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THE GUT IMMUNE SYSTEM AND THE MUCOSAL BACTERIA

AGNES E. WOLD

Department of Clinical Bacteriology, Göteborg University, Göteborg, Sweden.

SUMMARY

The gut associated immune system harbours the vast majority of all lymphoid cells in the human body. This corresponds to the fact that most antigens reach us via the gut. The mucosa of the upper airways and the gastro-intestinal tracts also host a rich normal bacterial flora, which serves as a stimulus to the immune system. There are many indications that the normal intestinal microflora affects the way in which other antigens, e.g. food antigens and other innocuous antigens, are handled. Thus, alterations in intestinal colonisation pattern might have predisposed for allergies and other hypersensitivity reactions. In this review, the immune system of the gut and its relation to the normal intestinal microflora will be discussed.

IgA

The lamina propria is seeded with as many as 10^{10} antibody producing cells/m of small intestine. Most of these are plasma cells that produce dimeric IgA, i.e. two IgA monomers held together by the polypeptide "joining chain" (*Brandtzaeg*, 1994). These IgA dimers bind to secretory component, also termed "polymeric immunoglobulin receptor", a trans-membrane protein that is exposed on the basolateral aspect of intestinal epithelial cells. The entire complex is transported through the epithelium to the lumenal side, where the transmembrane part of secretory component is cleaved off. The largest part of secretory component remains bound to IgA, together forming the secretory IgA complex (*Mestecky* et al., 1999).

The secretory IgA molecule is specially designed to afford protection on surfaces populated by microbes. It is hydrophilic and highly resistant to proteolysis, much due to its rich substitution with carbohydrate chains (*Brown* et al., 1970; *Underdown* and *Dorrington*, 1974). Secretory IgA efficiently prevents microbial attachment to host mucosal structures (*Freter*, 1969; *Williams* and *Gibbons*, 1972; *Svanborg-Edén* and *Svennerholm*, 1978) and, hence, strongly reduces translocation (*Albanese* et al., 1994; *Maxson* et al., 1995; *Dickinson* et al., 1998). Secretory IgA does not activate complement (*Russell* et al., 1997) and bacteria coated by secretory IgA are not killed. In contrast, IgA can counteract the inflammatory potential of IgG or IgM antibodies that are bound to the same target.

It is known that many of the commensal microbes in the large intestine are coated by IgA, which does not seem to affect them negatively (*van Saene* and *van der Waaij*, 1979; *van der Waaij*, 1996). In fact, IgA coating might even be advantageous to commensal bacteria. The carbohydrate chains of secretory IgA function as receptors for the mannose-specific adhesin of type 1 fim-

briae, the most common adhesin of *E. coli* and other enterobacteria (*Wold* et al., 1990). A complete lack of IgA in serum and secretions constitutes is the most common primary immunodeficiency, afflicting some 1/500 individuals, two thirds of whom are healthy. We have investigated the effect of absence of IgA in secretions on the commensal *E. coli* flora. In fact, individuals who lack IgA have a lower proportion of type 1-fimbriated *E. coli*, compared to age-matched controls, and the *E. coli* strains retrieved from IgA-deficient individuals also expressed less of the mannose-specific adhesin compared to *E. coli* from the control individuals (*Friman* et al., 1996; *Friman* et al., 2002). This suggests that the interaction between bacteria and secretory IgA is advantageous to the bacteria.

T CELLS

T cells are found in two compartments in the intestinal mucosa: in the lamina propria and between the epithelial cells (so-called intra-epithelial lymphocytes).

The T cells found in the villus lamina propria are mainly CD4-positive and display signs of activation (*deMaria* et al., 1993; *Schieferdecker* et al., 1992). They do not respond with proliferation to mitogens and other stimuli, indicating that they are terminally differentiated. They secrete cytokines spontaneously, especially interferon-γ (*Hauer* et al., 1997, 1998). It appears as the micromilieu in the mucosa favours development of a certain T cell phenotype. Transgenic T cells with the same antigen specificity produce predominantly IL-2 in the spleen and Peyer's patches, but IFN-γ or IL-10 in the gut *lamina propria* (*Saparov* et al., 1997). The function of *lamina propria* T cells under physiologic conditions is unknown.

The intra-epithelial cells are mainly of the CD8+ phenotype. They carry either the αβ or γδ type of antigen receptor. The exact function of the intraepithelial cells is unknown, but they can synthesise IL-2 and IFN-γ (*Lundqvist* et al., 1996) and lyse virus-infected cells (*Cebra* et al., 1989). Their T cell receptors are oligoclonal suggesting that the entire population derives from a limited set of T cell clones (*Blumberg* et al., 1993).

INDUCTION OF MUCOSAL IMMUNE RESPONSES

Intestinal immune responses are induced in the Peyer's patches, which are mucosal lymphoid nodules situated in the wall of the small intestine (*Craig* and *Cebra*, 1971). The patches are covered by a specialised epithelium, the follicle-associated epithelium. This epithelium contains specialised epithelial cells, termed M-cells, which are specialised in transporting material from the lumen into the patches, without degrading it. M-cells lack brush borders and the enzymatic machinery of the absorptive epithelial cell (*Neutra* et al., 1996). In the patches, particulate and soluble antigens are degraded and presented by macrophages and dendritic cells. T cells and B cells with the appropriate specificities proliferate, mature and leave the patches via the efferent lymph. After circulating in the blood for a few days, they return to the intestine, but not to the

Peyer's patches, but to the *lamina propria* of the intestine, and, to a lesser extent, to other mucosa. This process is termed "homing" (*Craig* and *Cebra*, 1971).

IMPORTANCE OF GUT FLORA ON THE SPECIFIC IMMUNE SYSTEM

The majority of all lymphoid cells in the gut are there because of the normal intestinal microflora. Germfree animals have only one tenth as many IgA-producing cells and T cells in the intestinal *lamina propria* as conventional animals (*Crabbé* et al., 1968, 1970; *Hashimoto* et al., 1978).

Mucosa not regularly colonised by microbes, for example the respiratory and urinary tracts, have comparatively more IgG in their secretions compared to the upper respiratory and gastrointestinal tracts (*Reynolds*, 1988; *Svanborg Edén* et al., 1985). IgG activates complement, thereby lysing bacteria but also eliciting inflammation. Thus, the continuous presence of a normal intestinal microflora in the upper respiratory tract and gut seems to promote development of immune effector functions that are non-inflammatogenic and mainly prevent too close contact between the commensal microbes and the host. How this regulation occurs is not known. In mice, it appears as if switch from IgM can occur in the lamina propria in the absence of T cell help (*Fagarasan* et al., 2001), but whether this is true in humans is unclear (*Brandtzaeg* et al., 2001).

When germfree animals are colonised by a normal flora, lymph nodes and Peyer's patches increase in weight and germinal centres develop, the serum IgG concentration rises and antibodies appear towards the colonising microorganisms (*Carter* and *Pollard*, 1971). These antibodies may often cross-react with other bacteria, and non-bacterial structures. For example, the "natural antibodies" directed against blood group antigens, that were thought by Landsteiner to occur spontaneously as part of the normal physiologic development, have been shown to result from immunisation by gut microbes in the normal flora (*Wiener*, 1951; *Springer* and *Horton*, 1969; *Scheffel* and *Kim*, 1979).

Both live and dead bacteria can induce mucosal immune responses, but live, colonising bacteria are better than dead ones, supposedly because more antigen is delivered to the immune system with a bacteria which replicates in the intestinal tract (*Hohmann* et al., 1979). Provided, however, that high enough doses were given over long enough periods of time, killed *E. coli* or *Bacteroides* can induce equally large amounts of IgA-containing plasma cells as colonisation by live bacteria of the same species (*Moreau* et al., 1978). The best inducers of antibody responses are bacteria, which are able to invade the mucosa, because larger doses of bacterial antigens will come into contact with the immune system (*Hohmann* et al., 1979). This raises the question whether bacteria in the intestinal lumen which are not coated by IgA avoid this type of immune response simply because they are not able to translocate and, hence, they may not be "seen" by the immune system. Anaerobes usually do not translocate (*Berg*, 1983), while live facultative bacteria can regularly be found in the mesenteric lymph nodes provided that their population levels in the large intestinal microflora reaches a certain level (*Wells* et al., 1987; *Berg* et

al., 1988; *Herías* et al., 1995, 1997).

Moreau and co-workers attempted to determine whether certain types of intestinal bacteria were better than others in triggering the mucosal immune system. She colonised germfree mice with a range of Gram-positive and Gram-negative bacteria and measured the density of IgA-containing plasma cells in the intestinal lamina propria. The best inducers of IgA plasma cells were *E. coli* and *Bacteroides*, while all tested Gram-positive species were inferior (*Moreau* et al., 1978). Quite to the contrary, Cebra and co-workers found most IgA to be produced in response to colonisation by Gram-positive *Listeria monocytogenes* or segmented filamentous bacteria, while the Gram-negative *Morganella morganii*, *Ochrobacterium atrophii* and *Helicobacter muridium* all gave less IgA stimulation (*Cebra*, 1999 and personal communication).

Intraepithelial cells carrying the αβ type of receptor increase in numbers in response to bacterial colonisation, while the γδ type does not (*Kawaguchi* et al., 1993). However, the γδ type of lymphocytes produces IFN-γ in response to lumenal bacteria, which in turn upregulates the expression of MHC class II molecules on intestinal epithelial cells (*Matsumoto* et al., 1999). The oligoclonality of intraepithelial cells does, however, not seem to be determined by distinct bacterial antigens, since both germfree and conventional mice exhibit such restricted clonality (*Regnault* et al., 1996).

People ingesting probiotic bacteria exhibit activation of cell-mediated immune effector functions, such as enhanced phagocytosis and secretion of IFN-γ by blood lymphocytes and IFN-α by blood mononuclear cells (*Wold*, 2001). In addition, immunoglobulin-producing cells with specificity against the administered strain appear in the blood (*Wold*, 2001).

THE TRANSIENT NATURE OF THE RESPONSE TO GUT BACTERIA

When a bacterial strain successfully colonises the intestine and reaches numbers high enough to permit translocation, germinal centres are formed in the Peyer's patches, B cells committed to IgA production seed the mucosa and secretory IgA is produced into the intestinal lumen. However, this immune response is self-limiting, in that the secretory IgA so produced, coats the bacteria in the intestinal lumen, preventing further translocation and, hence, stimulation of the gut lymphoid tissue (*Shroff* et al, 1995). Despite the continued presence of the microbe in the gut flora, there will be no, or only minimal, further stimulation of the gut associated lymphoid tissue (*Shroff* et al, 1995).

Because of this phenomenon, a persistent activation of the mucosal immune system requires a high turnover of bacterial strains in the microflora. In accordance, Pakistani infants, who are colonised by a never-ending succession of new enterobacteria (*Adlerberth* et al., 1991, 1998) have higher secretory IgA levels in their saliva, and higher anti-*E. coli* antibody levels than Swedish infants of the same age (*Mellander* et al., 1995). Bottle-fed infants, who have a more varied and less stable microflora than breast-fed infants and probably encounter more translocated bacteria, display signs of increased immune responsiveness (*Wold* and *Adlerberth*, 2000).

IMMUNE RESPONSE TO FOOD PROTEINS

Food antigens provide little stimulation to the immune apparatus. Although an estimated 0.01 to 0.1% of ingested food proteins are taken up into the circulation in an intact, theoretically fully immunogenic form (*Husby* et al., 1985a), the immune response to food proteins in humans is limited to low levels of serum antibodies of the IgG4 and IgG2 subclasses (*Husby* et al., 1985b). These subclasses are poor in fixing complement and interacting with phagocytes. Development of antibody responses dominated by IgG1 and IgG3, antibody isotypes with strong inflammatogenic properties, may result in food intolerance reactions (*Saalman* et al., 1995, 2001).

Accordingly, serum IgA and IgG are only slightly increased in germfree mice fed a commercial rat diet compared with those fed an "antigen-free" extensively hydrolysed liquid diet, but much lower than in conventional mice (*Hashimoto* et al., 1978). One must also bear in mind that even the sterilised feed given to germfree animals is contaminated by endotoxin and other bacterial components (*Midtvedt* and *Gustafsson*, 1981), which may contribute to this low-grade immune stimulation. Rats fed very high doses of protein antigens form serum IgG antibodies, but no secretory IgA response (*Peri* et al., 1982; *Wold* et al., 1987, 1989). Hence, food proteins lack the features that enable strong immune responses to develop. When a plasmid encoding production of the food protein ovalbumin was cloned into *E. coli* and this strain is used to colonise germfree rats, secretory IgA antibodies against ovalbumin were produced (*Dahlgren* et al., 1991).

ORAL TOLERANCE

Exposure of the mucosal immune system to food antigens normally results in development of specific immunological tolerance to these proteins. This means that if the food antigen is later administered systemically, it will evoke less of an immune response than it would in an individual who had not been fed the protein. Thus, local swelling will not be elicited by intradermal injection of the antigen (so called delayed-type hypersensitivity, which is a sign of the presence of memory T cells to the antigen), and T cells taken from blood or lymph nodes will fail to proliferate when stimulated with the same antigen *in vitro*. IgE-mediated hypersensitivity reactions do not develop. There may also be a weaker and/or more short-lived serum IgG antibody response to the antigen after systemic administration than in non-fed individuals. The original observations of oral tolerance were made in guinea pigs (*Wells*, 1911; *Chase*, 1946), but most of the work on tolerance has thereafter been done in rats and mice (*Thomas* and *Parrot*, 1974; *Hanson* et al., 1977). More recently it was also shown that humans who ingest KLH develop T cell tolerance to this protein, although the antibody response was intact (*Husby* et al., 1994). Oral tolerance may be seen as a way to economise the resources of the immune system by avoiding to react to a wealth of innocuous antigens entering via the mucosal membranes. Oral tolerance also protects us from dangerous inflammatogenic responses that would destroy mucosal architecture and func-

tion. Allergies and hypersensitivity reactions may be seen as a failure of the individual to develop and/or maintain tolerance to environmental, innocuous antigens.

MECHANISMS FOR ORAL TOLERANCE

Despite decades of research, it is still very unclear where and how oral tolerance is induced and by which mechanisms immune responses are suppressed in the tolerant animal or human being (*Smith* et al., 2000). Oral tolerance may operate through at least two different mechanisms: Anergy and suppressor (regulatory) T cells.

Anergy means that T helper cells are paralysed when they encounter their antigen on an antigen-presenting cell which does not simultaneously deliver the activating signals that the T cell needs in order to proliferate and mature to an effector cell. These so-called co-stimulatory signals may be T cell activating cytokines (for example IL-1 and IL-12), but also a direct binding between the antigen-presenting cell and the T cell via so called accessory molecules. Both T cell activating cytokines and accessory molecules are produced when antigen-presenting cells are exposed to microbial products. Thereby, all antigens in or on microbes will be presented in a highly immunogenic fashion. Food proteins, on the other hand, lack the capacity to elicit such signals in the antigen-presenting cell, because they possess no "danger signals". T cells that encounter their antigens in the absence of activating signals during antigen-presentation may be paralysed or even receive a death signal.

Suppressor, or regulatory, T cells are formed somewhere in the gut-associated immune system. They are antigen-specific and become activated by feeding the specific antigen. However, instead of helping other T cells, they will suppress their function by mechanisms yet to be defined – secretion of IL-10 and/or TGF-β, as well as contact-mediated mechanisms have been described (*Smith* et al., 2000). Suppressor cells induced in an animal fed a protein antigen may be transferred into a naïve recipient and suppress immune responses in this animal. Nothing is known about the requirements for inducing T-suppressor cells, but one might speculate that the gut mucosa provides a suitable environment for the maturation of T-suppressor cells.

A very interesting new model suggests that the intestinal epithelial cell plays a key role in oral tolerance (*Karlsson* et al, 2001). It has been known for a long time that serum from an animal fed a tolerogenic dose of a protein antigen can be transferred to a naïve animal, which will become tolerant to that protein without having eaten it. The serum factor appears a few hours after feeding. Telemo has suggested that the serum factor consist of membrane fragments, so called "tolerosomes" produced by the intestinal epithelial cell. According to this model, intact protein is taken up by intestinal epithelial cells, processed to peptide fragments and loaded onto MHC class II molecules within the epithelial cell. Membrane vesicles are then budded off from the baso-lateral facet of the enterocyte which on their surface carry MHC class II molecules with loaded peptides. These membrane vesicles may disperse themselves in the whole body via the lymph and blood stream and merge with membranes of antigen-presenting cells in the mucosa, liver, or lymph nodes. Supposedly, these vesicles contain information that ensures that the antigen is presented in a non-immunogenic, tolerogenic, fashion.

In addition to the above two mechanisms, antigen non-specific anti-inflammatory signals may down-regulate immune effector functions. For example, whether a delayed-type hypersensitivity reaction develops or not depends not only on the presence of memory T cells, but also on the local conditions in the skin where the antigen is injected. In the presence of anti-inflammatory cytokines or other mediators, recruitment of T cells to the site of antigen deposit may be counteracted. Many individuals, especially those living in developing countries with a high infectious burden, have high levels of specific IgE antibodies to environmental antigens, such as mites, and mount a wheal-and-flare reaction if mite antigen is injected in the skin. However, they do not have clinical symptoms of allergy, probably due to the existence of active anti-inflammatory mechanisms (*Yazdanbakhsh* et al, 2002).

THE NORMAL MICROFLORA AND ORAL TOLERANCE

Oral tolerance is more short-lived in germfree compared to conventional animals (*Moreau* and *Courthier*, 1988). In conventional animals, administration of cholera toxin or *E. coli* heat labile toxin breaks oral tolerance to food antigens (*Elson* and *Ealding*, 1984; *Gaboreau-Routhiau* and *Moreau*, 1996). Thus, it is clear that bacteria or their products profoundly interfere with responses to food and other environmental antigens. It is possible that the gut microenvironment, especially in the presence of the correct intestinal microflora, provides a milieu where it is difficult to activate T cells because the antigen-presenting cells in the gut do provide much of co-stimulatory signals. This might have to do with antigen processing. Accordingly, antigen presenting cells from germfree mice are stronger stimulators of naïve T cells than antigen presenting cells from conventional animals, and the greatest T cell activation is seen when the antigen presenting cells derive from germfree animals which are fed an antigen free liquid sterile diet (*Hooper* et al., 1995). A range of products secreted by macrophages in response to bacterial products have been shown to decrease the T cell stimulating capacity of dendritic cells, for example the cytokines TNF-α (*Holt* et al., 1993) and IL-10 (*Koch* et al., 1996), the prostaglandin E_2 (*Chouiab* et al., 1985), and nitrous oxide (*Holt* et al., 1993). Thus, animals from which alveolar macrophages have been removed display greatly enhanced immune responses to inhaled antigens (*Holt* et al., 1993). Similarly, depletion of macrophages from a preparation of dendritic cells from gut lamina propria also enhances their antigen presenting ability (*Pavli* et al., 1990).

INFLUENCE OF THE COMMENSAL FLORA ON INNATE IMMUNITY

Antigen presentation represents the crossroads between the ancient innate and the more modern acquired immune system. The innate immune system reacts in a fixed fashion to certain molecular constellations that present “danger” to the host, i.e. molecular patterns that are only found in prokaryotes. The acquired immune system can be activated by any structure, because the

variable regions of antibodies and T cell receptors have endless variability. However, because antigen-presenting cells, which are monocytes, macrophages or dendritic cells, belong to the innate immune system, they have kept the tendency to become activated by microbial products. When they digest microbes and simultaneously present the antigens contained therein to T cells, they will convey activating signals to the T cells. In this way, bacteria function as their own adjuvans.

There is no question that the normal intestinal microflora substantially influences the entire innate immune system (*Wold* and *Adlerberth*, 2000). Peritoneal macrophages from conventional animals have increased levels of cyclic AMP and lysosomal enzymes, phagocytose more avidly, secrete more of oxygen radicals, and display enhanced cytotoxic activity, compared to macrophages obtained from germfree animals (*Meltzer*, 1976; *Johnson* and *Balish*, 1980; *Podroprigora* et al., 1980; *Morland* and *Midtvedt*, 1984; *Mitsuyama* et al., 1986). Spleen and bone marrow macrophages from conventional animals produce more IL-1, IL-6 and TNF-α when stimulated by LPS than macrophages from germfree animals (*Nicaise* et al., 1993, 1995). Mono-colonisation of germfree animals with *E. coli*, but not bifidobacteria, was reported to prime their macrophages for such cytokine production (*Nicaise* et al., 1993).

The long ranging effects of the microflora on sites which will not be colonised by bacteria, such as the sterile peritoneal cavity, spleen or bone marrow, can be explained by the fact that bacterial products are taken up by mucosal macrophages, which then leave the mucosa carrying their microbial components with them. Bacterial LPS may persist within macrophages in a bioactive form for very long periods of time (*Duncan* and *Morrison*, 1984). Peptidoglycans from bacterial cell walls, probably deriving from the intestinal microflora, have been detected inside macrophages in the red pulp of the spleen in rats and humans (*Kool* et al., 1994; *Hoijer* et al., 1995). Breakdown products of peptidoglycans have been detected in the urine of healthy people, indicating a constant uptake, degradation and excretion of bacteria or their components from the intestine (*Johanssen* and *Kreuger*, 1988).

The pyrogenic and sleep-inducing properties of microbial break-down products (*Johanssen* et al., 1991; *Martin* et al., 1984) may account for the fact that germfree animals have lower body temperature than conventional ones, and are more difficult to anaesthetise (*Midtvedt*, personal communication).

DIFFERENT BACTERIA ELICIT DIFFERENT INNATE RESPONSES

We have recently found that Gram-positive and Gram-negative bacteria affect human monocytes very differently. Whereas Gram-positive bacteria stimulate secretion of very large quantities of IL-12 from human monocytes, this is not seen with Gram-negative bacteria, which instead stimulate production of large amounts of IL-10 (*Hessle* et al., 2000). These two cytokines have largely opposing properties. IL-12 is a T cell activating cytokine, which stimulates cell-mediated effector functions, such as production of IFN-γ in T cells and NK cells. IL-10 instead dampens T cell activation and IFN-γ production and reduces antigen-presentation.

Further, the two groups of bacteria elicit different patterns of pro-inflammatory cytokines and inflammatory mediators in human monocytes (*Hessle* et al., in manuscript). Gram-positive bacteria induce more TNF-α than Gram-negative bacteria, whereas the latter induce more of IL-6, IL-8 and PGE2. Probably, the two response patterns are optimally suited to facilitate the killing and removal of the two types of bacteria. Gram-positive bacteria, with their very thick and sturdy cell wall, may not be digested efficiently enough by unprimed monocytes/macrophages. If the phagocyte is primed by TNF-α and IFN-γ, their lytic capacity is enhanced. Gram-negative bacteria may preferentially require soluble factors (antibody and complement) for their elimination. Antibodies are produced by plasma cells that mature under the influence of IL-4, IL-6 and IL-10, the two latter being preferentially induced by Gram-negative bacteria. Complement factors are acute phase proteins, whose synthesis is stimulated above all by IL-6. Lastly, the strong PGE2 response seen to Gram-negative, but not Gram-positive, bacteria may facilitate leakage of plasma proteins, including antibody and complement, out of the microvasculature.

Gram-positive and Gram-negative bacteria may, considering the above indications, affect antigen presentation rather differently. Whereas Gram-positive bacteria induce IL-12 production in antigen-presenting cells that will enhance so called Th1 reactions, the large amounts of PGE2, and the comparatively low amounts of IL-12, produced in monocytes stimulated by Gram-negative bacteria would instead favour differentiation of the T cells into the Th2 pathway (*Hessle* et al., in manuscript).

ARE WE INTOLERANT BECAUSE OF A FAULTY INTESTINAL MICROFLORA?

There are several diseases that increase in societies where the standard of living and level of hygiene are high. This has been most clearly demonstrated for allergies (*Williams* et al., 1994; *von Mutius*, 1994), but is also seen with inflammatory bowel disease (*Langholtz* et al., 1991; *Munkholm* et al., 1992), and perhaps some autoimmune disorders which appear at an earlier age today (*Pundziute-Lycka* et al, 2002). These diseases have in common an uncontrolled damaging immune response, which can be seen as a lack of tolerance.

Allergies are strongly linked to excessively hygienic life-styles, such as those characterising modern Western societies. Good housing standard, small families (*Strachan* et al., 1989, *Strachan*, 2000) and absence of infections (*Matricardi* et al., 1997, 2000) have all been linked to high risk of developing allergies, while exposure to early day-care (*Kramer* et al., 1999), to pets (*Hesselmar* et al., 1999), or to a life-stock farm environment (*Braun-Farlander* et al., 1999) all protect against allergy development.

The hygienic life-style of modern society has also led to a change in the intestinal colonisation pattern in infancy. Infants born in Sweden are later colonised by *E. coli* and other enterobacteria than infants born in Pakistan are, and they have a lower strain turnover in their microflora over the first 6 months of life (*Adlerberth* et al., 1991, 1998). Colonisation by enterococci and lactobacilli are also delayed in Western, compared to African, infants (*Bennet* et al., 1991). This has led to the hypothe-

sis that a defect development of the normal intestinal microflora in infancy is the cause of the allergy epidemics in the Western world (*Wold*, 1988).

The present composition of the normal flora may not be adequate in promoting the induction of oral tolerance, through a number of potential mechanisms:

1) A delayed colonisation with certain key bacterial species might deprive the developing immune system of certain necessary signals,
2) Other bacteria that can expand in the microflora in the absence of certain bacteria, may provide the wrong signals to the immune system, preventing oral tolerance to develop against innocuous antigens,
3) The low overall antigenic stimulation afforded by an abnormally stable intestinal microflora may prevent sufficient T cell activation to generate suppressor T cells.

We have recently observed that in infants born in Sweden in the late 1990s, staphylococci have become the major colonisers of the newborn infants. This includes not only coagulase-negative staphylococci, but also *S. aureus* (*Lindberg* et al, 2000). The emergence of staphylococci as major intestinal colonisers suggests that there is reduced colonisation resistance afforded by the intestinal ecosystem, perhaps as a function of a poorly developed anaerobic flora. A low exposure of the Swedish infant to faecal bacteria is suggested by a delayed acquisition of *E. coli* and a low *E. coli* strain turn-over (*Nowrouzian* et al, 2002). *E. coli* is a bacterial species that is only found in the intestinal tract of man and animals and its presence is a sign of faecal contamination. Since it has no other reservoir in nature, its spread is greatly hampered by hygiene.

Thus, we have observed both a change in which bacterial groups that are most numerous in the microflora, and the turnover rate of strains in the intestinal microflora of infants. Interestingly, infant who have low secretory IgA levels in saliva during their first months of life are at increased risk of developing atopy (*Payette* et al., 1977; *van Asperen* et al., 1985; *Neffen* et al., 1986). It remains to be discovered whether the shift in microbial colonisation pattern that seems to have occurred as a result of a highly hygienic lifestyle, is the cause of the allergy epidemic characterising affluent societies.

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