastfeeding results in a low risk for allergic disorders in the long term. Such conclusions are supported by recent studies in human birth cohorts and by studies carried out in her laboratories with the induction of egg allergy in experimental (mouse) animal models.

Carine Blanchard made the final presentation in session I. Due to certain unavoidable circumstances, she could not provide a full-length manuscript of her presentation. Therefore, a more detailed summary of her talk is presented here. Her presentation focused on the immunologic evaluation of human milk oligosaccharides (HMO) with respect to disease expression in the neonate after exposure to allergens and infectious agents. She discussed in some detail the enzyme fucosyltransferase (FUT) and its genotypes FUT2 and FUT3. These enzymes are expressed on blood groups ABH and Lewis, intestinal mucosa, and other human body fluids. Recent studies have suggested that the early trajectory of neonatal microbial colonization is significantly influenced by the number of environmental factors. These include gestational age of the neonate, method of delivery, use of antibiotics, geographic location of birth, genetics, maternal stage...
of lactation, maternal diet, and specific immunologic components delivered to the neonate via breastfeeding. These factors appear to determine the outcome of colonization and the composition of the neonatal microbiome as healthy or aberrant based on the metabolites generated by the microbiome. An aberrant microbiome has been associated with the development of sustained inflammation, induction of asthma, atopy, obesity, inflammatory bowel disease, and other disease states. Employing FUT2 and FUT3 genotypes as proxy for the HMOs, several ongoing investigations have provided important information on the role of HMOs in human milk and colostrum;
1. 2′-Fucosylated HMOs in human milk alleviate the negative effects of cesarean section on infant gut microbiota.
2. HMOs in infant formula significantly improve the outcome of infections in infants.
3. Maternal FUT2- and FUT3-positive status is related to a lower risk of respiratory infections during the first 6 months of neonatal life.
4. Maternal HMOs are associated with the prevention of colonization and growth of the pathogenic microbiome.
5. Elevated serum IgE levels are associated with the absence of gut microbiota in experimental models of infection.

She summarized the results of the LIFE child cohort studies from Leipzig (Germany) and Bangkok (Thailand) and other investigations involving cholera toxin/ovalbumin-induced food allergy in experimental animal models. These studies have also demonstrated that the use of HMO and FUT2/FUT3 genotypes in infant feeding is associated with significantly decreased allergic sensitization. The mechanisms underlying such protection appear to be related to the modulation of regulatory T-cell function by HMOs and independent of the regular prebiotic effects associated with milk oligosaccharides and other soluble products in the milk.

Based on the information summarized above from the presentations in session I of the workshop, it is apparent that we have come a long way in understanding the evolutionary biology of mammalian lactation, the presence and role of specific innate and adaptive immunologic mechanisms associated with human milk, and the impact of breastfeeding on the systemic and mucosal immunologic development in the neonate. Recent information about the microbiology of the milk and lactation and its influence on gut colonization, presented in session II, and studies about the role of HMOs and other soluble components of the colostrum and milk, presented in session III, are summarized next by W. Allan Walker and Bo Lönnerdal, respectively.

It is gratifying to note the wealth of new information presented in this workshop, and it is hoped to generate further interest in exploring many unanswered
questions related to the mammary glands and lactation and its impact on the neonate. Finally, it would be appropriate to conclude this summary by recapitulating the statement made by Frank Oski (MD) several decades ago:

Imagine that the world had created a new dream product to feed and immunize everyone born on earth. Imagine also that it was available everywhere, required no storage or delivery and helped mothers plan their families and reduce the risk of cancer. Then Imagine that the world refused to use it.

Frank Oski (1932–1996)

Pearay L. Ogra