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BACTERIAL REGULATION OF IMMUNITY

AGNES E. WOLD, INGEGERD ADLERBERTH, and VERONICA HERÍAS

Department of Clinical Immunology, University of Göteborg, Sweden

INTRODUCTION

The gut-associated lymphoid tissue is confronted with a vast array of antigens, ranging from food antigens to pathogenic microorganisms. There is evidence that the gut immune system has the capacity to distinguish between potentially harmful antigens (microbial antigens) and harmless antigens (food antigens), the former inducing strong mucosal and systemic immunity, the latter inducing immunologic tolerance (oral tolerance). The difference in immunogenicity between bacterial and food antigens is probably related to how they are presented by antigen presenting cells. Thus, microbial antigens are probably mainly presented by macrophages capable of phagocytosing whole bacteria. Phagocytosis stimulates the macrophage to express surface-bound T cell costimulatory molecules and secrete cytokines capable of activating T cells. Soluble food antigens are chiefly presented by non-phagocytic dendritic cells and cannot stimulate the expression of costimulatory molecules or cytokines. Presentation of protein antigen by dendritic cells in the absence of such signals leads to T cell anergy (non-reactivity) or may stimulate T suppressor cells. Paradoxically, at the same time as bacteria enhance strong immunity to themselves, they seem to be able to down-regulate immune responses to other antigens, such as food proteins. Thus, animals lacking a normal intestinal microflora display enhanced immunity to fed proteins compared with conventional animals. This may be explained by the fact that interaction of macrophages with LPS or other bacterial antigens induces the release of a series of mediators which decrease antigen presentation by dendritic cells in the vicinity. Failure to develop oral tolerance to food antigens leads to allergies and hypersensitivity reactions, diseases which are constantly increasing in incidence in the Western world. The paper discusses the hypothesis that the increased incidence of allergies is related to a changed pattern of neonatal intestinal colonisation which has occurred during the last decades, for example a delayed colonisation with enterobacteria.

The gut-associated lymphoid system comprises the largest part of the immune system. This is not surprising, considering that most foreign substances reach us via the gut. Not only do we swallow food antigens and food-borne microbes, but also inhaled particles, which become trapped in the respiratory mucus and are transported to the pharynx (cilia beat upwards from the trachea and downwards from the nasal cavity). Furthermore, the gut-associated lymphoid system has intimate contact with the normal intestinal microflora which contains ten times more cells that there are eukaryotic cells in the body. The aim of the present paper is to discuss the regulation of the immune responses engendered against bacterial and food antigens and the impact of the normal microbial microflora on this regulation.

THE GUT IMMUNE SYSTEM

Bacterial and viral infections of the gastrointestinal tract generally induce strong immune responses. Intestinal immunity against gut microbial antigens is induced in the Peyer's patches, which are groups of lymphoid nodules in the gut wall of the small intestine. The epithelium overlying the Peyer's patches contains M cells, epithelial cells specialised in transporting antigen from the gut lumen to the underlying lymphoid cells (*Gebert* et al., 1996). In the Peyer's patches, bacteria are degraded and bacterial antigens are presented to B and T cells, which, provided they have specificity for the antigens in question, start to proliferate and mature. The activated cells leave the Peyer's patches via the lymphatics, circulate for a few days in the blood stream, and finally settle in the lamina propria of the gut and other mucosa, a process called "homing" (*Craig* and *Cebra*, 1971; *Guy-Grand* et al., 1978; *Parmely* and *Manning*, 1983).

Most of the homing B cells develop into plasma cells that produce dimeric IgA. The IgA dimers are held together by a protein named joining chain, which also permits the molecular complex to bind to secretory component, an integral membrane protein exposed on the basolateral side of epithelial cells of the intestine, as well as on ductal cells in salivary and respiratory glands, and lactating mammary glands (*Goldblum* et al., 1996). The complex between dimeric IgA and secretory component is transported through the epithelial cell to the lumenal side, where the secretory component is cleaved off from its transmembrane portion. In this way, secretory component remains attached to the IgA dimer, forming secretory IgA, which is highly resistant to low pH and proteolytic enzymes, and is therefore optimally designed to persist at mucosal surfaces (*Mostov* and *Blobel*, 1983; *Brandtzaeg*, 1985). Secretory IgA is the predominant antibody isotype produced in the intestine, but cells producing IgG and IgM antibodies are also found in the lamina propria of various mucosae (*Goldblum* et al., 1996) and are also part of the homing system (*Dahlgren* et al., 1990).

The T cells which are present in the gut lamina propria also originate in the Peyer's patches (Guy-Grand et al., 1978) and are mainly of the CD4 phenotype (*Selby* et al., 1981). They show signs of activation (*Schieferdecker* et al., 1992; *deMaria* et al., 1993) and spontaneously secrete cytokines (*Hauer* et al., 1997). Interspersed among the epithelial cells are the intraepithelial lymphocytes, which are mainly CD8-positive T cells with cytotoxic potential and unknown function (*Lundqvist* et al., 1996).

There are thousands of single lymph nodules in the colon, but their role in triggering gut immune responses has not been much studied. However, colonic lymphoid nodules seem to be able to function as induction sites for local immunity, since locally applied virus induces a secretory IgA response in the colon (*Ogra* and *Karzon*, 1969).

THE RESPONSE OF THE GUT IMMUNE SYSTEM TO MICROBIAL ANTIGENS

The presence of microbial pathogens in the gut does not only stimulate vigorous mucosal immune responses, but also strong systemic immunity, e.g. high titres of specific IgG and IgM antibodies in the blood (*Waldman* and *Ganguly*, 1974; *Bienenstock* and *Befus*, 1980).

A strong immune response is also seen when non-pathogenic gut bacteria colonise germ free animals (*Wold* et al., 1989; *Shroff* et al., 1995). In a conventional animal which already harbours a normal microflora, the capacity of a novel bacterial strain to induce immunity to itself depends on its ability to persist in high enough numbers for a period of time, i.e. to colonise. Dead microorganisms, or microorganisms transiently passing through the gastrointestinal tract without colonising it, may be taken up into the Peyer's patches and induce moderate immune responses (*Ogra* et al., 1968; *Goldblum* et al., 1975; *Ogra* et al., 1980; Wennerås et al., 1994), but the response will be much stronger with a colonising or invading strain (*Hohmann* et al., 1979). This is probably only a function of the amount of bacterial antigen reaching the immune system.

IMMUNE RESPONSE TO FOOD ANTIGENS

As opposed to microbial antigens, food proteins elicit a very weak and slow antibody response. The response is dominated by serum antibodies of the IgG4 and IgG2 subclasses (*Husby* et al., 1985a), which are poor in fixing complement and interacting with phagocytes. The normal immune response to food antigens is thus characterised by a low inflammatogenic potential. The weak responsiveness to food antigens is not due to their exclusion by the gut mucosal barrier, because an estimated 0.1% of ingested food proteins are taken up into the circulation in an intact, theoretically fully immunogenic form (*Kilshaw* and *Cant*, 1984; *Husby* et al., 1985b). In laboratory animals, the feeding of high doses of protein antigens does not result in a secretory IgA antibody response, but only in the formation of serum IgG antibodies (*Peri* et al., 1982; *Wold* et al., 1987).

EVIDENCE FOR A DIFFERENCE IN RESPONSIVENESS AGAINST FOOD AND BACTERIAL ANTIGENS

To be able to directly compare the responsiveness to food and bacterial antigens experimentally, we gave germfree rats a feed containing egg and milk whey powder (to which they had never been exposed before), and simultaneously colonised them with an E. coli strain (*Wold* et al., 1989). Antibodies of the IgG, IgA and IgM isotypes directed against E. coli LPS and type 1 fimbrial antigens rapidly occurred in secretions, concomitantly with IgM and IgG antibodies in serum. At a later stage, serum IgA antibodies occurred. In contrast, the food antigens induced only a very weak and slow IgG response in serum, and no IgA antibodies in secretions.

LPS and type 1 fimbriae are large polymers and could thus be expected to be more immunogenic than moderately sized monomeric food proteins such as ovalbumin and β-lactoglobulin. However, rat dams monocolonised with *E. coli* during pregnancy and lactation, displayed quite substantial titres of antibodies against the internal bacterial protein β-galactosidase in the milk, while antibody titres against food proteins were low or absent (*Wold* et al., 1989). Thus, a small protein within a bacterium induced better immunity than a protein of similar size consumed in large amounts via the food. This suggested that the "packaging" of an antigen was

crucial to its immunogenicity. To prove this, we transformed *E. coli* with an ovalbumin-encoding plasmid and colonised germ-free rats with this bacterium. Indeed, these rats made secretory IgA antibodies against ovalbumin, whereas rats fed ovalbumin did not (*Dahlgren* et al., 1991).

MECHANISM BEHIND STRONG IMMUNE RESPONSE TO BACTERIAL ANTIGENS

All protein antigens must be taken up, processed and presented on class II MHC molecules by antigen-presenting cells in order to stimulate T cells. Antigen-presenting cells derive from bone marrow precursors which differentiate to various types of macrophages (for example Kupffer cells in the liver, alveolar macrophages in the lungs, microglia in the brain), to dendritic cells in lymphoid tissue, or to Langerhans cells in the skin and buccal mucosa (*Szabolcs* et al., 1996). Whereas some antigen-presenting cells are good phagocytes (for example tissue macrophages), others are poor phagocytes, but excellent presenters of soluble antigens (dendritic cells).

Nevertheless, it is not enough for a T cell to recognise its specific antigen peptide on the proper MHC molecule, in order for it to be activated. The antigen presenting cell must also deliver additional, stimulating signals to the T cell, which are necessary for triggering naive T cells to produce IL-2 and IL-2 receptor, and hence to start proliferating. Certain cytokines produced by antigen presenting cells are stimulatory, e.g. IL-1 (*Rosenwasser* et al., 1979). In addition, antigen presentation becomes much more efficient if the T cell and antigen-presenting cell bind to each other via so called co-stimulatory molecules (*Geppert* et al., 1990). There are a number of co-stimulatory molecules on macrophages and dendritic cells, e.g. ICAM-1 which binds to LFA-1 on T cells, LFA-3 which binds T cell CD2 (*Geppert* et al., 1990), and CD80 (B7-1) and CD86 (B7-2) which bind to CD28 on T cells (*Hathcock* et al., 1994).

Different co-stimulatory molecules preferentially stimulate selected subsets of T cells, and thus direct the immune response into various pathways (*Shahinian* et al., 1993). Similarly, the cytokine milieu which prevails when a naive T cell first encounters its antigen determines the future cytokine repertoire of that T cell (*Mosmann* and *Sad*, 1996). Since different antigen presenting cells have different cytokine and co-stimulatory molecule repertoires, they are likely to deviate the immune responses in different directions. For example, macrophages, but not dendritic cells produce the T cell stimulatory cytokine IL-1 (*Steinman*, 1991). Differences in antigen presenting cell types in various organs is probably the reason for the varied immune responses evoked by a single antigen when given via different routes. For example, intradermal injection of an antigen is the superior mode of delivery to evoke delayed type hypersensitivity.

Bacterial products stimulate macrophages to produce IL-1 (*Keller* et al., 1994; *Hauschildt* and *Kleine*, 1995), and to express several co-stimulatory molecules (*Hathcock* et al., 1994; *Keller* et al., 1995). In this way, their antigen presenting capacity of the macrophage is increased (Ding et al., 1993). This enables all antigens contained in or on a bacterium to be presented to T cells in a fashion which leads to maximal stimulation, and hence immunity. Thus, ovalbumin present within a bacterium will be regarded by the gut immune system as a

bacterial, and hence potentially dangerous, antigen. By this mechanism, the immune system functions more economically in that it wastes little resources on harmless substances, and the risk of undesirable inflammatory reactions also decreases.

ORAL TOLERANCE

Food proteins are not only poor immunogens, but often induce a state of specific unresponsiveness to themselves, termed oral tolerance (*Weiner* et al., 1994; *Telemo* et al., 1997). If an orally tolerised animal is parenterally immunised with the same antigen to which it is tolerant, it will not respond with the expected activation of T helper cells. Thus, local swelling will not be elicited after intradermal injection of the antigen (so called delayed type hypersensitivity) and circulating T cells will not proliferate *in vitro* after addition of the antigen. In some cases, antibodies are formed after parenteral immunisation, but in other cases the antibody response is tolerised as well.

T helper cells may be divided into two more or less well defined subsets, according to their function. Th1 cells are T helper cells which preferentially secrete the cytokines IL-2 and IFN-γ and promote delayed type hypersensitivity reactions (such as the tuberculin reaction). Th2 cells, on the other hand, preferentially secrete IL-4, IL-5 and IL-10 and help B cells mature to antibody-producing cells (*Mosmann* and *Sad*, 1996). Th2 cells secreting IL-4 and IL-5 have been implicated in the pathogenesis of allergy, since IL-4 stimulates IgE production and IL-5 promotes the maturation of eosinophils in the bone marrow (*Jirapongsananuruk* and *Leung*, 1997). Th1 cells are more easily made tolerant than Th2 cells (*Burstein* et al., 1992; *de Wit* et al., 1992; *Melamed* and *Friedman*, 1994). Therefore, delayed type hypersensitivity reactions are more easily tolerised than antibody production, but both may be subject to the induction of oral tolerance (*Garside* et al., 1995b; *Lundin* et al., 1996; *Sudo* et al., 1997).

The original observations of oral tolerance were made in guinea-pigs (*Wells*, 1911; *Chase*, 1946), rats and mice (*Thomas* and *Parrott*, 1974; *Hanson* et al., 1977; *Kagnoff*, 1978), but a few years ago, it was shown that human beings may also become tolerant to ingested antigens (*Husby* et al., 1994). In the human experiment, tolerance was only demonstrated among T cells (i.e. T cells from tolerant individuals did not proliferate upon the addition of antigen) but not among B cells (cells producing antibodies to the fed antigen could still be demonstrated in the circulation).

MECHANISMS FOR TOLERANCE

To uphold a state of tolerance to certain antigens is an equally important task of the immune system as to respond with immunity to other antigens. Without mechanisms of tolerisation, we would react vigorously to our own tissues, as well as to harmless antigens such as food proteins which would create inflammatory and hypersensitivity states. The following mechanisms for achieving tolerance to autoantigens have been described:

1) Clonal deletion. This is the "classical" means by which autoreactive T cell clones are eliminated in the thymus during development (*Ramsdell* and

Fowlkes, 1990). T cells which bind strongly to antigens exposed in the thymus undergo apoptosis. Hence, autoreactive T cells are eliminated from the repertoire of mature T cells.

2) Anergy. Although it was for long thought that all autoreactive T cell clones were eliminated in the thymus, it was later found that there are T helper cells capable of reacting with self antigens in the circulation. However, when they encounter their antigens, they become paralysed du to inability to produce IL-2 and IL-2-receptor and hence to proliferate (IL-2 is an autocrine growth factor for T cells).
3) Active suppression by suppressor cells. According to this mechanism of tolerance, a helper T cell encounters its antigen, but is prevented to proliferate and develop into an effector cell by a regulatory, or suppressor, T cell, which secretes inhibitory cytokines. Suppressor cells may be CD8-postive (*Khoury* et al., 1992; *Miller* et al., 1992) or CD4-positive (*Chen* et al., 1995b; *Garside* et al., 1995a, 1995b), and often produce TGF-β (*Taguchi* et al., 1994; *Sakaguchi* et al., 1995; *Taguchi* and *Takahashi*, 1996).

All the above mechanisms have been shown to mediate oral tolerance in different experimental systems: clonal deletion (*Chen* et al., 1995a), anergy (*Whitacre* et al., 1991; *Melamed* and *Friedman*, 1993, 1994), and active suppression (*Miller* and *Hanson*, 1979; *Miller* et al., 1992). It has been suggested that a high dose of ingested protein tends to favour anergy and a low dose active suppression (*Weiner* et al., 1994), but there is also data to indicate that in young rats, anergy prevails, whereas adults rely more on active suppression (*Lundin* et al., 1996).

Anergy can result if the antigen presenting cell does not provide the proper co-stimulatory signals to the T cell, for example stimulating cytokines and co-stimulatory molecules (*Schwartz*, 1990). Hence, it is easy to understand how food antigens which lack the capacity to stimulate antigen presenting cells will tend to induce anergy and thereby tolerance, whereas bacteria induce immunity. Probably, similar mechanisms exist for the induction of suppressor cells. Thus, presentation of antigen by antigen presenting cells from the lamina propria has been shown to preferably stimulate the generation of CD8-positive cells (which may constitute suppressor cells), while antigen presenting cells from other organs preferentially promote the generation of CD4- positive cells (*Williams* et al., 1992).

CAN BACTERIA REGULATE IMMUNITY TO OTHER THINGS THAN THEMSELVES?

An interesting question is whether bacteria might affect antigen presenting cells in such a profound way that immune reactivity to other antigens, e.g. food antigens or autoantigens could be altered. The answer is "Yes". The presence or absence of a bacterial normal flora affects the immune response to food antigens. Thus, it is difficult to achieve oral tolerance in germ-free animals lacking a normal intestinal microflora (*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). Conversely, cholera toxin and *E. coli* heat labile toxin may break oral tolerance to food antigens (*Elson* and *Ealding*, 1984; *Gaborieau-Routhiau* and *Moreau*,

1996). Thus, it is clear that the presence of bacteria or their products not only promotes immunity to themselves, but may in addition strongly influence immune responses to other antigens occurring concomitantly in a human being or experimental animal.

How does this come about? The presentation of soluble protein antigens is probably chiefly a function of dendritic cells, which are antigen presenting cells with high density of class II MHC antigens on their surface, and with a great capacity to present protein antigens, but with no or very poor phagocytic ability (*Steinman*, 1991). Macrophages, on the contrary, are good presenters of bacteria (*Ziegler* et al., 1987), but poor presenters of soluble proteins antigens (*Crowley* et al., 1990). However, it is conceivable that dendritic cells are regulated by neighbouring macrophages. A number of macrophage-derived products have been shown to decrease the antigen presenting capacity of the dendritic cell, for example the cytokines TNF-α (*Holt* et al., 1993) and IL-10 (*Koch* et al., 1996), the prostaglandin PGE2 (*Chouaib* et al., 1985), and nitrous oxide (*Holt* et al., 1993). All these mediators are secreted by macrophages upon phagocytosis of bacteria or interaction with bacterial products. Holt and co-workers have shown that alveolar macrophages profoundly suppress the antigen presenting function of the dendritic cells of the lung (*Holt* et al., 1993). In fact, if macrophages are removed from the lung alveoli of rats, the rats display greatly enhanced immune responses to all 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). Thus, if we were to lack macrophages (or if our macrophages were not activated by microbial products), it is likely that our immune system would overreact to many harmless environmental antigens, including food proteins and airborne allergens.

Intestinal colonisation of germ-free rats, or even feeding of large numbers of bacteria to conventional mice, induces a state of activation of the macrophages in the peritoneal cavity as evidenced by an up-regulation of lysosomal enzymes (*Morland* and *Midtvedt*, 1984; *Perdigón* et al., 1986) and altered cytokine production (*Nicaise* et al., 1995). It is, thus, clear that the normal flora has the capacity to regulate the immune reactivity of the whole organism, including the handling of dietary antigens in the small intestine, despite the fact that the normal flora itself chiefly inhabits the large intestine. It is likely that the constant uptake of bacterial products via the portal blood, and the more or less constant translocation of low numbers of bacteria does prime a global low grade inflammatory response, which affects e.g. antigen presentation.

We can guess that certain bacterial groups in the intestinal microflora have a greater potential to affect the immune system than others. Bacterial species which are able to translocate, i.e. pass viable over the epithelial barrier, e.g. E. coli and other enterobacteria, lactobacilli and staphylococci (*Berg*, 1983), will presumably have a greater chance to influence the cells of the gut immune system than most obligate anaerobes, which do not translocate. Certain non-translocating bacteria may affect the immune system in an indirect fashion, e.g. by triggering mediator release from epithelial cells or by stimulating cells of the enteric nervous system. For example, lactic acid bacteria, which change the physical milieu in the intestine by production of H_2O_2 and hydrogen ions may affect enterochromaffin cells which are constantly monitoring the intestinal physico-chemical milieu, and which in turn interact with the enteric nervous system. A number of neuropeptides are

likely to interfere with immune functions, e.g. substance P which generally enhances inflammatory reactions and VIP and somatostatin which inhibits them.

Clearly, different bacterial groups will have different impact on the immune system. Thus, Gram-positive and Gram-negative bacteria have different ability to affect various co-stimulatory molecules on macrophages, and to stimulate the secretion of different cytokines (*Keller* et al., 1994). We have recently observed that colonisation of germ-free rats with *Lactobacillus plantarum* and *E. coli* altered T cell populations of the intestinal lamina propria, as compared with *E. coli* colonisation alone, and that *Lactobacillus plantarum*-colonised rats displayed reduced T cell proliferative responses to Con-A (*V. Herías*: Personal communication).

IS THE RISE IN THE FREQUENCY OF ALLERGIES ASSOCIATED WITH ALTERATIONS OF THE NEONATAL INTESTINAL MICROFLORA?

The incidence of atopic allergy, i.e. IgE-mediated hypersensitivity, is steadily on the increase in Western European countries, and vastly supersedes the incidence in the former socialist countries and in the third world (*Strachan*, 1989; *Björkstén*, 1994; *von Mutius* et al., 1994). Simultaneously, the intestinal bacterial colonisation pattern of neonates has changed gradually over the last decades. For example, in a study of the intestinal microflora of Swedish newborn infants born during the early eighties, 25% of the infants did not acquire any enterobacterial species during their first week of life (*Adlerberth* et al., 1991). In earlier studies (*Gareau* et al., 1959; *Bettelheim* et al., 1974), and in studies performed in developing countries (*Mata* and *Urrutia*, 1971; *Rotimi* et al., 1985; *Adlerberth* et al., 1991), infants are as a rule always colonised by day 3 with E. coli or other Gram-negative enterobacteria. It is probably a combination of the strict hygiene applied during Western hospital deliveries, and a reduced spread of enterobacteria in the hospital which results in the low enterobacterial colonisation rate. Later on, the Western babies continue to lead an overly hygienic life, with a low exposure to bacteria via the food or the environment in general. This will certainly reduce the risk of neonatal infections, and thus infant mortality, but may also result in an "abnormally" stable microflora. Thus, Swedish infants often carry a single *E. coli* strain in their microflora for months and years (*Kühn* et al., 1986), while Pakistani infants are colonised with a multitude of different *E. coli* strains in a rapid succession during their first six months (*Adlerberth* et al., manuscript in preparation).

Instead of enterobacteria, whose spread is severely restricted in a very clean milieu, Western infants will be colonised by other bacteria, which are less affected by hygienic measures. *Staph. epidermidis* and *Staph. aureus* are part of the normal flora of the skin and are transferred to the infants from the caretakers. They are also present on the nipple, and breast-fed infants suckle staphylococci together with their milk. Therefore, staphylococci, which are traditionally thought of only as inhabitants of the skin and nasal cavity, are nowadays the dominant species in the intestinal microflora of quite a few newborn infants, for example in Sweden (*Bennet* et al., 1991) and in the USA (*El Mohandes* et al., 1993). Other bacteria whose presence in the normal flora has increased, are the clostridia (*Sepp* et al., 1997), which are spore-formers and

therefore survive the hygienic measures applied in a modern hospital.

The slow colonisation of the intestine of Swedish children with enterobacteria may drastically reduce the exposure of the developing immune system to LPS. When the Swedish infant is finally colonised with enterobacteria, the anaerobes may already have become established, in which case the enterobacteria cannot reach equally high numbers as they do in the absence of competition (*Hoogkamp-Korstanje* et al., 1979; *Stark* and *Lee*, 1982). This, in turn, will hamper their ability to translocate across the intestinal mucosal barrier and influence the developing intestinal immune system (*Berg*, 1980; *Herías* et al., 1995). Furthermore, translocation occurs only during the establishment of a particular bacterial strain in the intestine. Once a specific IgA response to the bacterium has evolved, coating of the bacteria with secretory IgA will namely prevent further translocation (*Shroff* et al., 1995). The extended periods of carriage of individual E. coli strains among Swedish children will therefore contribute to the low exposure of their immune systems to LPS. As pointed out above, LPS favours the induction of oral tolerance to food antigens, and consequently, too little LPS may impede the ability of the infant's immune system to mature into being capable of distinguish between harmful and harmless antigens.

Equally possible is that the bacteria which have in part replaced E. coli and enterobacteria as dominant in the newborn infant's intestine may have untoward effects on the capacity to react with oral tolerance. One can speculate that Clostridium difficile, via its elaboration of toxins might be able to break oral tolerance, even if the toxin of this specific bacterium has not been tested in this respect. Many *Staph. aureus* strains elaborate toxins which function as so called superantigens, i.e. molecules which bind to and activate a large fraction of the T cells (*Herman* et al., 1991). All *Staph. aureus* strains also possess the immunoglobulin-binding molecule protein A, which may function as a B cell superantigen and activate a broad range of B cells (*Seppälä* et al., 1990; *Silverman*, 1992). It remains to be tested whether any of the above changes in the neonatal colonisation pattern is responsible for the continuous rise in allergies in the Western world.

As outlined above, the normal intestinal microflora is not an innocent bystander of the intestinal immune system, but an important modulator of intestinal immune functions. This opens the possibility that hypersensitivity and autoimmune phenomena may be related to disturbances in intestinal colonisation. Fortunately, it also opens the possibility of a remedy to these conditions - a controlled colonisation by probiotic bacteria.

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