Developmental Aspects of Food Allergy

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It is a well-recognized fact that the risk of developing an immediate- or delayed-type food allergic disease is higher in children than in adults. This observation implies that developmental factors operative during gestation and after birth are likely to play an important, although as yet poorly defined, role in the development of food allergic disease. The present review will summarize clinical and experimental evidence and will outline the importance of pre-, peri- and postnatal host responses to fed antigens which are presented to the gut-associated lymphoid tissues (GALT) either directly or via the breast milk.

After a brief discussion of ontogenetic aspects of neonatal immunity, I will concentrate on the afferent part of the immune response (antigen handling and absorption) (Table 1) and will secondly focus on the efferent immune responses that are initiated after the antigen has reached the systemic circulation or the GALT. Furthermore, conditions that are likely to induce or interfere with the induction of oral tolerance (oral tolerance being defined as a specific hyporesponsive immunological state following the first antigen presentation via the gut and the GALT) will be outlined.

THE IMMUNE SYSTEM OF THE HUMAN NEONATE

Cell-Mediated Immunity (T-Lymphocyte Development)

T-cell-mediated immune responses are comparatively mature at birth, and responses to antigens have been documented with T lymphocytes of fetal tissue at 15 to 16 weeks of gestation (1). (Table 2). A well-known clinical example of the maturity of the T-cell system at birth is the mature immune response to early postnatal BCG vaccination. Human newborn cells show a mature proliferative response to mitogens and normal lymphokine production and are capable of cytotoxic activity. This contrasts with the functional immaturity of nonspecific systems such as phagocytosis, antigen-presenting activity, and activity of the complement system (2).

A unique suppressor cell function has been demonstrated by several investiga-
Immune responses after oral antigen encounter can be divided into an afferent limb and an efferent limb. The afferent limb includes a wide variety of luminal and/or mucosal factors which are often interdependent. After the antigen has gained access to the gut-associated lymphoid tissues (GALT), the efferent limb of the immune response will be initiated. A disturbance of the normal immune response at both levels could lead to food-related diseases.

TABLE 2. **Cell-mediated immunity of the human neonate**

<table>
<thead>
<tr>
<th>Mature at birth (BCG vaccination)</th>
<th>MLC/PHA: Responsive after 15 weeks of gestation</th>
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</thead>
<tbody>
<tr>
<td>Nonspecific suppressor cells (cord blood):</td>
<td></td>
</tr>
<tr>
<td>Inhibition of adult T-cells</td>
<td>No effect on neonatal cells (prevention of GVHR?)</td>
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Most of the cell-mediated immune responses are mature at birth, and lymphocytes respond—for example, in a mixed lymphocyte culture—or the lectin phytohemagglutinin (PHA) after 15 weeks of gestation. Phagocytosis and lymphokine production at birth are, however, reduced as compared with that of older children.

tors in the cord blood (3). These cells exert a cytostatic influence on adult T-cell functions but not on allogeneic (neonatal) cells. This antigen nonspecific suppression does not affect autologous B-cells and has been implicated in the prevention of graft-versus-host reactions (GVHR) after mutual lymphocyte exchange between mother and child during late pregnancy and birth. According to different authors, this nonspecific suppression lasts between 5 and 14 days after birth (4).

Humoral Immunity

It has been shown that B-cell functions are immature at birth (Table 3). *In vitro* studies measuring single-cell immunoglobulin production have indicated that the IgM production is fully mature at birth, whereas IgG and IgA production are not.
Within 24 months, B-cell immunity has usually reached adult levels (IgA production may still be immature). IgG subclasses mature at different times; IgG1 and IgG3 responses reach adult levels within 12 months, whereas IgG2 and IgG4 may not have reached mature levels even 24 months after birth. The lack of T-cell help for the IgG production (90% of neonates) is not caused by active suppression (2).

DEVELOPMENTAL ASPECTS OF INTESTINAL PERMEABILITY AND ABSORPTION

The permeability of the intestine of the premature and term newborn to intact proteins (macromolecules) (5) and to inert sugar molecules (6) such as lactulose and rhamnose has been investigated for several years, yet there is still controversy as to how long the increased uptake of the cow’s milk antigens bovine serum albumin (BSA) and beta-lactoglobulin (BLG) persists. Roberton et al. (5) presented data showing that infants born before 37 weeks of gestation demonstrate higher concentrations of BLG 5 days after birth than do infants of higher gestational age. A similar transitional period, from an increased to a normal sugar permeability at around 36 to 40 weeks of gestation, has been reported by another group (6). From these data, it seems that the gastrointestinal permeability of the premature infant (less than 36 weeks of gestation) is uniformly increased both to high- and low-molecular-weight marker molecules (Fig. 1).

Under different clinical conditions in later life, changes in permeability to sugars do not, however, necessarily reflect increased macromolecular absorption. Evidence that sugar permeability and macromolecular permeability of the gastrointestinal tract do not necessarily correlate—for example, during an immunologically mediated anaphylactic response—has been presented by Turner et al. in a rat model (7).

An important new dimension of macromolecular absorption has been presented

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**TABLE 3. Humoral immunity of the human neonate**

<table>
<thead>
<tr>
<th>Immature B-cell function</th>
<th>IgA, IgG</th>
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<tbody>
<tr>
<td>Differential maturation of IgG</td>
<td>IgG1 + IgG3 &lt; 12 months</td>
</tr>
<tr>
<td></td>
<td>IgG2 + IgG4 &gt; 24 months</td>
</tr>
<tr>
<td>Lack of T help for IgG (not suppression)</td>
<td></td>
</tr>
</tbody>
</table>

*B-cell functions are usually immature at birth (IgA, IgG), and the IgG subclass maturation extends well into infancy. Neonates born after 36 weeks of gestation are, for example, capable of producing anti-BSA and anti-beta-lactoglobulin antibodies. Under certain circumstances, specific IgE antibody production can be triggered *in utero* after prenatal sensitization.*
LACTULOSE

RHAMNOSE

PROTEINS (BSA,BLG)

FIG. 1. Changes in intestinal permeability. The intestinal permeability to lactulose, rhamnose, milk, and soy proteins and human lactalbumin in human neonates is increased before 36 weeks of gestation. According to most investigators, permeability reaches normal levels at 40 (±3) weeks of gestation. This reduction in permeability has been termed "gut closure," although there is no evidence of morphologic changes during that period in the human neonate.

WEEKS OF GESTATION

POSTNATAL

by Müller et al. (9). Antigenic BLG and alpha-casein were measured in the serum of 45 5- and 10-day-old formula-fed infants born at 31 to 41 weeks of gestation. As expected, BLG was detected at 5 days of age in 14 of 19 infants born before 37 weeks of gestation but in only 1 of 10 infants born after 37 weeks of gestation. In contrast, however, casein was only present in 4 of 17 infants born before 37 weeks of gestation but in 10 of 12 infants of the more mature group.

If these observations are confirmed by other groups, these findings could point to a different (antigen-specific?) absorptive and/or clearing capacity of BLG and casein in premature and normal infants during the early postnatal period. Differential antigen handling at this age may be an important factor in modulating the immune responsiveness after ingestion. Although this study was well controlled for the presence of circulating (maternal) antibodies, specific immune complex formation could have affected their results. For obvious reasons, antigenic absorption in neonates is restricted to the measurements of cow's milk or soy proteins. The advantage of using a normal dietary antigen is reduced by the influences of passive and/or active immunity transferred during birth and/or lactation.

A different approach was taken by a Swedish group (10) that measured the serum concentration of human lactalbumin, which is a normal constituent of human milk and is taken up unaffected by local or systemic immune responses. Levels of human lactalbumin have been demonstrated to be 10 times higher in premature infants (26–31 weeks of gestation). Serum concentrations fell to normal levels at approximately 39 weeks (10). It remains to be proven whether this reflects increased uptake or delayed clearance.

To test the interesting hypothesis that an increased uptake of macromolecules may lead to food allergic disease (8) (Fig. 2), it would be important to investigate, in a prospective study, the incidence and prevalence of food allergic disease in premature, artificially fed infants. Alternatively, it is equally possible that the increased intestinal permeability is not sufficient (on its own) to induce a sensitization. Other genetic factors—for example, those affecting the antigen-processing
DEVELOPMENTAL ASPECTS OF FOOD ALLERGY 103

Immaturity

Viruses
Bacteria
Alcohol
Detergents

INCREASED GUT PERMEABILITY

Antigen A

Sensitization

Antigen A (+ B + C etc)

Eczema
Colic
Migraine
Behaviour disturbance

FIG. 2. Hypothetical role of an increased intestinal permeability on subsequent development of food-induced hypersensitivities (I). An increased intestinal permeability to macromolecular antigens may lead to sensitization to antigen A. Continuing administration of A further increases the permeability based on an immunologic mechanism to antigen A but also to the unrelated antigens B, C, etc. This chain of events may lead to sensitization to a wide range of food antigens. This mechanism could explain the existence of multiple food allergies but does not explain the initial event that leads to sensitization to antigen A.

capacity and/or the immune responsiveness of the host (I-r genes)—may play a more important role in tipping the balance toward sensitization (Fig. 3).

EFFECTS OF HUMAN MILK AND IMMUNITY OF LACTATION

Generally, human milk is the normal infant food; it represents optimal nutrition and transfers active and passive protective immunity for the neonate. Paul Ehrlich (11) demonstrated in 1892 that immunity could be transferred by breast milk (in mice), and later studies clearly demonstrated that human colostrum (and milk) provide important immune defenses during early neonatal development. Among the substances transferred are nonspecific agents such as: lysozyme; lactoferrin; specific secretory antibodies (and possibly antiidiotypic antibodies) against viruses, bacteria, and food antigens; and immunocompetent cells (macrophages, T- and B-lymphocytes) (12–14). High levels of IgA antibodies in human milk prevent bacterial attachment and may also protect against potentially harmful immune responses to ingested antigens (15). On the other hand, human milk has been known for a long time to contain food antigens (such as milk proteins, wheat, and ovalbumin) (16,17), which may also trigger food allergic symptoms in the infant; sensitization
Gut permeability (normal or increased)

Antigen A

DEFECTIVE PROCESSING OF ANTIGEN

Genetic factors

Sensitization to a range of food antigens

Intestinal hypersensitivity reaction with increased uptake of A (+ B + C)

Antigen A + B + C

FIG. 3. Hypothetical role of defective antigen processing and/or genetic factors in the development of food-induced hypersensitivities (II). Regardless of changes in intestinal permeability, alterations in the antigen handling and processing mechanisms of the gut-associated lymphoid tissues (GALT) could lead to sensitization to a variety of food antigens. The immune responsiveness is likely to be governed by genetic factors, and the increased incidence of atopic diseases in children with a parental history of allergy points in this direction. An increased macromolecular permeability may be necessary but is not sufficient on its own to cause a sensitization.

via breast milk has also been described (18,19). These observations would suggest that dietary manipulations in the mother might be helpful in preventing food allergic disease in the neonate. Early clinical studies seem to point to a protective role of antigen avoidance during pregnancy (20). These studies, however, need confirmation.

On balance, it still seems that breast-feeding is not an important source of sensitization in a population at risk, and it is more likely to protect the population of infants against food allergic and infectious diseases (21–23).

A recent study (24) casts some doubts on the beneficial effects of prolonged breast-feeding (>3 months) on the reduction of allergic symptoms in infants at risk of developing an allergy (family history of allergy). This study, however, also demonstrates that breast-feeding reduces diarrheal episodes in infancy. Further studies are needed, and this study certainly cannot be taken as an argument against breast-feeding.
EFFECTS OF IMMATURETH AND LACTATION ON INTESTINAL ANTIGEN HANDLING

*In vitro* studies, using the everted gut-sac technique, demonstrated that intestinal antigen handling and uptake was changed during the lactation period in the rat. Observed changes were reversible at the end of lactation and could be reproduced by the injection of an equivalent amount of the hormone prolactin. Details will be discussed by W.A. Walker (*this volume*).

The same group demonstrated an increased binding of BSA and BLG and an increased uptake in newborn animals using microvillous membrane preparations (25). These microvillous membranes of newborn animals also showed a decreased protein breakdown capability which could account for this phenomenon. However, it remains to be proven whether these disturbances in the immunophysiology of antigen handling alone might increase the susceptibility to develop adverse reactions to foods in the early postnatal period. Further work is clearly needed to clarify whether this potentially important and complementary mechanism of the regulation of macromolecular antigen uptake exists in humans too and whether it is of physiological importance.

EFFECTS OF PASSIVELY TRANSFERRED ANTIBODY ON THE HUMORAL IMMUNE RESPONSE

Placental transfer of specific antibodies protects the human neonate effectively against pathogenic organisms and toxins before he or she is capable of producing an active humoral immune response. It was tempting to speculate that a high level of circulating antifood antibody may similarly be protective against potentially hazardous oral sensitization in the neonate. High levels of IgG antibodies to milk and egg in cord sera reflect the maternal concentration, and there is a slight correlation with protection against atopic (IgE-mediated) disease during the first 2 years of life (26,27). The influence of circulating antibody on the transfer of antigen into milk in mice has been studied in animals. The findings suggest that circulating maternal antibodies can limit the transfer of a dietary protein from mother to newborn (28). However, it remains to be established whether the reduction of “maternally processed” antigen in this experimental system has beneficial effects on the offspring in reducing the chances of sensitization, or whether the presentation of small amounts of antigen is necessary for the induction of tolerance. Further studies are needed in order to answer these questions.

Studying the effects of passively transferred maternal anti-BSA antibodies on the development of anti-BSA antibodies in the human neonate (29) (with and without passively transferred antibody), Rieger’s group showed, over a 6-month period, that there was no difference in either group. This was also true when high and low BSA intake (via cow’s milk formulas) was taken into account (30).
These findings suggest that the initiation of IgG + IgM antibody formation to ingested antigens occurs relatively unaffected by the presence of circulating maternal antibodies (31). This important observation is at variance with the well-known fact that circulating antibodies can modulate systemic immune responses to parenterally administered antigens (see, for example, the effects of circulating specific antibodies on the reduced "take rate" after diphtheria and measles vaccinations in the young infant). This further highlights the important differences observed in immune responses following enteral or systemic antigen administration.

NEONATAL CAPACITY TO PRODUCE SPECIFIC ANTIBODY

Because of obvious ethical restrictions, the neonate’s capacity to produce specific antibody has mainly focused on the capacity to produce BSA or BLG antibodies after oral administration of a cow’s-milk-containing formula. The highest incidence of anti-BSA antibodies (above 80%) was found in children between the age of 4 months and 5 years (32). Premature infants born at around 35 to 36 weeks of gestation were able to produce detectable anti-BSA antibodies (33,34). The authors hypothesized that the failure of B cells to respond to ingested antigens in infants born before 30 weeks of gestation may represent the functional immaturity of the B-cell system and that antigens reaching the Peyer’s patches before the 34th week may preferentially stimulate the suppressor T-cell system, which would then suppress the systemic B-cell response (34). The above observations, however, lend themselves to a variety of different explanations, which will be discussed later. The hypothesis does not take the digestive, immunologic, and mucosal immaturity, as well as the existence of a nonspecific suppressor cell population (around birth), into account. It has also been shown that fetal B and T lymphocytes at 14 to 15 weeks of gestation are capable of responding to mitogens and to HLA-DR antigens (1–3). The capacity of the neonate to respond to ingested antigens will be discussed below.

PERINATAL INFLUENCES ON IgE RESPONSES

Limited studies in humans have demonstrated that circulating maternal anti-BSA antibodies (IgG or IgM) have no effect, in the child, on the further development of the immune response to ingested antigens of the same type (30,31,34). However, this phenomenon may not be true for the regulation of immunoglobulin E (IgE) synthesis. Evidence to support a different way of immune regulation for IgE has been presented by the work of the late E. Jarrett and her group (35–38). In a series of experiments, rats were used to explore factors influencing the development of IgE regulation during early life, when the immune system of the neonate most probably differs intrinsically from that of mature animals.

Stimulation of adult rats with antigens, whether injected or fed, led more often to reduced, as opposed to enhanced, IgE responses to subsequent challenge. This
capacity to suppress IgE production is activated by even minute amounts of antigen (nanogram quantities) which are frequently absorbed via the gastrointestinal tract. It was hypothesized that this would maintain the down-regulation of the IgE response in the neonatal rat (35). Further experiments demonstrated that there is a marked suppression of IgE responsiveness in the offspring of parenterally immunized female rats and that this state of suppression in the neonatal rat persists even when the circulating maternal antibody is no longer detectable (36,37). This protective effect could also be produced by injection of a small amount of antigen-specific IgG after birth and would suggest that transferred maternal antibody can suppress and modulate IgE responsiveness of neonatal rats. To summarize, it seems that both specific maternal IgG and specific antigen have a profound effect on the regulation of the IgE response (38) (Table 4). It has to be stressed that the existence of a similar regulating pathway for human IgE responses has not been established.

**IMMUNE RESPONSES TO INGESTED ANTIGENS**

An antigen can be defined as any substance (bacterial, viral, food) that elicits a specific immune response when introduced into the tissues of a person or animal. Single-protein molecules may have several antigenic determinants, and any antigen may evoke several immune responses that are not mutually exclusive. In the case of antigen administered via the gut, both systemic and mucosal immune reactions occur, and there may be either induction or suppression of a particular immune response (antibody-mediated or T-cell-mediated) (39–41). Active immunity, in which antigen-reactive cells and specific antibody develop, must be distinguished from immunologic tolerance, which is a specific immune response leading

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**TABLE 4. Perinatal influences on IgE responses**

<table>
<thead>
<tr>
<th>Suckling mother</th>
<th>Neonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunized with OVA</td>
<td>IgE low</td>
</tr>
<tr>
<td>Control</td>
<td>IgE high</td>
</tr>
<tr>
<td><strong>Cross-foster control neonates</strong></td>
<td></td>
</tr>
<tr>
<td>Immunized with OVA (lactating)</td>
<td>IgE low</td>
</tr>
<tr>
<td>IV antibody (lactating)</td>
<td>IgE low</td>
</tr>
<tr>
<td></td>
<td>(Jarrett)</td>
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</tbody>
</table>

*This table summarizes the work of the late E. Jarrett in rodents. If pregnant animals are immunized with hen’s egg albumin (ovalbumin), the IgE responses in the neonate will be suppressed. Normal neonates cross-fostered by lactating animals previously immunized (OVA) or injected with specific anti-OVA antibodies also show a reduced IgE response, and the subsequent suppression is suggestive of an IgG-mediated mechanism. (A similar IgE regulatory mechanism has not been established in humans.)*
to a specific hyporesponsiveness if the antigen is subsequently given parenterally (39,42). Active immune responses can readily be detected and measured in humans and in animals. The phenomenon of immunologic tolerance to ingested proteins has been studied mainly in small laboratory rodents (39,42-44), although circumstantial evidence implies that it also exists in humans (45,46). The experiments described below illustrate how the pattern of immune responses to ingested antigens of an individual animal, or possibly of a person, is critically dependent on the route of first exposure. An irreversible chain of events is set in motion, depending on the primary presentation of an antigen. The subsequent immune response may be modified, but never completely reversed, as a result of further antigen exposure or by the use of cytotoxic drugs (42). Chronic antigen exposure after initial immunization can, however, modulate the immune response, which has been shown in adult (47) and neonatal animals (48), but this treatment does not usually reinduce a complete state of tolerance. There is, however, some preliminary evidence (49) that mice immunized with ovalbumin in complete Freund's adjuvant and re-fed 1 week after immunization show a substantial reduction of their cell-mediated immune responses to ovalbumin ($p<0.01$) as well as reducing serum antibody levels to a lesser extent ($p<0.05$). The above-mentioned observations stress the fact that the humoral and cell-mediated limbs of the immune system are under different control and are also affected differently by oral antigen exposure (50,51).

Crucial for the understanding of the immunologic events triggered by oral antigen exposure is the knowledge of the recirculation pathway of (Peyer's patch) lymphocytes and of immune regulation within the GALT. This will not be discussed in detail here, but it has been the subject of recent reviews (52,53). Briefly, immune responses that develop after gastrointestinal antigen exposure are associated with stimulation of several types of immune regulatory T-lymphocytes in the GALT. Within the Peyer's patches and other organized lymphoid tissues of the gut, antigen-specific T-helper and T-suppressor cells are activated. There appears to be a dual activation of the T-lymphocytes which regulate the B-lymphocyte population; there seems to be an induction of T-helper cells for the IgA system and suppressor cells for IgM and IgG synthesis (41,54). Activation of antigen-specific T-helper cells for IgA probably leads to stimulation of B-lymphocytes which have recirculated to the mucosa and produces a mucosal IgA response. The simultaneous induction and activation of T-suppressor cells for IgG and IgM leads to the specific systemic tolerance. At the same time, T-suppressor cells for suppression of cell-mediated immune response in the gastrointestinal tract are also induced. Several groups have shown that the administration of antigens at an early stage in the postnatal development can cause diarrheal diseases, which are probably due to a cell-mediated immune response within the gastrointestinal tract. The sensitizing capacity of antigen administration has been shown in the preruminant calf, in piglets, and in mice (55-57).
EXPERIMENTS IN NEONATAL MICE

Based on clinical experience that suggests a vulnerable period in the human neonate within the first 4 to 6 weeks of life, potentially hazardous effects of early antigen administration on the development of food allergic disease have been investigated by studying the effects of age at first feed on the development of subsequent specific systemic immunity (in mice). Suppression (tolerance) or priming (enhancement of the immune response) of the immune system was investigated in animals that had been fed at various times after birth (1–42 days). Full details of the methods and results have been published (48,57,59). All animals were age-matched and were fed 1 mg of antigen ovalbumin per gram of body weight. Control animals were handled the same way but were given water instead. The findings are summarized in Fig. 4. Mice that had been fed OVA at ages 1, 3, 7, 14, and 42 days were immunized with ovalbumin and an adjuvant 28 days later. Animals fed at 14 and 42 days were tolerant (as expected); however, as shown in Fig. 4, animals fed OVA between age 1 and 7 days after birth did not develop oral tolerance, and mice fed on the day of birth repeatedly and consistently developed signs of priming, both for antibodies and cell-mediated immunity. This priming was always consistent in a series of experiments but did not reach significant levels within any individual experiment. Thus we argued that antigen administration, even earlier in life, may increase the priming effect.
PRENATAL ANTIGEN EXPOSURE

Based on the above-mentioned results, we exposed individual fetuses to 1 mg of OVA by intra-amniotic injection. Controls were injected with either saline or, as an unrelated antigen, BSA (Fig. 5). After the mice were born and normally reared, they were systemically immunized at 28 days of age (following the normal experimental protocol). Significant priming, both for antibody and DTH responses, was obtained in mice fed OVA before birth. No effects on the fetus were seen after systemic antigen exposure of only the mother on the 19th day of gestation.

EFFECTS OF WEANING ON THE INDUCTION OF TOLERANCE TO OVA

Usually by the time the mice were 14 days of age, the magnitude of immunologic suppression was as complete as in adult animals. This pattern was shortly disturbed when the animals were fed during the weaning period. In an experimental design in which the separate effects of age and weaning could be examined, animals were fed OVA or saline on the day of weaning as well as 3 and 7 days before or after the weaning day. The results are summarized in Fig. 6. When mice were weaned at 28 days of age and given a feed of OVA on that day, they showed no subsequent oral tolerance for antibody responses and less than usual suppression of cell-mediated immune responses. However, when littermates were weaned 3 days after a feed of ovalbumin or were fed OVA 3 days after weaning, they had
normal oral tolerance for both limbs of the immune system. Mice fed 7 days before or after the weaning day became tolerant in the normal way. It seems, therefore, that the transient reduction of oral tolerance was not age-related but was, instead, the result of the weaning process.

INDUCTION OF MUCOSAL CELL-MEDIATED IMMUNITY BY NEONATAL FEEDING

Administration of OVA to the gut of neonatal animals failed consistently to induce a mucosal cell-mediated immune response in the gut. However, feeding neonatal animals on day 1, followed by a challenge at 28 days of age, led to an increase in intraepithelial lymphocytes within the jejunal mucosa. This finding suggests induction of mucosal cell-mediated immune response (Fig. 7).

CONCLUSION

As briefly outlined, our knowledge about developmental aspects of food allergy is still scanty, and the conclusions drawn from published reports of animal and human studies are sometimes contradictory, if not confusing. It is, however, obvious that immune responses after oral antigen encountered in the neonate (or young infant) are different from adult immune responses, and they cannot be dissociated from developmental aspects. The final (immunologic) outcome after antigen pre-
FIG. 7. Effects of a neonatal ovalbumin feed and an ovalbumin challenge in mice. Animals were fed either water or OVA on the first day of life and were challenged for 10 days with OVA or water when they were 4 weeks of age. Only animals fed and challenged with OVA exhibited a significantly increased intraepithelial lymphocyte count (IEL, p<0.01) compatible with a cell-mediated immune response in the gut.

presentation to the GALT is an equation with a variety of interrelating variables, some of which are listed below:

1. Genetic background (parental history of atopy).
2. Environmental factors (e.g., smoking, pollution).
3. Time of intestinal antigen exposure.
4. Immaturity of digestion (creation of tolerogens versus immunogens?).
5. Immaturity of gut-associated immune regulation (e.g., HLA-DR expression).
6. Effects of breast milk on the neonate's immune system.
7. Age-related differences in binding, uptake, and mucosal permeability of macromolecules.
8. Immunosuppressive effects of (virus) infections.

For didactic reasons, the events that finally lead to oral sensitization or oral tolerance can be divided into an afferent limb (antigen binding uptake, digestion, processing, and permeability) and an efferent limb (which comprises the subsequent immune response). The final immune response can also be modulated by circulating antibodies.

The transmission of antigen and antibody via breast milk is likely to affect both limbs. It remains to be proven whether the reduction of antigen transmission into breast milk, be it through circulating maternal antibodies or by antigen avoidance, has a protective effect on the human neonate at risk (21,24). Early clinical reports investigating the effect of antigen avoidance during pregnancy are, however, encouraging but need confirmation on a larger scale (20).

ACKNOWLEDGMENTS

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**DISCUSSION**

*Dr. Freier:* In my animal experiments designed to produce hypersensitivity to food proteins, my adjuvant is *B. pertussis*. Do you think immunization with *B. pertussis* might predispose to the development of allergy in infancy?

*Dr. Strobel:* I do not know of any human study which has addressed this problem. It has been demonstrated in animal studies that feeding of an antigen, together with the injection of an adjuvant [N-acetyl-muramyl-dipeptide (MDP)] or *B. pertussis*, can prevent the induction of oral tolerance (1) or may lead to priming of the IgE response in rats (2). Adjuvant factors could obviously play a role in oral sensitization in the infant, but I would not like to speculate any further.

*Dr. Frick:* Is there any information on the avidity—that is, the strength—of antibodies in the newborn as compared with older children or adults?

*Dr. Strobel:* That is an important question. There is obviously a different maturation pattern of IgG in the infant; IgG2 and IgG4 subclasses reach adult levels at a later stage (at around 2 years of age) than do IgG1 and IgG3 subclasses. There is also an increase of antibody affinity with age within the IgG compartment. IgM antibodies, which were discussed, have a lower affinity and may not be particularly potent in providing a barrier against antigen entry via mucosal sites. Apart from differing secretory piece (SC) binding patterns when compared with IgA, I do not know whether the influences of antibody affinity on antigen entry have been investigated systematically.

*Dr. Schmitz:* I was very interested in your concluding hypothesis that cell-mediated immunity might be due to an imbalance of helper and suppressor T-cells in the gut mucosa. What evidence is there for such imbalance in human babies?

*Dr. Strobel:* The evidence in human neonates is still only by deduction. Nobody has actually shown that neonatal nonspecific suppressor cells do play a role in the pathogenesis of disease, but if you go back to the animal system there seems to be quite good evidence. For example, if you give cyclophosphamide before feeding an antigen, you can reverse tolerance induction, while on the other hand if you give an adjuvant which leads to increased antigen presentation, this will also abrogate tolerance. Thus it is not only the T-cell system which is important, it is the macrophage system as well, and I am sure we shall find other systems which have to be tuned in the right way to achieve tolerance rather than
sensitization and allergy. But the evidence in the human is, as you might expect, very scanty.

**Dr. Ring:** Following up the question of balance in immune-regulating systems, is there any evidence that children with HIV infection get more (or less) food allergies than other children?

**Dr. Strobel:** We have considered this possibility, but we do not have enough children with HIV infections or similar clinical conditions who are well enough to study this question. As far as I am aware, this question has also not been addressed in long-term follow-up studies from the United States. A model of immune dysregulation provided by haplo-identical mismatched bone marrow transplantation in children with severe immune deficiency after conditioning with high-dose cyclophosphamide (200 mg/kg) and busulphan (4 mg/kg) may provide some answers to your question. We have seen three children who were not previously allergic (and neither were their bone marrow donors), who developed a food-sensitive enteropathy which disappeared later during the reconstitution phase after transplantation. The diagnosis of the food-allergic disease, however, is not always easy under these conditions, and mucosal damage and infections after chemotherapy have to be excluded. These patients, however, responded more than three times to introduction and elimination of the offending foods (milk, wheat). All their serum IgE levels were elevated. I do not see why there could not be a more vulnerable period after a bone marrow transplantation or during and after virus infections which are known to suppress T-cell-mediated immune response.

**Dr. Chouraqui:** My question refers to predisposing factors. Do you think that neonatal ischemic gut injury leading to a necrotizing enterocolitis (NEC) is a consequence of cow's milk allergy rather than a predisposing factor?

**Dr. Strobel:** It has not been convincingly shown whether NEC precedes food-allergic diseases or whether food-allergic diseases occur after or are caused by NEC. There are observations to support either hypothesis. Certainly, if you have intestinal ischemia the gut becomes leaky and there are also changes in the distribution of T-cells and their homing pattern, which is also dependent on blood flow and perfusion. It is conceivable that a disturbance of the normal homing pattern of T-lymphocytes could be an important factor, but this remains speculative at the moment.

**Dr. Rieger:** I should like to emphasize one point. It has several times been implied that increased absorption of antigen is somehow related to an increased immune response, or even to allergy. This is not so. For example, when you feed newborn rabbits they absorb huge amounts of protein antigen, but it has been shown that they remain tolerant to these proteins for a long time afterwards. You showed that premature infants absorb more beta-lactoglobulin on the fifth day of life than do term infants, yet there is no evidence that these infants have increased immune responsiveness; on the contrary, they have a reduced IgG response. So I think we must stop associating increased antigen absorption with an automatically heightened immune response.

**Dr. Strobel:** I was trying to make the point that while permeability may be important, it is certainly not the whole story. There are many other factors to take into consideration, and I have tried to indicate how complex they are.

**Dr. Hadorn:** For clinicians there are two populations of children—those who are members of allergic families and those who are not. Let us call the latter normal children. In the normal children, do you think it may be in their interest to be given a chance to develop tolerance to cow's milk as early as possible?
Dr. Strobel: I do not think we need to worry about the development of tolerance in normal infants, because their usual immune response is directed to tolerance—that is, they tolerate the foods given to them. These children will become and usually stay tolerant, whatever we do. It may be unwise to introduce cow's milk earlier to children without a family history of atopy, since those who may still go on to develop an allergy at a later stage are put on the road to sensitization most likely as soon as they are exposed to the potential allergen.

DISCUSSION REFERENCES