Old Herborn University Seminar Monograph 13:
Polyspecific immunoglobulins, their possible role in the normal (physiologic) clearance of microorganisms and tissue fragments.
Editors: Peter J. Heidt, Volker Rusch, and Dirk van der Waaij.
Herborn Litterae, Herborn-Dill, Germany: 47-65 (2000).

THE NEONATAL INTESTINAL MICROFLORA AND THE IMMUNE SYSTEM

INGEGERD ADLERBERTH

Department of Clinical Immunology, Göteborg University, Göteborg, Sweden

SUMMARY

Directly after birth, bacteria commence to colonise the skin and the mucous membranes of the respiratory, urogenital and intestinal tract. With time, more or less complex bacterial ecosystems are established at various body surfaces, of which the normal intestinal microflora is the largest and most diverse. Thus, an adult individual harbours more than 400 bacterial species at this site. Most of these bacteria are obligatory anaerobic.

The establishment of the intestinal microflora after birth precedes in a sequential manner, and a complete microflora is not obtained until after several years of age. A number of factors may influence the establishment of the microflora, including delivery and feeding mode, social contacts and the degree of environmental hygiene.

Many bacteria that inhabit the intestine, especially facultatively anaerobic bacteria, are potential pathogens, since they may spread to extra-intestinal sites. High population levels of these bacteria in the intestine in early life make the new-born infant vulnerable to infections, for instance septicaemia. On the other hand, these bacteria may be of great importance for the maturation of the developing immune system. Bacteria colonising the intestine activate both mucosal and systemic immunity, and may contribute to development of oral tolerance towards harmless antigens.

This paper describes the establishment of the intestinal microflora in early life, factors affecting the colonisation process, and influence of the intestinal microflora on the infant's immune system.

ESTABLISHMENT OF THE MAJOR BACTERIAL GROUPS IN THE INTESTINE

The new-born infant is exposed to a wide range of different bacteria, but not all are able to establish and colonise in the neonatal intestine. The implantation of various bacteria into the intestinal microflora is regulated through limitations in the intestinal milieu, which may change with the successive establishment of different bacteria. In summarising what is known of the pattern of colonisation in the neonatal period, it is important to realise that there are considerable variations between studies as to when different bacterial groups settle in the intestine. This likely reflects both differences in methodology as well as study populations.

Aerobic and facultatively anaerobic bacteria

The intestinal milieu is characterised by a positive oxidation-reduction potential during the first days of life (*Grutte* et al., 1965), which favours the growth of aerobic or facultative bacteria, such as *E. coli* and other enterobacteria, enterococci and staphylococci (*Mitsuoka* and *Kaneuchi*, 1977; *Balmer* and *Wharton*, 1989; *Bennet* et al., 1991). These bacteria often reach population levels of 10^{10} bacteria/g faeces in the new-born infant, which is roughly 100 times more than those found in the microflora of adults (*Hoogkamp-Korstanje* et al., 1979; *Stark* and *Lee*, 1982).

E. coli strains that colonise the neonate may derive from the mother's faecal flora, in which case they are often transferred during delivery. Such transfer was frequently observed by *Bettelheim* and co-workers in the seventies (*Bettelheim* et al., 1974), whereas most other studies from Western countries (*Gothefors* et al., 1976; *Fryklund* et al., l992), and even a study of home delivered infants in an urban slum area in Pakistan (*Adlerberth* et al., 1999a) report that only a minority of neonates acquire their dominant intestinal *E. coli* strains from their mothers. It is likely that transfer of bacteria from mother to infant during delivery is markedly reduced when mothers give birth laying on their back and faecal soiling of the infant is avoided, whereas a standing or kneeling delivery position, as practised by certain indigenous populations, almost invariably leads to faecal contamination of the infant and most likely to transfer of large amounts of maternal bacteria to the neonate (*Mata* and *Urrutia*, 1971).

In maternity and neonatal wards, *E. coli* strains may be spread between infants via the nurses' hands (*Bettelheim* and *Lenox-King*, 1976; *Gothefors* et al., 1976). This type of transmission is greatly reduced when "rooming in" is practised, i.e. when the mothers themselves and not the staff handle the babies (*Bettelheim* et al., 1983). Later on, *E. coli* may be acquired from other family members or other persons in contact with the neonate.

Other enterobacteria, such as *Klebsiella*, *Enterobacter* and *Citrobacter*, are isolated from 20-60% of neonates, and in colonised infants reach similar population numbers as *E. coli* (*Lundequist* et al., 1985; *Balmer* and *Wharton*, 1989). These enterobacteria are less common than *E. coli* in the flora of adult individuals, and the strains colonising neonates are therefore rarely of maternal origin. Instead they derive from the intestinal microflora of other neonates, transferred via the staff, or from environmental sources (*Fryklund* et al., 1992; *Adlerberth* et al., 1999a).

Enterococci (*Enterococcus faecalis* or *E. faecium*), are isolated from almost all neonates and commonly reach population levels of 10^{10} CFU/g of faeces (*Rotimi* and *Duerden*, 1981; *Stark* and *Lee*, 1982). Some neonates may acquire these bacteria from their mothers, although this has never been particularly studied. In addition, enterococci are very good at spreading and surviving in the hospital milieu.

Staphylococci, e.g. *Staphylococcus epidermidis* or *S. aureus*, also establish in the intestine of many neonates during the first days of life and may reach population levels of 10^{10} bacteria/g faeces (*Balmer* and *Wharton*, 1989). Although commonly found in low numbers in the intestinal flora of adults, staphylococci are mostly regarded as members of the skin flora, which is probably the origin of many strains colonising the infant.

Aerobic streptococci are also found in the early neonatal microflora (*Rotimi* and *Duerden*, 1981), and strains of the Gram-negative genera *Aeromonas*, *Pseudomonas* and *Acinetobacter* may be transiently isolated from neonates dur-

ing the first week of life (*Rotimi* et al., 1985; *Adlerberth* et al., 1999a).

Anaerobic bacteria

The establishment and expansion of aerobic and facultatively anaerobic bacteria lower the redox-potential to negative values and "makes way" for obligatory anaerobic bacteria (*Hoogkamp-Korstanje* et al., 1979; *Stark* and *Lee*, 1982). Anaerobes recognised as early colonisers include *Bacteroides*, bifidobacteria and clostridia, which may reach populations of 10^{9-11}/g faeces within a week after birth (*Mata* and *Urrutia*, 1971; *Rotimi* and *Duerden*, 1981; *Stark* and *Lee*, 1982).

Infants delivered by caesarean section, who do not come into contact with the maternal faecal, vaginal or perineal flora at delivery, show a delayed colonisation with anaerobic bacteria, especially *Bacteroides* (*Neut* et al., 1987; *Grönlund* et al., 1999). *Bacteroides* die rapidly in contact with environmental oxygen and, since this limits transfer in the hospital milieu, acquisition of *Bacteroides* from the mother is probably of great importance.

Bifidobacteria are considerably more aerotolerant than *Bacteroides*. They are probably spread between neonates in maternity wards, since bifidobacterial colonisation pattern is ward-dependent (*Mitsuoka* and *Kaneuchi*, 1977; *Lundequist* et al., 1985). However, transfer of bifidobacteria from mother to infant also occurs. *Tannock* and coworkers demonstrated bifidobacterial strains with the same ribotype in mother and infant in two out of five mother/infant pairs (*Tannock* et al., 1990).

Clostridia are commonly isolated from the intestinal flora of neonates. These bacteria form spores which are spread in the environment and which are resistant to hygienic measures. In accordance, clostridial species are usually the first anaerobes to colonise infants after sectio deliveries (*Neut* et al., 1987). Strains of e.g. *C. difficile* colonising neonates are almost never acquired from the mother, but are frequently spread between neonates in maternity and neonatal wards (*Martirosian* et al., 1995).

In many recent studies, lactobacilli have been detected in as many as 60-80% of one month old infants (*Hall* et al., 1990; *Kleessen* et al., 1995), whereas such colonisation was infrequently observed in earlier studies (*Mata* and *Urrutia*, 1971; *Ellis-Pegler* et al., 1975; *Stark* and *Lee*, 1982). It is likely that improved bacteriological methods have facilitated the identification of these bacteria, which could have been grouped with e.g. bifidobacteria in earlier studies. Although lactobacilli dominate the vaginal microflora of fertile women, these lactobacilli do not seem to colonise the intestine of the baby (*Tannock* et al., 1990). The mother's faecal flora is a more likely source of infants' intestinal lactobacilli, as the dominating species in adults' intestine, *L. plantarum* (*Ahrné* et al., 1998), dominates also in neonates (*Bennet* and *Nord*, 1987). However, lactobacilli are probably easily acquired also from other sources. Within one month, sectio-delivered infants carry lactobacilli at a rate similar to vaginally delivered infants (*Hall* et al., 1990; *Grönlund* et al., 1999).

It may take a long time before many other intestinal anaerobic bacteria, including *Veillonella*, *Eubacterium*, *Peptostreptococcus*, *Peptococcus* and *Ruminococcus*, establish in the intestine, although there are large variations between different studies (*Mata* and *Urrutia*, 1971; *Ellis-Pegler* et al., 1975; *Mitsuoka* and *Kaneuchi*, 1977; *Rotimi* and *Duerden*, 1981; *Stark* and *Lee*, 1982; *Benno* et al., 1984; *Lundequist* et al., 1985; *Kleessen* et al., 1995; *Sepp* et al., 1997).

Many of the strict anaerobic bacteria

colonising the intestine can not be cultured *in vitro*. Little is known about these bacteria and their time of establishment in the microflora. In mice, segmented filamentous bacteria, a group of non-culturable, strictly anaerobic, spore-forming, Gram-positive bacteria (*Klaasen* et al., 1992), colonise the intestinal tract and become the dominating microbes around the time of weaning (*Garland* et al., 1982). Related bacteria occur also in humans (*Klaasen* et al., 1992).

When the anaerobic bacterial populations expand in the intestine, facultative bacteria are suppressed and decline in numbers (*Mata* and *Urrutia*, 1971; *Stark* and *Lee*, 1982). Within a few weeks or months, reduced counts of e. g. *Klebsiella*, *Enterobacter* and staphylococci are observed (*Ellis-Pegler* et al., 1975; *Rotimi* and *Duerden*, 1981; *Balmer* and *Wharton*, 1989; *Kleessen* et al., 1995; *Adlerberth* et al., 1999a). Other facultative bacteria, such as *E. coli* and enterococci retain quite high population numbers for longer periods of time (*Bennet*, 1987). Thus, high levels of both facultatives and anaerobes may co-exist during the first months (*Hoogkamp-Korstanje* et al., 1979; *Stark* and *Lee*, 1982), or even years of life (*Ellis-Pegler* et al., 1975). This relates to the fact that a diversified anaerobic microflora is required to suppress e.g. the numbers of *E. coli* in the intestine. In mice mono-associated with *E. coli*, population levels of $10^{10\text{-}11}$ bacteria/g faeces are obtained. To bring down the *E. coli* population to the levels found in conventional animals, 95 different anaerobic strains isolated from conventional mice are required (*Freter*, 1992). In humans, the successive establishment of different anaerobic species into the intestinal microflora proceeds over a period of several years (*Ellis-Pegler* et al., 1975; *Midtvedt*, 1994), finally resulting in a pronounced anaerobic predominance. The ratio of anaerobes to aerobes has been calculated to be 1.5 before 4 month's age, 10 between 4 and 12 month's age, 50 between 1 and 4 year's age and 200 in an adult (*Ellis-Pegler* et al., 1975).

THE INFLUENCE OF BREASTFEEDING ON THE INTESTINAL MICROFLORA

A great number of studies have investigated the intestinal microflora in breastfed and bottlefed infants. The results vary considerably between studies, but some differences between breastfed and bottlefed infants are quite consistently observed. Thus, most studies find lower counts of clostridia and enterococci in breastfed than in bottlefed infants (*Stark* and *Lee*, 1982; *Benno* et al., 1984; *Lundequist* et al., 1985; *Balmer* and *Wharton*, 1989; *Kleessen* et al., 1995), whereas breastfed infants tend to have higher counts of staphylococci than bottlefed infants, especially during the neonatal period (*Lundequist* et al., 1985; *Balmer* and *Wharton*, 1989). The reason may be that staphylococci colonising the nipple are swallowed during breastfeeding (*Gothefors*, 1975).

High bifidobacterial counts were a long time regarded as the most characteristic feature of the intestinal flora of the breastfed infant (*Bullen* et al., 1976). However, most studies from the eighties and onwards report similar counts of bifidobacteria in breastfed and bottlefed infants (*Lundequist* et al., 1985; *Balmer* and *Wharton*, 1989; *Balmer* et al., 1994; *Kleessen* et al., 1995). Nevertheless, more breastfed than bottlefed infants may harbour a flora dominated by bifidobacteria, most-

ly due to lower levels of other bacterial groups (*Balmer* and *Wharton*, 1989). Lactobacilli, which is another group of acid tolerant bacteria, are not favoured in the intestine of breastfed babies (*Benno* et al., 1984; *Kleessen* et al., 1995).

Only a minority of studies report higher counts of *Bacteroides* or enterobacteria in bottlefed than in breastfed infants (*Bullen* et al., 1976; *Benno* et al., 1984; *Balmer* and *Wharton*, 1989). However, the composition of the enterobacterial flora differs at the species and strain level between breastfed and bottlefed infants. Breastfed infants less often carry enterobacteria other than *E. coli*, for example *Klebsiella* or *Enterobacter* (*Örskov* and *Biering-Sörensen*, 1975; *Bullen* et al., 1976; *Balmer* and *Wharton*, 1989; *Adlerberth* et al., 1991). They also have a more stable enterobacterial flora than bottlefed infants, with fewer different *E. coli* serotypes being present concomitantly (*Örskov* and *Biering-Sörensen*, 1975; *Mevissen-Verhage* et al., 1985) and over time (*Mevissen-Verhage* et al., 1985). It seems reasonable to assume that differences at the species and strain level between breastfed and bottlefed infants may exist also for other major bacterial groups in the intestinal microflora.

A number of different factors in breastmilk has been suggested to contribute to differences in the bacterial flora between breastfed and bottlefed infants. These include the low buffering capacity of human milk (*Bullen* et al., 1976), and factors like secretory IgA, lactoferrin, lysozyme, complex oligosaccharides and nucleotides (*Wold* and *Hanson*, 1994). However, feeding with formulas supplemented with e.g. lactoferrin or nucleotides or formulas with reduced buffering capacity have not resulted in a "breastfed" type of microflora in the infants studied (*Balmer* et al., 1989a; 1989b; 1994).

GLOBAL DIFFERENCES IN INTESTINAL COLONISATION PATTERN - IMPACT OF HYGIENIC CONDITIONS

The intestinal colonisation pattern is strongly influenced by the degree of bacterial exposure and, thus, varies considerably between infants in developing and industrialised societies.

In general, infants born in poor areas in developing countries are earlier colonised with a number of different bacteria than infants in rich and highly developed societies. In indigenous Guatemalan infants, meconium passed at 4-7 hours after birth often contained bacteria, most commonly enterobacteria and streptococci (*Mata* and *Urrutia*, 1971). The mothers gave birth in a kneeling position, and maternal faeces commonly contaminated the infant during delivery (*Mata* and *Urrutia*, 1971), facilitating direct transfer of bacteria from mother to infant. However, under poor hygienic conditions, sectio-delivered neonates as well acquire bacteria almost immediately after birth (*Rotimi* et al., 1985; *Adlerberth* et al., 1991), reflecting heavy environmental exposure to bacteria. In Pakistan, infants from underprivileged groups, whether delivered in hospital or at home and regardless of delivery mode, acquire enterobacteria earlier and harbour a more diverse enterobacterial flora than Swedish infants (*Adlerberth* et al., 1991; 1999a). In an ongoing study, we have observed than at least 40% of Swedish neonates have not yet acquired any enterobacteria at one week of age, and that it takes a month before all infants harbour enterobacteria in their faecal flora (*Adlerberth* et al., 1999b). This shows the severely restricted circulation of enterobacteria in

a highly hygienic modern society.

Pronounced environmental exposure to enterobacteria also leads to a high turn over of enterobacterial strains in the microflora. Thus, different *E. coli* strains replace each other in rapid succession in the intestinal flora of Pakistani infants (*Adlerberth* et al., 1999a) whereas in Swedish infants a single *E. coli* strain usually dominates the enterobacterial microflora for prolonged periods of time (*Kuhn* et al., 1986).

Colonisation with many other bacterial groups, e.g. enterococci, lactobacilli and eubacteria is similarly delayed in Western infants (*Bennet* et al., 1991; *Sepp* et al., 1997). The fact that more Swedish than Estonian one year old infants carry *C. difficile* (*Sepp* et al., 1997) indicate that Swedish infants have a poorly developed intestinal microflora by that age, as *C. difficile* is common in the intestinal microflora of young infants, but usually disappear when a complex anaerobic microflora is established.

In the absence of competition from e.g. enterobacteria, intestinal colonisation with "skin bacteria" like *S. epidermidis* have become more prominent among neonates in Western societies. Thus, early colonisation with *S. epidermidis* is more common in Sweden than in Ethiopia (*Bennet* et al., 1991). Similarly, in French neonates (*Borderon* et al., 1996), *S. epidermidis* rather than *E. coli* or enterococci are the first bacteria to colonise the intestine. Today, all Swedish infants seem to be colonised with staphylococci within three days after birth (*Adlerberth* et al., 1999b).

BACTERIAL TRANSLOCATION

Certain bacteria that colonise the intestinal tract have the capacity to translocate, i.e. pass viable over the intestinal barrier to reach mesenteric lymph nodes, blood or other organs (*Berg*, 1983b). Translocating bacteria include *E. coli* and other enterobacteria, staphylococci, enterococci and lactobacilli. Most obligate anaerobes seem unable to translocate - most likely they do not survive in the oxygen-rich milieu of viable tissues. Translocation may occur when bacteria with the capacity to translocate reach high population levels in the intestine, i.e. more than 10^8 bacteria/g faeces (*Berg*, 1995), as occur e.g. during treatment with antibiotics (*Berg*, 1983b). It is further promoted by deficiencies in the host immune system and damage to the intestinal mucosal barrier (*Berg*, 1995). Although mostly studied in experimental animals, bacterial translocation has been shown to occur also in humans (*Brooks* et al., 1993), and has been suggested to be the mechanism behind septicaemia due to enteric bacteria in immunocompromised patients (*Tancrede*, 1992). Furthermore, bacterial translocation may explain the propensity of enterobacteria to cause septicaemia in new-born, especially premature infants (*Van Camp* et al., 1994). As discussed above, facultative bacteria reach high population levels in the intestinal flora during the neonatal period which, in combination with immature immune functions, may predispose for bacterial translocation and subsequent septicaemia (*Van Camp* et al., 1994). A considerable frequency of asymptomatic bacteraemia, occurring at the time for intestinal colonisation, has been observed in neonates (*Albers* et al., 1966).

Neonatal septicaemia is especially common in developing countries (*Dawodu* and *Alausa*, 1980; *Khan* et al., 1993), where intestinal bacteria such as *E. coli*, *Klebsiella* and other enterobacteria, enterococci and *Pseudo-*

monas are responsible for up to 80% of the cases (*Dawodu* and *Alausa*, 1980; *Bhutta* et al., 1991).

The mechanisms behind bacterial translocation are only partly understood. It is likely that bacteria are taken up via the Peyer's patches and other lymphoid follicles lining the intestinal tract, in which case translocation may be regarded as a physiological process related to the sampling of lumenal contents by the gut immune system, causing disease only if the host defence systems are overridden. In addition, enterocytes under certain conditions may permit passage of live bacteria.

THE NEONATAL IMMUNE SYSTEM

The new-born infant is more susceptible to infections than older children and adults because certain functions of the immune system are to a greater or lesser extent immature in the neonate.

Some aspects of innate immunity are not fully developed at birth. Concentrations of the complement factors C8 and C9 only reach 20 and 10%, respectively, of adult levels (*Ballow* et al., 1974), and the plasma levels of C1, C2, C3, C4, C6 and C7 are also low in the new-born infant (*Fireman* et al., 1965; *Adinolfi* and *Beck*, 1976). In accordance, the sera of neonates show decreased bactericidal activity (*Lassiter* et al., 1992).

The number of phagocytes in the blood are as high in infants as in adults, but the total pool of granulocytes that can be mobilised during infection is small. Neutrophils show reduced adherence and chemotaxis and lower enzymatic activity than in adults (*Klein* et al., 1977; *Kovarik* and *Siegrist*, 1998). Chemotactic activity of monocytes may be impaired, and there is a clearly diminished influx of monocytes into sites of inflammation (*Wilson* et al. 1996). Natural killer cells are present in normal numbers at birth, but show decreased cytotoxic activity compared with cells from adults (*Wilson* et al., 1996).

The specific immune system is fairly well developed at birth. However, although the neonate has the capacity to mount a specific immune response to most antigens, a primary immune response has to take place for each new antigen that is encountered. Thus, neonatal T cells are almost entirely of the naive phenotype (*Lewis* et al., 1991), which means that they are more difficult to activate than memory cells (*Wilson* et al., 1996). Like naive T cells from adults, neonatal T lymphocytes produce high levels of IL-2 when stimulated with mitogens, but very little IFN-γ and IL-4 (*Bodeker* et al., 1982; *Lewis* et al., 1991). They also show an inability to express the CD40 ligand following activation (*Nonoyama* et al., 1995), which limits their capacity to deliver help to B cells (*Wilson* et al., 1996).

The cytotoxic capacity of sensitised T cells, defined as the capacity to lyse allogeneic lymphocytes, is not fully developed in term neonates (*Granberg* and *Hirvonen*, 1980). At least partly, this could reflect the absence of memory CD8+ T cells in neonates, as such cells kill more efficiently than naive CD8+ T cells (*McFarland* et al., 1992; *Wilson* et al., 1996).

Neonatal B cells function is fully mature at birth with regard to IgM formation but not with regard to IgG or IgA formation (*Andersson* et al., 1981). Thus, B cell activation *in vivo* in response to antigens mainly results in IgM production (*Gathings* et al., 1977). This is most likely at least partly related to the inability of neonatal T cells to provide efficient help for B cell differentiation and isotype switch, as discussed above. Neonatal B cells respond poorly to bac-

terial polysaccharides, which are antigens not requiring contact-dependent T cell help (*Wilson* et al., 1996). However, the limited capacity of neonatal T cells to produce IFN-γ could also be of importance in this matter (*Wilson* et al., 1996), as IFN-γ enhances the B cell response to bacterial capsular polysaccharides (*Peeters* et al., 1992).

Another specific feature of the foetal and neonatal B-cell repertoire is the relative preponderance of B cells expressing CD5 (*Bhat* et al., 1992). B cells of the CD5+ or B1 phenotype represent a separate lineage, precursors for which are found only early during development (*Herzenberg* et al., 1986). They typically produce polyspecific antibodies, most often of the IgM isotype, which are often reactive with self-antigens as well as bacterial antigens (*Casali* and *Notkins*, 1989; *Barbouche* et al., 1992). These antibodies may be produced independently of exogenous antigen stimulation, and are thought to play a role in regulation and development of the immune system in early ontogeny and also to act as a first line of defence against invading micro-organisms, before specific immune responses have evolved (*Casali* and *Notkins*, 1989; *Avrameas*, 1991).

The serum levels of immunoglobulin, except IgG, are low in neonates (*Allansmith* et al., 1968). Due to the active transport of IgG antibodies over the placenta, IgG levels in cord blood exceed those found in maternal blood (*Allansmith* et al., 1968). IgG synthesised by the baby is present only in low amounts (*Martensson* and *Fudenberg*, 1965). Immunoglobulins of the IgA class may also be found in cord blood, in concentrations of less than 50 μg/ml (*Allansmith* et al., 1968; *Wilson* et al., 1996). IgM is present in sera of all healthy neonates, in concentrations of 110-150 μg/ml, which resemble approximately 10% of adult levels (*Allansmith* et al., 1968; *Wilson* et al., 1996). Much of the IgM antibodies present at birth are likely to be polyspecific antibodies, produced by CD5+ B cells in the absence of exogenous antigenic stimulation.

THE MUCOSAL IMMUNE SYSTEM IN THE NEONATE

The gut associated immune system contains the vast majority of all lymphoid cells in the human body. This include lymphoid cells dispersed in the lamina propria, intraepithelial lymphocytes, and organised lymphoid tissue, such as Peyer's patches and colonic lymphoid follicles as well as the mesenteric lymph nodes that drain the intestinal tract. At birth, lymph nodes and Peyer's patches contain only primary follicles with mainly IgM+ and IgD+ cells, but very few IgA+ cells (*Russell* et al., 1990). The lamina propria contains very few immunoglobulin-containing cells (*Perkkiö* and *Savilathi*, 1980; *Russell* et al., 1990), which are mainly IgM+ and almost never IgA+ (*Iwase* et al., 1987; *Russell* et al., 1990). In accordance, no or only small amounts of secretory IgA are detected in foetal gut content or in meconium (*Rule* et al., 1971; *Petit* et al., 1973).

CD4+, CD3+ and CD8+ cells are readily identified in the lamina propria of both foetal and neonatal intestine. Many of the CD4+ cells have the morphological appearance of macrophages or dendritic cells (*Russell* et al., 1990). Very few intraepithelial lymphocytes are present at the time of birth, and no epithelial MHC class II expression is observed (*Russell* et al., 1990).

DEVELOPMENT OF THE IMMUNE SYSTEM AFTER BIRTH - RELATION TO INTESTINAL COLONISATION

Systemic immunity

In healthy neonates, there is a rapid increase in serum IgM during the very first weeks after birth (*Allansmith* et al., 1968). Thereafter the levels increase more slowly and 60-100% of adult levels are achieved at one year of age (*Stiehm* and *Fudenberg*, 1966; *Allansmith* et al., 1968). IgG production in the neonate is most likely initiated during the first weeks of life (*Allansmith* et al., 1968). By two months of age, the amount of circulating IgG synthesised by the infant equals the amount derived from transplacental transfer, and by one year of age, almost all circulating IgG is synthesised by the infant (*Wilson* et al., 1996). Sixty to 80% of adult IgG levels are reached by one year's age (*Allansmith* et al., 1968; *Stiehm* and *Fudenberg*, 1966) and adult levels by 7 years' age (*Allansmith* et al., 1968). Of the IgG subclasses, the production of IgG2 and IgG4 increase more slowly than that of IgG1 and IgG3 (*Morell* et al., 1972). The levels of IgA in serum also increase gradually after birth. At one year of age, serum levels of IgA are approximately 20-25% of adult levels, which are not achieved before 12 years of age (*Stiehm* and *Fudenberg*, 1966; *Allansmith* et al., 1968). The most pronounced increase in serum IgA levels is observed during the first 3 months after birth (*Stiehm* and *Fudenberg*, 1966).

The initiation of IgG and IgA production after birth is likely to be a response to bacterial colonisation of the gastrointestinal tract and other mucosae. The importance of the intestinal microflora as a stimulus for antibody production is illustrated by the fact that serum immunoglobulin levels in germfree animals are only 10 to 20% of those in conventional animals (*Sell* and *Fahey*, 1964; *Kim* et al., 1966; *Wostmann* et al., 1971). When germfree animals are colonised by an intestinal flora, immunoglobulin concentrations in serum and secretions rise and antibodies appear towards the colonising micro-organisms (*Carter* and *Pollard*, 1971). In comparison with the strong stimulus afforded by the microflora, the diet contributes very little antigenic stimulation, despite the fact that bacterial components are present in standard animal feed (*Midtvedt* and *Gustafsson*, 1981). Thus, serum IgA and IgG are slightly increased in germfree mice fed a commercial diet compared with those fed an antigen-free diet, but much lower than in conventional mice (*Hashimoto* et al., 1978).

It is less clear how serum IgM levels depend on exogenous antigenic stimulation. A high proportion of the IgM in serum may represent "natural" antibodies, which may be formed as a consequence of endogenous antigenic stimulation by self antigens or antibodies of maternal origin (*Berg*, 1983a). Thus, germfree mice fed an antigen free diet possess normal levels of serum IgM (*Hooijkaas* et al., 1984). However, the very rapid increase in serum IgM observed in human infants during the first weeks of life is most likely a response to antigenic, probably microbial stimulation after birth (*Allansmith* et al., 1968).

Mucosal immunity

Secretory IgA responses develop earlier and independently of serum IgA antibody responses (*South*, 1971). Neonatal secretions contain no or only low levels of secretory IgA (*Rule* et al., 1971; *Petit* et al., 1973; *Burgio* et al., 1980; *Gleeson* et al., 1982; *Mellander* et al., 1984) but relatively more IgM than in older children (*Gleeson* et al., 1982; *Mellander* et al., 1984). This is due to the fact that IgM, in the absence of

dimeric IgA, can bind to secretory component and be transported out into mucosal secretions (*Hanson* et al., 1999).

During the first weeks or months of life, there is a marked increase in secretory IgA in saliva, where-after the levels may decrease slightly and then remain fairly constant for years (*Gleeson* et al., 1982). Adult levels of secretory IgA are reached at the age of 6-8 years (*Burgio* et al., 1980).

Immunohistochemical examinations of human postnatal intestine show the appearance of secondary lymphoid follicles, increasing numbers of IgA positive cells in the lamina propria, increasing numbers of intra-epithelial lymphocytes and the expression of MHC class II antigen on enterocytes (*Perkkiö* and *Savilathi*, 1980; *Russell* et al., 1990; *Rognum* et al., 1992; *Machado* et al., 1994). Expansion of mucosal dendritic cells also occurs postnatally (*MacDonald*, 1996). Levels of soluble IL-2 receptors, secreted by activated T cells and macrophages (*Rubin* and *Nelson*, 1990), rise within days after birth presumably reflecting mucosal immune responses (*Spear* et al., 1995).

The appearance of IgA positive cells in the lamina propria and the rapid increase in secretory IgA levels after birth is most likely primarily the result of colonisation of mucosal surfaces by commensal bacteria. Germfree animals have approximately one tenth as many IgA-producing cells in the intestinal lamina propria as conventional animals (*Crabbé* et al., 1968; *Hashimoto* et al., 1978). *Koopman* et al. (1982) demonstrated an increase in IgA-producing cells in the ileal mucosa and Peyer's patches after associating germfree mice with an intestinal microflora. In contrast, food antigens are very poor inducers of secretory IgA production (*Wold* et al., 1989).

Animal studies have shown that also the presence and activation state of intra-epithelial lymphocytes as well as the expression of MHC class II molecules on enterocytes are strongly dependent on the presence of a normal microflora (*Umesaki* et al., 1993; 1995). In response to lumenal bacteria, intra-epithelial lymphocytes start to produce IFN-γ, which in turn upregulates the expression of MHC class II molecules on intestinal epithelial cells (*Matsumoto* et al., 1999).

In humans, 24-74% of intestinal bacteria are coated with IgA *in vivo* (*van der Waaij* et al., 1996). The capacity of a bacterial strain to induce strong immunity is linked to its ability to colonise and to invade the Peyer's patches (*Hohmann* et al., 1979). When a bacterial strain successfully colonises the intestine and reaches numbers high enough to permit translocation, a stimulation of the Peyer's patches resulting in the formation of germinal centres occurs. Activated B cells leave the patches and home to the lamina propria where they differentiate into IgA-producing plasma cells. The secretory IgA so produced coats the bacteria in the intestinal lumen, which most likely prevents further translocation. Thus, despite the continued presence of the microbe in the gut flora, there will be no, or only minimal, further stimulation of the immune system (*Shroff* et al., 1995). Therefore, it is likely that a persistent activation of the mucosal immune system depends on the continuous acquisition of new bacterial strains in the microflora.

Only a limited portion of the secretory IgA produced in response to a microbe colonising the intestine seem to be specific for this microbe (*Cebra*, 1999). A significant proportion of the secretory IgA produced may, in fact, represent polyreactive antibodies (*Vassilev* and *Veleva*, 1996; *Quan* et al., 1997). It is possible that a portion of intestinal lamina propria IgA plasma cells are derived from B1 cells residing in the peritoneal cavity (*Kroese* et al., 1995). In addition, it has been shown that IgA secret-

ing hybridoma clones obtained from mouse Peyer's patches may also produce polyreactive antibodies (*Shimoda* et al., 1999). Polyreactive antibodies could possibly act as a first line of defence at mucosal surfaces, before the induction of specific immune responses (*Quan* et al., 1997).

The pattern of intestinal colonisation may influence the "maturation" of the immune system

As mentioned above, it is likely that a persistent activation of the mucosal immune system requires a high turn-over of bacterial strains in the intestinal microflora. Pakistani infants, who are early colonised and constantly acquire new enterobacterial strains in their intestinal flora, have higher secretory IgA levels in saliva, and higher anti-*E. coli* antibody levels than Swedish infants of the same age (*Mellander* et al., 1985). Also, *Nagao* and co-workers (1993) found that children living in slum areas in Sao Paulo had higher levels of salivary IgA than Brazilian middle-class children, most likely reflecting differences in microbial exposure between the groups.

Breastfeeding, the intestinal microflora and the immune system

Breastfed infants may experience less translocation, partly because they are colonised with fewer different strains, but mainly because secretory IgA, which is present in high concentrations in the milk, directly prevents translocation of gut bacteria (*Maxson* et al., 1995). A reduction of translocation may be a major reason for the strong protection against sepsis afforded by breastfeeding (*Winberg* and *Wessner*, 1971; *Ashraf* et al., 1991). Thus, it is likely that breastfeeding reduces the load of different microbial antigens reaching the intestinal immune system. Accordingly, immunocompetent mouse pups nursed by SCID/SCID mothers, and therefore are not supplemented with IgA via the milk, undergo an accelerated development of IgA responses (*Kramer* and *Cebra*, 1995; *Cebra*, 1999).

Many prospective studies show a more rapid and prominent increase of salivary IgA after birth in bottlefed than in breastfed infants (*Stephens*, 1986; *Gleeson* et al., 1986). Serum IgA responses may also be elevated in bottlefed neonates (*Sarrinen* et al., 1979), and *Stephens* et al. (1984) demonstrated significantly higher levels of IgM anti-*E. coli* antibodies in bottlefed compared to breastfed babies from the sixth day of life. The baseline activation of lymphocytes also seems to be higher in bottlefed than in breastfed infants, as reflected by a higher integrin expression and pronounced proliferative responses in the absence of antigen (*Pabst* et al., 1997). The antibody response to mucosal vaccines (e.g. live poliovirus and rotavirus) is often lower in breastfed than in bottlefed infants, which is generally attributed to an inhibition of virus replication in the gut or an enhanced clearance of virus from mucosal surfaces due to antiviral secretory IgA antibodies and other factors in breastmilk (*Pichichero*, 1990; *Rennels*, 1996).

However, breastmilk contains a host of factors that might modulate the developing immune system (*Wold* and *Hanson*, 1994). Large amounts of both inflammatory (IL-1, TNF-α) and anti-inflammatory (TGF-β, IL-10) cytokines are present in breastmilk (*Wold* and *Hanson*, 1994). Animal experiments show that cytokines survive and retain biologic activity during the passage through the gastrointestinal tract and that they may even be taken up into the circulation and thus affect immune functions (*Rollwagen* and *Baqar*, 1996). Breastmilk also contains large numbers of macrophages and activated T lymphocytes (*Wold* and *Hanson*, 1994) which could also influence the infant's immune system. Indeed, radiolabelled

human breastmilk leukocytes fed to new-born baboons are taken up into the circulation (*Jain* et al., 1989). The transient tuberculin positivity observed in breastfed infants born to tuberculin-positive mothers provides indirect evidence that functionally active T cells are taken up from the maternal milk by the infant (*Schlesinger* and *Covelli*, 1977). Furthermore, a range of hormones, such as thyroxin, insulin and corticosteroids, and growth factors, e.g. epidermal growth factor, nerve growth factor and insulin-like growth factor, are present in human milk (*Koldovsky* and *Thornburg*, 1987). Human milk also contains considerable amounts of mononucleotides, which may augment proliferative responses by lymphocytes (*Carver* et al., 1990). Thus, although reducing antigenic stimulation, breastfeeding may have other stimulating effects on the neonatal immune system.

The intestinal microflora and oral tolerance

Food proteins and other harmless soluble antigens usually induce a state of specific unresponsiveness, termed oral tolerance, when presented to the immune system via the oral route (*Telemo* et al., 1997). To uphold a state of tolerance to these antigens is an important task of the immune system; in the absence of such mechanisms inflammatory and hypersensitivity reactions might occur.

The presence of a normal bacterial flora in the gut facilitates the induction of oral tolerance to food antigens (*Moreau* and *Corthier*, 1988; *Sudo* et al., 1997). Further, the administration of LPS together with food antigens increases the tolerising effect of feeding (*Kim* and *Ohsawa*, 1995). The mechanisms behind these interactions have not been defined but could possibly involve effects of the intestinal microflora on antigen presenting cells (*Wold* et al., 1998). Several factors secreted by macrophages in response to bacterial products have been shown to decrease the antigen presenting capacity of dendritic cells (*Holt* et al., 1993; *Chouaib* et al., 1985), which are the cells likely to present soluble protein antigens to T cells (*Steinman*, 1991; *Wold* et al., 1998). A downregulation of the antigen presenting capacity of dendritic cells could be crucial for the induction of oral tolerance to soluble protein antigens (*Wold* et al., 1998).

It is possible that the early establishment of high population levels of certain bacteria in the neonatal intestine provides stimulus for the developing immune system of importance for the induction of tolerance to e.g. food proteins and inhaled environmental antigens. Thus, in Western societies, where colonisation with certain bacterial groups is delayed and the intestinal microflora of infants seem to be less diverse than in developing countries, too little stimulation of the immune system could hamper tolerance induction (*Sepp* et al., 1997; *Wold* et al., 1998). The incidence of atopic allergy is steadily increasing in Western countries (*Björkstén*, 1994). An interesting speculation is that the high incidence of allergy relates to an inadequately developed intestinal microflora of infants in these societies (*Sepp* et al., 1997; *Wold* et al., 1998).

LITERATURE

Adinolfi, M. and Beck, S.: Human complement - C7 and C9 - in fetal and newborn sera. Arch. Dis. Child. 50, 562-564 (1976).

Adlerberth, I., Carlsson, B., de Man, P., Jalil, F., Khan, S.R., Larsson, P., Mellander, L., Svanborg, C., Wold, A.E., and Hanson,

L.Å.: Intestinal colonization with *Enterobacteriaceae* in Pakistani and Swedish hospital delivered infants. Acta Paediatr Scand. 80, 602-610 (1991).

Adlerberth, I., Jalil, F., Carlsson, B., Mellander, L., Hanson, L.Å., Larsson, P., Khalil, K., and Wold, A.E.: High turn over rate of *Escherichia coli* strains in the intestinal flora of infants in Pakistan. Epidemiol. Infect. 121, 587-598 (1999).

Adlerberth, I., Åberg, B., Aberg, N., Hesselmar, B., Saalman, R., Strannegård, I.-L., and Wold, A.E.: Staphylococci dominate the neonatal intestinal flora. Submitted for publication (2000).

Ahrné, S., Nobaek, S., Jeppson, B., Adlerberth, I., Wold, A.E., and Molin, G.: The normal Lactobacillus flora of healthy human oral and rectal mucosa. J. Appl. Microbiol. 85, 88-94 (1998).

Albers, W.H., Tyler, C.W., and Boxerbaum, B.: Asymptomatic bacteremia in the newborn infant. J. Pediatr. 69, 193-197 (1966).

Allansmith, M., McClellan, B.H., Butterworth, M., and Maloney, J.R.: The development of immunoglobulin levels in man. J. Pediatr. 72, 276-290 (1968).

Andersson, U., Bird, A.G., Britton, S., and Palacios, R.: Humoral and cellular immunity in humans studied at the cell level from birth to two years of age. Immunological Rev. 57, 5-38 (1981).

Ashraf, R.N., Jalil, F., Zaman, S., Karlberg, J., Khan, S.R., Lindblad, B.S., and Hansons L.Å.: Breastfeeding and protection against neonatal sepsis in a high risk population. Arch. Dis. Child. 66, 488-490 (1991).

Avrameas, S.: Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton'. Immunol. Today 12, 154-159 (1991).

Ballow, M., Fang, F., Good, R.A., and Day, N.K.: Developmental aspects of complement components in the newborn: The presence of complement components and C3 proactivator (properdin factor B) in human colostrum. Clin. Exp. Immunol. 18, 257-266 (1974).

Balmer, S.E., Hanvey, L.S., and Wharton, B.A.: Diet and faecal flora in the newborn: Nucleotides. Arch. Dis. Child. 70, F137-F140 (1994).

Balmer, S.E., Scott, P.H., and Wharton, B.A.: Diet and faecal flora in the newborn: Casein and whey proteins. Arch. Dis. Child. 64, 1678-1684 (1989a).

Balmer, S.E., Scott, P.H., and Wharton, B.A.: Diet and fecal flora in the newborn: Lactoferrin. Arch. Dis. Child. 64, 1685-1690 (1989b).

Balmer, S.E., and Wharton, B.A.: Diet and faecal flora in the newborn: Breastmilk and infant formula. Arch. Dis. Cild. 64, 1672-1677 (1989).

Barbouche, R., Forveille, M., Fischer, A., Avrameas, S., and Durandy, A.: Spontaneous IgM autoantibody production *in vitro* by B lymphocytes of normal human neonates. Scand. J. Immunol. 35, 659-667 (1992).

Bennet, R.: The faecal microflora of newborn infants during intensive care management and its relationship to neonatal septicaemia. Ph.D. Thesis. Stockholm University, Stockholm, Sweden (1987).

Bennet, R., Eriksson, M., Tafari, N., and Nord, C.-E.: Intestinal bacteria of newborn ethiopian infants in relation to antibiotic treatment and colonization with potentially pathogenic Gram-negative bacteria. Scand. J. Infect. Dis. 23, 63-69 (1991).

Bennet, R., and Nord, C.-E.: Development of the faecal anaerobic microflora after caesarean section and treatment with antibiotics in newborn infants. Infection 15, 332-336 (1987).

Benno, Y., Sawada, K., and Mitsuoka, T.: The intestinal microflora of infants: composition of the fecal flora in breastfed and bottlefed infants. Microbiol Immunol 28, 975-986 (1984).

Berg, R.D.: Host immune response to antigens of the indigenous intestinal flora. In: Human intestinal microflora in health and disease. (Ed.: Hentges, D.). Academic Press, New York, 101-126 (1983a).

Berg, R.D.: Translocation of indigenous bacteria from the intestinal tract. In: Human intestinal microflora in health and disease. (Ed.: Hentges, D.). Academic Press, New York, 333-351 (1983b).

Berg, R.D.: Bacterial translocation from the gastrointestinal tract. Trends Microbiol. 3, 149-154 (1995).

Bettelheim, K., Peddie, B., and Chereshsky, A.: The ecology of *Escherichia coli* in a maternity ward in Christ Church, New Zealand. Zbl. Bakt. Hyg., l Abt. Orig. B

178, 389-393 (1983).
Bettelheim, K.A., Breadon, A., Faiers, M.C., O'Farell, S.M., and Shooter, R.A.: The origin of O-serotypes of *Escherichia coli* in babies after normal delivery. J. Hyg. Camb. 72, 67-70 (1974).
Bettelheim, K.A. and Lenow-King, S.M.J.: The acquisition of *Escherichia coli* by newborn babies. Infection 4, 174-179 (1976).
Bhat, N.M., Kantor, A.B., Bieber, M.M., Stall, A.M., Herzenberg, L.A., and Teng, N.N.H.: The ontogeny and functional characteristics of human B-1 (CD5+ B) cells. Int. Immunol. 4, 243-252 (1992).
Bhutta, Z.A., Naqvi, S.H., Muzaffar, T., and Farooqui, B.J.: Neonatal sepsis in Pakistan. Presentation and pathogens. Acta Paediatr. Scand. 80, 596-601 (1991).
Björkstén, B.: Risk factors in early childhood for the development of atopic diseases. Allergy 49, 400-407 (1994).
Bodeker, B.G.D., Korturann, C., Peter, H.H., Pichler, W.J., and Muhlradt, D.F.: Interleukin 2 in the ontogeny of human lymphoid tissues. Immunobiology 162, 66-77 (1982).
Borderon, J.C., Lionnet, C., Rondeau, C., Suc, A.L., Laugier, J., and Gold, F.: Current aspects of the fecal flora of the newborn without antibiotherapy during the first seven days of life: *Enterobacteriaceae*, enterococci, staphylococci. Pathol. Biol. Paris 44, 416-422 (1996).
Brooks, S.G., Sedman, J.M.P., Tring, I., Johnstone, D., Mitchell, C.J., and MacFie, J.: Translocation of enteric bacteria in humans. Br. J. Surg. 80, 901-902 (1993).
Bullen, C.L., Tearle, P.V., and Willis, A.: T. Bifidobacteria in the intestinal tract of infants - an *in vivo* study. J. Med. Microbiol. 9, 325-333 (1976).
Burgio, G.R., Lanzavecchia, A., Plebani, A., Jayakar, S., and Ugazio, A. G.: Ontogeny of secretory immunity: Levels of secretory IgA and natural antibodies in saliva. Pediatr. Res. 14, 1111-1114 (1980).
Carter, P.B., and Pollard, M.: Host responses to "normal" microbial flora in germfree mice. J. Reticuloendothel. Soc. (RES) 9, 580-587 (1971).
Carver, J.D., Pimentel, B., Cox, W.I., and Barness, L.A.: Dietary nucleotide effects upon immune function in infants. Pediatrics 88, 359-363 (1990).
Casali, P., and Notkins, A.L.: CD5+ B lymphocytes, polyreactive antibodies and the human B-cell repertoire. Immunol. Today 10, 366-368 (1989).
Cebra, J.J.: Influences of microbiota on intestinal immune system development. Am. J. Clin. Nutr. 69 (suppl.), S1046-S1051 (1999).
Chouaib, S., Welte, K., Mertelsmann, R., and Dupont, B.: Prostaglandin E2 acts at two distinct pathways of lymphocyte activation: Inhibition of interleukin 2 production and downregulation of transferrin receptor expression. J. Immunol. 135, 1172-1179 (1985).
Crabbe, P., Bazin, H., Eyssen, H., and Heremans, J.: The normal microbial flora as a major stimulus for proliferation of plasma cells synthesizing IgA in the intestine. Int. Arch. Allergy Appl. Immunol. 34, 362-375 (1968).
Dawodu, A.H. and Alausa, O.K.: Neonatal septicaemia in the tropics. Afr. J. Med. Sci. 9, 1-6 (1980).
Ellis-Pegler, R.B., Crabtree, C., and Lambert, H.P.: The faecal flora of children in the United Kingdom. J. Hyg. Camb. 75, 135-142 (1975).
Fireman, P., Zuchowski, D.A., and Taylor, P.M.: Development of human complement system. J. Immunol. 103, 25-31 (1965).
Freter, R.: Factors affecting the microecology of the gut. In: Probiotics - the scientific basis. (Ed.: Fuller, R.). Chapman & Hall, London, 111- 144 (1992).
Fryklund, B., Tullus, K., Berglund, B., and Burman, L. G.: Importance of the environment and the faecal flora of infants, nursing staff and parents as sources of Gram-negative bacteria colonizing newborns in three neonatal wards. Infection 20, 253-257 (1992).
Garland, C.D., Lee, A., and Dickson, M.R.: Segmented filamentous bacteria in the rodent small intestine: Their colonization of growing animals and and possible role in host resistance to *Salmonella*. Microb. Ecol. 8, 181-190 (1982).
Gathings, W.E., Lawton, R.A., and Cooper, M.D.: Immunofluorescent studies of the development of pre-B cells, B lymphocytes and immunoglobulin isotype diversity in humans. Eur. J. Immunol. 7, 806-809 (1977).

Gleeson, M., Cripps, A.W., Clancy, R.L., Hensley, M.J., Dobson, A.J., and Firman, D.W.: Breastfeeding conditions and differential developmental pattern of mucosal immunity. Clin. Exp. Immunol. 66, 216-222 (1986).

Gleeson, M., Cripps, A.W., Clancy, R.L., Husband, A.J., Hensley, M.J., and Leeder, S.R.: Ontogeny of the secretory immune system in man. Austr. N. Z. J. Med. 12, 255-258 (1982).

Gothefors, L.: Studies of antimicrobial factors in human milk and bacterial colonization of the newborn. Ph.D. Thesis. Umeå University, Umeå, Sweden (1975).

Gothefors, L., Carlsson, B., Ahlstedt, S., Hanson, L.Å., and Winberg, J.: Influence of maternal gut flora and colostral and cord serum antibodies on presence of *Escherichia coli* in faeces of the newborn infant. Acta Paediatr. Scand. 65, 225-232 (1976).

Granberg, C. and Hirvonen, T.: Cell-mediated lympholysis of fetal and neonatal lymphocytes in sheep and man. Cell. Immunol. 51, 13-22 (1980).

Grutte, F.K., Horn, R., and Haenel, H.: Ernahrung und biochemisch-mikroökologische Vorgange in Enddarm von Sauglingen. Z. Kinderheilkd. 93, 28-39 (1965).

Grönlund, M.M., Lehtonen, O.P., Eerola, E., and Kero, P.: Fecal microflora in healthy infants born by different methods of delivery: Permanent changes in intestinal microflora after cesarean delivery. J. Pediatr. Gastroenterol. Nutr. 28, 19-25 (1999).

Hall, M.A., Cole, C.B., Smith, S.L., Fuller, R., and Rollers, C.J.: Factors influencing the presence of faecal lactobacilli on early infancy. Arch. Dis. Child. 65, 185-188 (1990).

Hanson, L.Å., Dahlman-Höglund, A., Karlsson, M., Lundin, S., Dahlgren, U., and Telemo, E.: Normal microbial flora of the gut and the immune system. In: Probiotics, other nutritional factors and intestinal microflora. Nestlé Nutrition Workshop Series, Vol. 42 (Eds.: Hanson, L.Å. and Yolken, R.H.). Vevey/Lippincott-Raven Publishers, Philadelphia, 217-228 (1999).

Hashimoto, K., Handa, H., Umehara, K., and Sasaki, S.: Germfree mice reared on an "antigen-free" diet. Lab. Anim. Sci. 28, 38-45 (1978).

Herzenberg, L.A., Stall, A.M., Lalor, P.A., Sidman, C., Moore, W.A., Parks, D.R., and Herzenberg, L.A.: The LY-1 B cell lineage. Immunol. Rev. 93, 81-102 (1986).

Hohmann, A., Schmidt, G., and Rowley, D.: Intestinal and serum antibody responses in mice after oral immunization with *Salmonella*, *Escherichia coli* and *Salmonella-Escherichia coli* hybrid strains. Infect. Immun. 25, 27-33 (1979).

Holt, P.G., Oliver, J., Bilyk, N., McMenamin, C., McMenamin, P.G., Kraal, G., and Thepen, T.: Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells *in vivo* by resident alveolar macrophages. J. Exp. Med. 177, 397-407 (1993).

Hoogkamp-Korstanje, J.A.A., Lindner, J.G.E.M., Marcelis, J.H., den Daas-Slagt, H., and de Vos, N.M.: Composition and ecology of the human intestinal flora. Antonie van Leeuwenhoek 45, 335-340 (1979).

Hooijkaas, H., Preesman, A.A., Pleasants, J.R., and Wostmann, B.S.: Isotypes and specificities of immunoglobulins produced by non-immunized germfree mice fed chemically defined utrafiltered "antigen free" diet. Eur. J. Immunol. 14, 1127-1133 (1984).

Iwase, T., Moro, I., and Mestecky, J.: Immunohistochemical study of the ontogeny of the secretory immune system. Adv. Exp. Med. Biol. 216B, 1359-1368 (1987).

Jain, L., Vidyasagar, D., Xantou, M., Ghai, V., Shimada, S., and Blend, M.: *In vivo* distribution of human milk leukocytes after ingestion by newborn baboons. Arch. Dis. Child. 64, 930-933 (1989).

Khan, S.R., Jalil, F., Lindblad, B.S., and Karlberg, J.: Early child health in Lahore, Pakistan: X. Mortality. Acta Paediatr Suppl. 390, 109-117 (1993).

Kim, J.H. and Ohsawa, M.: Oral tolerance to ovalbumine in mice as a model for detecting modulators of the immunologic tolerance to a specific antigen. Biol. Pharm. Bull. 18, 856-858 (1995).

Kim, Y.B., Bradley, S.G., and Watson, D.W.: Ontogeny of the immune response. I. Development of immunoglobulins in germfree and conventional colostrum-deprived piglets. J. Immunol. 97, 52-63 (1966).

Klaasen, H.L.B.M., Koopman, J.P., Poelma,

F.G.J., and Beynen, A.C.: Intestinal, segmented, filamentous bacteria. FEMS Microbiol. Rev. 88, 165-180 (1992).

Kleessen, B., Bunke, H., Tovar, K., Noack, J., and Sawatzki, G.: Influence of two infant formulas and human milk on the development of the faecal flora in newborn infants. Acta Paediatr. Scand. 84, 1347-1356 (1995).

Klein, R.B., Fischer, T.J., Gard, S.E., Biberstein, M., Rich, K.C., Stiehm, E.R.: Decreased mononuclear and polymorphonuclear chemotaxis in human newborns, infants and young children. Pediatrics 60, 467-472 (1977).

Koldovsky, O. and Thornburg, W.: Hormones in milk. J. Pediatr. Gastroenterol. Nutr. 6, 172-176 (1987).

Koopman, J.P., Mullink, J.W.M.A., Prins, R.A., Welling, G.W., and Hectors, M.C.: Association of germfree mice with intestinal microflora obtained from "normal" mice. Lab. Anim. 16, 59-64 (1982).

Kovarik, J. and Siegrist, C.-A.: Immunity in early life. Immunol. Today 19, 150-152 (1998).

Kramer, D.R. and Cebra, J.J.: Early appearance of "natural" mucosal IgA responses and germinal centers in suckling mice developing in the absence of maternal antibodies. J. Immunol. 154, 2051-2062 (1995).

Kroese, F.G.M., Cebra, J.J., van der Cammen, M.J.F., Kantor, A.B., and Bos, N.A.: Contribution of B1 cells to intestinal IgA production in the mouse. Methods 8, 3743 (1995).

Kuhn, I., Tullus, K., and Mollby, R.: Colonization and persistence of *Escherichia coli* phenotypes in the intestines of children aged 0 to 18 months. Infection 14, 7-12 (1986).

Lassiter, H.A., Tanner, J.E., and Miller, R.D.: Inefficient bacteriolysis of *Escherichia coli* by serum from human neonates. J. Infect. Dis. 165, 290-298 (1992).

Lewis, D.B., Yu, C.C., Meyer, J., English, B.K., Kahn, S.J., and Wilson, C.B.: Cellular and molecular mechanisms forreduced interleukin 4 and interferron-γ production by neonatal T cells. J. Clin. Invest. 87, 194-202 (1991).

Lundequist, B., Nord, C.E., and Winberg, J.: The composition of faecal microflora in breastfed and bottlefed irlfants from birth to 8 weeks. Acta Paediatr. Scand. 74, 54-58 (1985).

MacDonald, T.T.: Accessory cells in the human gastrointestinal tract. Histopathology 29, 89-92 (1996).

Machado, C.S., Rodrigues, M.A., and Maffei, H.V.: Gut intraepithelial lymphocyte counts in neonates, infants and children. Acta Paediatr. 83, 1264-1267 (1994).

Martirosian, G., Kuipers, S., Verbrugh, H., van Belkum, A., and Meisel-Mikolajczyk, F.: PCR ribotyping and arbitrary primed PCR for typing strains of *Clostridium difficile* from a Polish maternity hospital. J. Clin. Microbiol. 33, 2016-2021 (1995).

Mata, L.J. and Urrutia, J.J.: Intestinal colonization of breastfed children in a rural area of low socio-economic level. Ann. N.Y. Acad. Sci. 176, 93-109 (1971).

Matsumoto, S., Nanno, M., Watanabe, N., Miyashita, M., Amasaki, H., Suzuki, K., and Umesaki, Y.: Physiological roles of γ δ T-cell receptor intraepithelial lymphocytes in cytoproliferation and differentiation of mouse intestinal epithelial cells. Immunology 97, 18-25 (1999).

Maxson, R.T., Jackson, R.J., and Smith, S.D.: The protective role of enteral IgA supplementation in neonatal gut origin sepsis. J. Pediatr. Surg. 30, 231-233 (1995).

McFarland, H.I., Nahill, S.R., Maciaszek, J.W., and Welsh, R.M.: CD11b (Mac-1): A marker for CD8+ cytotoxic T cell activation and memory in virus infection. J. Immunol. 149, 1326-1333 (1992).

Mellander, L., Carlsson, B., and Hanson, L.Å.: Appearance of secretory IgM and IgA antibodies to *Escherichia coli* in saliva during early infancy and childhood. J. Pediatr. 104, 564-568 (1984).

Mellander, L, Carlsson, B., Jalil, P., Söderström, T., and Hanson, L.Å.: Secretory IgA antibody response against *Escherichia coli* antigens in infants in relation to exposure. J. Pediatr. 107, 430-433 (1985).

Mevissen-Verhage, E.A.E., Marcelis, J.H., Guiné, P.A.M., and Verhoef, J.: Effect of iron on serotypes and haemagglutination patterns of *Escherichia coli* in bottle-fed infants. Eur. J. Clin. Microbiol. 4, 570-574 (1985).

Midtvedt, A.-C.: The establishment and development of some metabolic activities asso-

ciated with the intestinal microflora in healthy children. Ph.D. Thesis, Stockholm University, Stockholm, Sweden (1994).

Midtvedt, T. and Gustafsson, B.E.: Digestion of dead bacteria by germ-free rats. Curr. Microbiol. 6, 13-15 (1981).

Mitsuoka, T. and Kaneuchi, C.: Ecology of the bifidobacteria. Am. J. Clin. Nutr. 30, 1799-1810 (1977).

Moreau, M.C., and Corthier, G.: Effect of gastro-intestinal microflora on induction and maintenance of oral tolerance to ovalbumin in C3H/HeJ rnice. Infect. Immun. 56, 2766-2768 (1988).

Morell, A., Skvaril, F., Hitzig, W.H., and Barandun, S.: IgG subclasses: Development of the serum concentration in 'normal' infants and children. J. Pediatr. 80, 960-964 (1972).

Martensson, L. and Fudenberg, H.H.: Gm genes and gγ-globulin synthesis in the human fetus. J. Immunol. 94, 516-520 (1965).

Nagao, A.T., Pilagallo, M.I.D.S., and Pereira, A.B.: Quantitation of salivary, urinary and faecal sIgA in children living in different conditions of antigenic exposure. J. Trop. Pediatr. 39, 278-283 (1993).

Neut, C., Bezirtzoglou, E., Romond, C., Beerens, H., Delcrois, M., and Noel, A. M.: Bacterial colonization of the large intestine in newborns delivered by caesarean section. Zbl. Bakt. Hyg. A 266, 330-337 (1987).

Nonoyama, S., Penix, L.A., Edwards, C.P., Aruffo, A., Wilson, C.B., and Ochs, H.D.: Diminished expression of CD40 ligand (gp39) by activated neonatal T cells. J. Clin. Invest. 95, 66-75 (1995).

Örskov, F., and Biering-Sörensen, K.: *Escherichia coli* serogroups in breastfed and bottlefed infants. Acta Path. Microbiol. Scand. Sect. B 83, 25-30 (1975).

Pabst, H.F., Spady, D.W., Pilarski, L.M., Carson, M.M., Beeler, J.A., and Krezolek, M.P.: Differential modulation of the immune response by breast or formula feeding of infants. Acta Paediatr. 86, 1291-1297 (1997).

Peeters, C.C.A.M., Tenbergen-Meekes, A.-M., Heijnen, C.J., Poolman, J.T., Zegers, B.J.M., and Rijkers, G.T.: Interferon-γ and interleukin-6 augment of the human *in vitro* antibody response to the *Haemophilus influenzae* type B polysaccharide. J. Infect. Dis. 165, Suppl 1, S161-S162 (1992).

Perkkio, M. and Savilathi, E.: Time of appearance of immunoglobulin-containing cells in the mucosa of the neonatal intestine. Pediatr. Res. 14, 953-955 (1980).

Petit, J.C., Galinha, A., and Salomon, J.C.: Immunoglobulins in the intestinal content of the human fetus with special reference to IgA. Eur. J. Immunol. 3, 373-375 (1973).

Pichichero, M.E.: Effect of breast-feeding on oral rhesus rotavirus vaccine seroconversion: A meta-analysis. J. Infect. Dis. 162, 753-755 (1990).

Quan, C.P., Berneman, A., Pires, R., Avrameas, S., and Bouvet, J.-P.: Natural polyreactive secretory immunoglobulin A autoantibodies as a possible barrier to infections in humans. Infect. Immun. 65, 3997-4004 (1997).

Rennels, M.B.: Influence of breastfeeding and oral poliovirus vaccine on the immunogenicity and efficacy of rotavirus vaccines. J. Infect. Dis. 174, Suppl. 1, S107-S111 (1996).

Rognum, T.O., Thrane, P.S., Stoltenberg, L., Vege, A., and Brantzaeg, P.: Development of intestinal mucosal immunity in fetal life and the first postnatal months. Pediatr. Res. 32, 145-149 (1992).

Rollwagen, F.M., and Baqar, S.: Oral cytokine administration. Immunol. Today 17, 548-550 (1996).

Rotimi, V.O. and Duerden, B.I.: The development of the bacterial flora in normal neonates. J. Med. Microbiol. 14, 51-62 (1981).

Rotimi, V.O., Olowe, S.A., and Ahmed, I.: The development of bacterial flora in premature neonates. J. Hyg. Camb. 94, 309-318 (1985).

Rubin, L.A. and Nelson, D.L.: The soluble interleukin-2 receptor: Biology, function and clinical application. Ann. Intern. Med. 113, 619-627 (1990).

Rule, A.H., Lawrence, D., Hager, H.J., Hyslop, N., and Shwachman, H.: IgA: presence in meconium obtained from patients with cystic fibrosis. Pediatrics 48, 601-604 (1971).

Russell, G.J., Bhan, A.K., and Winter, H.S.: The distribution of T and B lymphocyte populations and MHC class II expression in human fetal and postnatal intestine. Pediatr.

Res. 27, 239-244 (1990).
Saarinen, U.M., Pelkonen, P., and Siimes, M.A.: Serum immunoglobulin A in healthy infants: An accelerated postnatal increase in formula fed compared to breast fed infants. J. Pediatr. 95, 410-412 (1979).
Schlesinger, J.J. and Covelli, H.D.: Evidence for transmission of lymphocyte response to tuberculin by breast-feeding. Lancet ii, 529-532 (1977).
Sell, S. and Fahey, J.L.: Relationship between gammaglobulin metabolism and low serum gammaglobulin in germfree mice. J. Immunol. 93, 81-87 (1964).
Sepp, E., Julge, K., Vasar, M., Naaber, P., Björkstén, B., and Mikelsaar, M.: Intestinal microflora of Estonian and Swedish infants. Acta Paediatr. 86, 956-961 (1997).
Shimoda, M.Y.I., Azuma, N., and Kanno, C.: Natural polyreactive immunoglobulin A antibodies produced in mouse Peyer's patches. Immunology 97, 9-17 (1999).
Shroff, K.E., Meslin, K., and Cebra, J.J.: Commensal enteric bacteria engender a self-limiting humoral mucosal immunoe response while permanently colonizing the gut. Infect. Immun. 63, 3904-3913 (1995).
South, M.A.: IgA in neonatal immunity. Ann. N. Y. Acad. Sci. 176, 40-48 (1971).
Spear, M.L., Stefano, J.L., Fawcett, P., and Proujansky, R.: Soluble interleukin-2 receptor as a predictor of neonatal sepsis. J. Pediatr. 126, 982-985 (1995).
Stark, P.L. and Lee, A.: The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. J. Med. Microbiol. 15, 189-203 (1982).
Steinman, R.M.: The dendritic cell system and its role in immunogenicity. Ann. Rev. Immunol. 9, 271-296 (1991).
Stephens, S.: Development of secretory immunity in breast fed and bottle fed infants. Arch. Dis. Child. 61, 263-269 (1986).
Stephens, S., Kennedy, C.R., Lakhani, P.K., and Brenner, M.K.: *In-vivo* immune responses of breast- and bottle-fed infants to tetanus toxoid antigen and to normal gut flora. Acta Paediatr. Scand. 73, 426-432 (1984).
Stiehm, E.R. and Fudenberg, H.H. Serum levels of immune globulins in health and disease: A survey. Pediatrics 37, 715-726 (1966).
Sudo, N., Sawamura, S., Tanaka, K., Aiba, Y., Kubo, C., and Koga, Y.: The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J. Immunol. 159, 1739-1745 (1997).
Tancrede, C.: Role of human microflora in health and disease. Eur. J. Clin. Microbiol. Infect. Dis. 11, 1012-1015 (1992).
Tannock, G.W., Fuller, R., Smith, S.L., and Hall, M.A.: Plasmid profiling of members of the family *Enterobacteriaceae*, lactobacilli, and bifidobacteria to study the transmission of bacteria from mother to infant. J. Clin. Microbiol. 28, 1225-1228 (1990).
Telemo, E., Karlsson, M., Dahlman-Hoglund, A.U.D., and Lundin, S.: Oral tolerance in experimental animals. Int. Archs. Allergy Appl. Immunol. 113, 219-223 (1997).
Umesaki, Y., Okada, Y., Matsumoto, S., Imaoka, A., and Setoyama, H.: Segmented filamentous bacteria are indigenous bacteria and induce MHC class II molecules and fucosyl asialo GM1 glycolipids on the small intestinal epithelial cells in the ex-germ-free mouse. Microbiol. Immunol. 39, 555-562 (1995).
Umesaki, Y., Setoyama, H., Matsumoto, S., and Okada, Y.: Expansion of α,β T-cell receptor-bearing intestinal intraepithelial lymphocytes after microbial colonization in germ-free mice ant its independence from thymus. Immunology 79, 32-37 (1993).
Van Camp, J.M., Tomaselli, V., and Coran, A.G.: Bacterial translocation in the neonate. Curr. Op. Pediatr. 6, 327-333 (1994).
van der Waaij, L., Limburg, P., Mesander, G., and van der Waaij, D.: *In vivo* IgA coating of anaerobic bacteria in human feces. Gut 38, 348-354 (1996).
Vassilev, T.L. and Veleva, K.V.: Natural polyreactive IgA and IgM autoantibodies in human colostrum. Scand. J. Immunol. 44, 535-539 (1996).
Wilson, C.B., Lewis, D.B., and Penix, L.A.: The physiologic immunodeficiency of immaturity. In: Immunologic disorders of infants & children. 4th Edition (Ed.: Stiehm, E.R.). W.B. Saunders Company, Philadelphia, 253-295 (1996).
Winberg, J. and Wessner, G.: Does breastmilk protect against septicaemia in the newborn? Lancet, 1091-1094 (1971).
Wold, A.E., Adlerberth, I., and Herias, V.:

Bacterial regulation of imrnunity. In: Immunomodulation of the gastrointestinal mucosa. Old Herborn University Seminar Monographs, Vol. 11 (Eds.: Heidt, P.J., Cebra, J.C., Rusch, V.D., van der Waaij, D., and Walker, R.I.). Herborn Litterae, Herborn-Dill, 14-27 (1998).

Wold, A.E, Dahlgren, U., Hanson, L.Å., Mattsby-Baltzer, I., and Midtvedt, T.: Difference between bacterial and food antigens in mucosal immunogenicity. Infect. Immun. 57, 2666-2673 (1989).

Wold, A.E. and Hanson, L.Å.: Defence factors in human rnilk. Curr. Opin. Gastroenterol. 10, 652-658 (1994).

Wostmann, B.S., Pleasants, J.R., and Bealmear, P.M.: Dietary stimulation of immune mechanisms. Fed. Proc. Fed. Am. Soc. Exp. Biol. 30, 1779-1784 (1971).