Development of Oral Tolerance

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INTRODUCTION

"Oral (food) tolerance" usually describes a clinical situation that is not necessarily synonymous with the immunological definition of "tolerance," that is, as observed during development, transplantation or neonatal tolerance. In the context of this chapter, "oral tolerance" is defined as an antigen-specific immunological hyporesponsiveness after prior enteral administration.

Oral exposure to foods or other ingested antigens has two major effects on the immune system. These reactions can be generalized (i.e., simplified) as leading to (a) induction of systemic immunological hyporesponsiveness (tolerance) or (b) sensitization and priming. Although the suppressive effects of oral antigen ingestion have been described in anecdotal form for over 150 years, the underlying immunoregulatory mechanisms are still poorly understood and remain controversial at times.

The development of oral tolerance is thought to be the basic immunologic mechanism that prevents adverse effects (hypersensitivity/food allergy) after intestinal antigen exposure. There is little doubt that systemic suppression of specific immune responses exists in humans, but for ethical reasons most reports in humans are circumstantial (1,2). A great deal of basic work has been performed on laboratory rodents for only a limited range of food and other T-cell-dependent antigens (e.g., milk and egg proteins, sheep erythrocytes, and organ-specific antigens) (3–5).

A breakdown or modulation of these important homeostatic mechanisms is likely to be one of the reasons for the development of sensitizations after food intake. Suppression of systemic immunity after a feed usually does not include B cell responses. Serum antibody production of infants (or adults) against food proteins is a universal phenomenon and is usually not related to an intolerance phenomenon to the antigen in question. Most low-level food antibodies of clinically tolerant individuals are of the immunoglobulin G (IgG) class. Increased serum or mucosal antibodies to food proteins or tissue constituents of the IgA or IgE class are more likely to be an indicator of a possible underlying pathological process (e.g. celiac disease, acute onset cow's milk allergy) (6,7).
Genetic Background

It is evident from prospective clinical studies that the genetic background is one of the most important single risk factors for the development of an allergic reaction, although currently it is still difficult to quantify. The importance of this factor is undisputed (8), however, chromosomal location of the genetic susceptibility is far from clear and may vary in extended kindreds (9). The risk of developing allergic symptoms (eczema and asthma) increases in infants of allergic parents (uni- or biparental history with or without high cord blood IgE levels). When designing prophylactic (dietary) interventional studies, these have to be carefully controlled for the family (genetic) background and confounding environmental variables such as smoking, house dust mite and pet exposure.

POSTNATAL ACQUISITION OF TOLERANCE TO INGESTED ANTIGENS

The acquisition of tolerance is a multistep process that is determined by a variety of difficult quantifiable variables. Although the genetic "make up" cannot be modified, important contributing factors can be studied and are discussed in more detail later. Based on published work, it is likely that induction and maintenance of oral tolerance are modified by factors summarized in Fig. 1.

A modification of any of these variables under experimental and possibly under clinical conditions may have a profound effect on the acquisition of tolerance (Fig. 1). Careful interpretation of experimental data is needed, especially when extrapolating to human conditions and disease.

Circumstantial Evidence for Oral Tolerance in Humans

Dakin (10) cited the practice of American Indians to chew and ingest the leaves of the poison ivy plant to prevent later contact sensitivity, a habit that Dakin assumed would be ineffective. Recently published retrospective studies (11) clearly demonstrate a reduction in nickel allergy after ear piercing in those individuals who have had previous oral exposure to nickel (through dental braces) prior to the ear piercing. Allergies to other chemicals were not affected. Earlier studies in humans (2) revealed that intradermal injection of bovine serum albumin (BSA) only led to increased antibody production in those individuals who had exhibited anti-BSA antibodies beforehand. Others, who had previously been exposed to milk and who had not produced antibodies against BSA (? tolerant) also failed to produce serum antibodies after intradermal immunization.

The obvious lack of successful oral vaccines with nonreplicating (protein) antigens is further circumstantial evidence for powerful immunosuppressive effects exercised after oral antigen presentation to mucosal surfaces (Fig. 2).
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Food proteins

Lumen
- secretory antibody
- motility
- digestion
- microbial colonization
- mucus coat

Mucosa
- digestion
- permeability
- antigen-antibody interactions
- inflammation
- immaturity
- binding
- uptake
- permeability

Circulation
- antibody
- antigen
- antigen-antibody complexes

Systemic Immunity:
- immaturity
- congenital deficiency
- acquired deficiency
- "processed" antigen
- food antigen
- unaltered

FIG. 1. Specific and nonspecific factors affecting the afferent and efferent limbs of intestinal immunoregulation and the development of tolerance. A variety of luminal and mucosal factors can affect the antigen handling of complete and degraded food proteins and modulate the subsequent immune responses. Host factors such as immaturity at time of exposure, inflammation, and preexisting antibodies are likely to contribute to the overall immunological effects of antigen presentation at mucosal sites. PP, Peyer's patch.

EVIDENCE FOR MODULATORY EFFECTS OF ORAL ANTIGENS ON THE IMMUNE SYSTEM

Regulation of IgE Production in the Rat

Jarrett (12) hypothesized that increased IgE production (in rats) is a manifestation of an IgE immunosuppressive immunodeficiency caused by a lack of suppression (specific or nonspecific). According to this hypothesis, the IgE response would be suppressed by absorption of small amounts of antigen, which would be above the threshold concentration required to stimulate suppressor cells to suppress IgE responses. Further regulatory influence could be exerted by maternal IgG antibody transmission via breast milk (13). To me, there is no convincing clinical evidence that this mechanism is a major route of suppression of IgE production in humans. A preferential activation of a T cell subpopulation (TH2 cell) with predominant release
FIG. 2. Immunological mechanisms operative during the induction of oral tolerance and their potential relevance for treatment of autoimmune disease via oral antigen administration. Food (but conceivably also other "self") proteins are presented via the intestinal mucosa and activate lymphocytes that preferentially "home to the gut" but also reach other mucosal sites. Antigen reaches the underlying immunocompetent cells via the Peyer’s patch or via the epithelium. Presentation of the antigens (or peptides) will, depending on the mode of presentation, induce suppression (tolerance) or possibly sensitization. T lymphocytes may be tolerized by presentation of antigen without a co-stimulatory signal via the CD28/B7 pathway. A negative signal may be provided by CD8 cells (and ?macrophages) and secretion of transforming growth factor-β (TGF-β) and other cytokines. Although the triggering of the suppressive cytokine secretion is antigen dependent, the suppressive effects via this route could also be nonspecific.
of IgE stimulatory cytokines [interleukin-4 (IL4), IL-5] may be the underlying immuno- 
regulatory abnormality in atopic individuals (13).

ANTIBODY AND ANTIGEN TRANSFER VIA BREAST MILK

Breast milk is a major source of nutritive, anti-infective, and other immunological 
 factors for the infant, and its protective activity is undisputed. Clinical studies have 
failed to demonstrate a simple correlation between maternal food antibody levels (in 
serum or breast milk) on the subsequent development of allergic symptoms in the 
infant. Positive (14) as well as negative results (15) regarding the protective efficacy 
have been published.

There is good evidence that a wide variety of nutritional and even parasitic antigens 
(16,17) are excreted in the breast milk. Prophylactic effects of a maternal elimination 
diet (milk, egg, fish) on early atopic symptoms may be related to a reduction in antigen 
(and antigen–antibody complex) transfer during breast-feeding. These studies—by 
inference—would also indicate that sensitization via breast milk is a distinct possibil-
ity. Indeed, prolonged breast-feeding (more than 9 months) and heredity have been 
found in one study to be major predictors of later atopic disease (18). The antigen 
content of human breast milk is variable and on the order of micrograms/liter (mg/
ml). Effects of these amounts on the developing immune system of the infant are 
unknown and both protective and potentially sensitizing properties are conceivable.

EXPERIMENTAL STUDIES

Studies in neonatal mice indicate that antigen transfer via breast milk occurs in 
small amounts (ng/ml), which can have a profound effect on the suckling offspring 
(19–21). The effects of antigen administration via breast milk follow to some extent 
the same pattern as if the antigen were fed by intragastric gavage (at a comparable 
age) (see Fig. 3). Specific immune responses in the suckling offspring can be primed 
or suppressed, depending on the time, choice, and quantity of antigen and frequency 
of suckling. One interesting aspect in these studies is the observation that immune 
suppression (oral tolerance) can be induced via breast-feeding with antigen doses 
that otherwise would sensitize (prime) the immune system.

REGULATION OF THE IMMUNE RESPONSE AFTER ORAL ANTIGEN 
EXPOSURE—ORAL TOLERANCE

As mentioned before, most experimental animals develop a state of specific sys-
temic hyporesponsiveness after oral antigen exposure (4,22,23), which affects hu-
moral and cellular immunity. The induction of this tolerant state seems to be an 
antigen dose-dependent phenomenon that modulates humoral and cell-mediated im-
munity at different levels. The mucosal (secretory) IgA (IgM) production is generally
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not suppressed (24). The gut-associated lymphoid tissues (GALT) play a crucial role in creating an immunological environment that is able to handle, process and present antigen in an immunosuppressive way (25,26).

Suppressor Mechanisms

T cells with suppressive activity have been identified in the intestinal mucosa, mesenteric lymph nodes, and spleen after feeding an antigen (27–29). Modulation of T cell function and antigen presentation by hormones, adjuvants, immunosuppressants, and immunological immaturity can prevent tolerance induction (22,30). Interestingly, no lymphocytes with suppressive activity can be identified in a tolerant host several weeks after tolerance induction, unless stimulated with IL-2 before analysis in an antigen-specific assay. Suppressor mechanisms are generally thought to be antigen specific, although it has recently been shown that this may only partially be the case (31). Suppression can also be mediated in a non-food-antigen model via suppressive cytokines [e.g., transforming growth factor-β (TGF-β)], which could also act on (suppress) immune cells that are naive to the antigen (32) (Fig. 2).

Humoral Factors

Inhibitory serum factors (antigen–antibody complexes or antibody) have been reported for particulate antigens (33) (sheep red blood cells) but evidence for antibody-mediated suppression in experimental systems using soluble antigens is unconvincing.

OTHER MECHANISMS FOR ORAL TOLERANCE

Despite clear evidence of transferable tolerance with T cells (mostly, but not exclusively CD8+) isolated from mesenteric lymph nodes or spleens of fed mice (possibly even isolated from the intestinal intraepithelial lymphocyte population) (34), there is still some controversy about the importance of clonal anergy (35). Without entering into detailed critique about the experimental details that could partially account for the observed differences, it seems that the immunological development of oral tolerance is at least a two-step process and both steps are not mutually exclusive: early generation of T-lymphocytes with suppressive activities is followed by a “tolerant” state in which other immunological mechanisms such as clonal anergy and clonal deletion may be operative. The exact role of the antigen dose in determining suppression or anergy or both is unresolved.

ORAL TOLERANCE AND THE ROLE OF INTESTINAL ANTGEN “PROCESSING”

We and others (36,37) have described the existence of a circulating “tolerogen” in the serum of naive mice 60 minutes after a feed of a protein antigen such as
TABLE 1. Suppression of delayed hypersensitivity in recipients of serum retrieved under different experimental conditions

<table>
<thead>
<tr>
<th>Donor</th>
<th>Serum retrieved after feed at</th>
<th>Antibody responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 minutes</td>
<td>60 minutes</td>
</tr>
<tr>
<td>BALB/c saline</td>
<td>No</td>
<td>No</td>
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<tr>
<td>BALB/c ovalbumin</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>BALB/c# saline</td>
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<td>BALB/c# ovalbumin</td>
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<td>SCID saline</td>
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<td>SCID ovalbumin</td>
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* Germ-free animals served to control for the possible effects of intestinal colonization (SCID animals are kept under sterile conditions). The results indicate that the failure to generate a "tolerogen" in SCID animals was not due to colonization of the gut but more likely was due to the immunological abnormality of the GALTs in those animals.

In this adoptive transfer model, serum of ovalbumin-fed animals (and saline-fed controls) was recovered 5, 30, and 60 minutes after a feed of ovalbumin, (1 mg/g), and injected into naive recipients in which humoral and cell-mediated immunity was investigated 3 weeks after systemic immunization with ovalbumin in complete Freund's adjuvant. Table 1 summarizes some of the results of a series of investigations.

The most striking results were (a) that only serum retrieved after 60 minutes conferred "tolerance" for delayed hypersensitivity; (b) that serum retrieved after 5 minutes did not confer tolerance despite identical amounts of immunoreactive ovalbumin; and (c) that mice with a severe combined immunodeficiency phenotype (SCID, i.e., lacking B and T lymphocytes) were unable to generate a tolerogen 60 minutes after a feed, indicating that a functioning mucosal immune system may be a necessary requirement. Further immunochemical analysis of the serum containing the tolerogenic activity revealed that the suppressive activity was associated with the presence of 21/24-kDa-sized product of ovalbumin [molecular weight (MW) 43 kDa] (31; E. Furrie, M.W. unpublished observation).

ORAL SENSITIZATION

Systemic (oral) sensitization as well as oral tolerance is influenced by the above-mentioned variables. Feeding of very small doses of antigens (in the nanogram to microgram range per animal) to rodents often leads to priming of the immune system, whereas doses in the range of 1 mg/g body weight lead to tolerance induction. Intermittent feeds of small antigen doses are likely to cause priming, whereas the total dose administered as a single feed would be tolerogenic (38). Before tolerance is
established, some animal species (? humans) go through a priming (sensitisation) phase before suppression is established (39).

**Age (Maturity vs. Immaturity) at First Antigen Exposure**

Immunological effects of the frequency, dose, and age at first antigen exposure are generally difficult to evaluate and to separate in a clinical setting. In experiments in neonatal rodents, it could be established that antigen administration of cow’s milk bovine serum albumin (BSA) or hen’s egg albumin (ovalbumin) in a dose that would induce tolerance in adult animals had sensitizing properties during the first 1 to 3 days of life (19). Induction of normal oral tolerance was unrelated to gut closure or weaning (Fig. 3).

Studies in infants seem to indicate a similar vulnerable neonatal period. A high-risk (biparental history of atopy) infant seems to be more susceptible to sensitizing

![Diagram of immune response and age in days]

**FIG. 3.** Effect of age at first feed on the induction of oral tolerance. Several experiments (mainly in rodents) have demonstrated that the induction of systemic hyporesponsiveness after a feed (oral tolerance) is an age- and dose-related phenomenon. Oral antigen administration only leads to immunological suppression at about 7 to 10 days of life. Exposure during the first 3 days or 1 day before delivery via intra-amniotic injection induces priming. The tolerance observed in animals fed at about 14 days of life is long lasting and not related to gut closure. Antibody responses are suppressed for 3 to 6 months, whereas suppression of delayed hypersensitivity responses is maintained for at least 17 months.
influences. In a prospective study, 5/9 infants who developed milk allergy while breast-fed were from a high-risk population and all had received a formula feed during the first week of life (40). Clinical studies in infants at risk that evaluate the effects of long-term oral antigen exposure indicated sensitizing effects (increased frequency of atopic disease) of a cow’s milk formula given from birth (41,42).

**Dietary Antigen Exposure of the Mother During Pregnancy and Breast-feeding**

Examination of the effects of dietary elimination in atopic mothers during pregnancy alone has failed to show a reduction of atopic symptoms in their infants. Major problems associated with such studies are the risks of suboptimal nutrition, awkward diets, and poor compliance. Most studies combine dietary elimination during pregnancy with dietary restrictions during breast-feeding. Recent studies indicate a prophylactic role (delayed onset of atopic eczema) of antigen avoidance combined with delayed and staged introduction of solids in a high-risk population. Confounding factors, such as smoking, pets, house dust mite, and pollutant exposure, need to be taken into consideration (43–45).

**Desensitization by Antigen Feeding**

Suppression of an ongoing immune response in a sensitized host by feeding the antigen has been partially achieved for humoral and cell-mediated immune responses (23,46). Desensitization was most effective when large antigen doses were fed close to the sensitization events or repeatedly. The implications of these observations for human (allergic) diseases are unknown but are likely to be of great importance and are the subject of continuing research. Recent reports describing the therapeutic use of orally induced suppression in the management of autoimmune diseases are encouraging (47–49).

**CONCLUSION**

Antigen-specific acquisition of immunological hyporesponsiveness (oral tolerance) after oral administration remains poorly understood. Multiple variables have been established and are summarized in Fig. 1. A breakdown or inhibition of this protective process is likely to be the underlying pathogenic mechanism for the development of food-sensitive (allergic) and possible autoimmune diseases. Systemic sensitization can affect all limbs of the immune system and may cause clinical symptoms involving all organs. Dissection of the underlying mechanisms of oral tolerance induction at the molecular and cellular level are still outstanding.

Experimental evidence and clinical experience currently favor the notion that very early postnatal oral antigen exposure is more likely to induce sensitization than tolerance. There is evidence from human and experimental studies that “large” antigen doses are more likely to be tolerogenic than “low” doses (50–52). The physiological
role of antigen transfer by breast-feeding is unresolved. Under certain conditions, antigen transfer via breast milk is capable of sensitization and can also cause symptoms in a previously sensitized child.

Acquisition of tolerance and its opposite, priming, are multifactorial processes that are dependent on a susceptible genetic background that can be modified by a variety of ill understood environmental factors. Recently, new roles for intestinally induced immune suppression as therapeutic options in, for example, autoimmune disease (see also Fig. 2), by activating increasingly better defined suppressive mechanisms are currently being explored in experimental systems and clinical trials (47,49,53).

REFERENCES


35. Mowat AM. Depletion of suppressor T cells by 2'-deoxyguanosine abrogates tolerance in mice fed ovalbumin and permits the induction of intestinal delayed-type hypersensitivity. *Immunology* 1986; 58: 179–84.


**DISCUSSION**

*Dr. Moneret-Vautrin:* I did not fully understand what you meant by linking autoimmunity and tolerance. You said that you created oral tolerance to ovalbumin and that you observed bystander suppression to bovine serum albumin. But where does autoimmunity take place in this model?

*Dr. Strobel:* The model described does not directly address the question of autoimmunity. It deals with the suppressive mechanisms induced after oral administration of antigens and the role of “gut processing.” Recently, other groups have also shown that the suppressive effects observed toward food proteins can be demonstrated with self-antigens in experimental autoimmune disease. Weiner *et al.* fed myelin basic protein (MBP) to animals with autoimmune encephalomyelitis (EAE) and demonstrated *in vitro* that feeding specific MBP peptides could reduce the immune response to other nonrelated peptides, which were given at the same time. The authors believe that one does not necessarily have to identify the complete autoimmune antigen (or tolerogen) as long as one has an epitope that will lead to (suppressive) cytokine secretion, for example, TGF-β. If one triggers secretion of this TGF-β by a cross-reacting epitope, one may induce tolerance (or suppression) on a much broader basis than that related just to this one specific epitope.

*Dr. Fritsche:* You mentioned that the dose of antigen is very important for inducing tolerance. Have you tried to induce tolerance with more than 1 mg/g body weight in order to suppress the B cell compartment antibody?

*Dr. Strobel:* If you feed 1 mg/g body weight in the mouse, you suppress delayed-type hypersensitivity and also antibodies to ovalbumin and bovine serum albumin. You cannot go much lower than this, because if you do, you get priming; for example, if you feed microgram or nanogram quantities to these animals, they may get primed and you can transfer the priming effect with T cells. The dose of 1 mg/g definitely induces tolerance, and if you give a higher dose, say, 2 or 6 mg, one gets slightly more suppression but not a lot more. It is surprising how little additional suppression one achieves with increasing doses above the tolerogenic level.

*Dr. Brandtzaeg:* I wonder if you could elaborate on TGF-β as a suppressive factor? So
many activities are claimed for this cytokine. It is supposed to be the principal switching
factor for IgA, and now it is also a suppressive factor. It is confusing!

Dr. Strobel: I do find the highly pleiotropic effect of cytokines confusing as well. It is the
factor that has been associated with suppression in an in vitro model in an antigen-specific
way. When myelin basic protein (MBP) is added to the sensitized T cells, they secrete TGF-
β, which can then suppress the other (cocultured) cells. There is certainly a range of other
factors that are likely to induce tolerance. I have not looked at the effects of TGF-β in vivo
myself, so I cannot comment any further.

Dr. Aalberse: I was interested in your negative results with respect to antibodies during
the serum transfer experiments. You said there is no suppressive effect on antibodies, but
you did not specify the antibody you looked at. Did you actually look at IgE or did you look
at IgG?

Dr. Strobel: In this study I did not look at IgE. If you look into the IgE system, you really
have to focus on that and get all the conditions absolutely right. At this stage, we were looking
at total IgG levels and IgG subclasses. It is interesting that the immunoglobulin subclass
response—IgG1, 2a, and 3 in mice—was not related so much to the treatment the animals
received as to the frequency the animals had been exposed to the antigen. There was a paper
published in Immunology (1) showing that the subclass response in humans seems to be
related to the frequency of antigen exposure.

Dr. Aalberse: I am just curious to know whether I am more like a rabbit or a rat!

Dr. Strobel: I believe that you are certainly more like a rat than a rabbit. Rodents usually
go through a very brief sensitization phase immediately after being fed an antigen, after which
they develop tolerance. Rabbits are sensitized early and can remain sensitized indefinitely.
In humans, like in rodents, the basic phenomenon is tolerance, and sensitization occurs only
in a relatively small number of individuals, considering the vast exposure to foreign antigens.

Dr. Moneret-Vautrin: Could you induce better tolerance by modifying the physical presenta-
tion of antigens by including them in liposomes or other vesicles, which are not easily
digested in the upper part of the intestine and which would be processed directly in Peyer’s
patches?

Dr. Strobel: We have used ISCOMs (immune stimulating complexes). But if you use IS-
COMs you can get sensitization in the gut and also delayed-type hypersensitivity responses.
We have not used other types of vesicles, but I know that if you use some liposomal carriers
you may get stimulation rather than tolerance. This is an important point that has to be borne
in mind when using ISCOMs for vaccination, because if antigen exposure occurs by chance
at the time an oral vaccine is given, you may well sensitize the individual to that antigen
instead of vaccinating him, for example, against polio.

Dr. Brandtzaeg: There was a publication by Nicklin and Miller (2) 10 years ago showing
that the epithelial barrier seems to be very important for the induction of tolerance. If there
was even slight damage to this barrier, tolerance could not be induced. Did you try to repro-
duce these findings?

Dr. Strobel: I believe we showed this in 1987 (3). Whenever you break the integrity of the
mucosal surface, either by inflammation, as in graft-versus-host disease, or by radiation,
during which you may also get disruption of the epithelial layer, or by using ISCOMs, which
may also disrupt the epithelial layer, you abrogate tolerance in this model. It appears that
"tolerogenic" processing of the antigen becomes impossible under these circumstances, even
though other parts of the immune system are still intact.

Dr. Brandtzaeg: But do you actually think that has to do with the epithelial cells? Could
it be that you are disrupting the antigen-presenting cells beneath the epithelium?
Dr. Strobel: If you alter the structure of the epithelium, you alter the local immune system. I cannot tell whether the local antigen-presenting cells are also disrupted in these models.

Dr. Borel: Did you try to use the tolerogenic peptide to induce oral tolerance?

Dr. Strobel: We used the tolerogen to transfer tolerance once into neonatal mice because we thought neonates were unable to process the antigen in a "tolerogenic" way. It only worked in neonatal mice when we additionally gave adult T lymphocytes.

Dr. Rey: Savilahti et al. (4) have recently published a very interesting paper about the higher incidence at a mean age of 11 years of allergic manifestations in preterm infants fed exclusively with human milk to the age of 4 months than in preterm babies fed with adapted cow's milk formula. They speculate that it could be explained by the very low amount of antigen in human milk. You said that with 1 mg/g body weight you can induce tolerance but with 0.1 to 1.0 µg/g you obtain a priming effect. Can you comment on the preventive effect of a high or low degree of protein hydrolysate?

Dr. Strobel: I would not like to speculate and extrapolate on the prophylactic effects of hydrolyzed infant feeds based on the experiments that I presented.

REFERENCES


