Session IV: Discussion

STEPHAN STROBEL, MD, PhD, MRCP: Dietary manipulation and induction of tolerance **(see pp. S74-S79)**

DR. FERGUSON: Most experiments that showed that the passage of an antigen from the gut lumen into the blood very subtly alters the antigen were done with ovalbumin. Haven't you also shown that you could produce oral tolerance to gum arabic, which is an entirely different substance? The phenomenon of rendering an antigen tolerogenic through gut processing has recently been confirmed for gliadin by Ricardo Troncone. Richard Kay showed that cholera toxin, which has an entirely different shape, was also processed during absorption across the gut. Therefore, if one can identify the molecular changes in ovalbumin, it is likely to be something that can apply to other disparate molecular forms.

DR. STROBEL: Yes, the phenomenon is not limited only to ovalbumin or bovine serum albumin. Now the problem is to identify the peptide, or whatever it is, and test it in vivo. It is not easy to get enough material so that we can sequence it.

DR. FERGUSON: A normal gut immune system is needed for gut processing. We did some experiments with irradiated mice. After whole body irradiation, the same amount of immunoreactive ovalbumin reached the bloodstream after feeding as in mice that were not irradiated, but it was no longer tolerogenic. This capacity could be restored by early repletion of lymphocytes in the irradiated animals. Thus lymphocytes are needed in and around the gut epithelium for this phenomenon.

DR. STROBEL: This experiment was unique, and we did not believe the findings at first. Our work was based on personal communications with Morimoto and Hansen. Then Hansen and I showed that we could obtain this "tolerogenic" effect only when 7 days had elapsed since we replenished the irradiated animals with normal lymphocytes; a "functioning" intestinal immune system was probably needed to process it. This suppressive effect might actually be related to the epithelial cell rather than to the lymphoid system, but I am not certain.

DR. FERGUSON: We know, for example, that viruses frequently produce a brief immunodeficiency. There may be analogies between Dr. Strobel's model and secondary cow milk protein sensitization after infections or iatrogenic processes such as irradiation or chemotherapy. His model surely will be clinically relevant.

DR. WALKER: Are you suggesting that peptides that are produced by intraluminal antigen hydrolysis and that cross the epithelium are the tolerogens?

DR. STROBEL: Yes, that is the only difference we have found in the serum.

DR. WALKER: As you have mentioned, it is difficult to apply mouse data to human beings. One may be dealing with very different processes. However, Ian Sanderson, in our laboratory, has found that the timing of class II antigen expression is directly related to weaning. Class II antigens are expressed not before weaning but afterward. In addition, inflammatory mediators, such as interferon gamma, can increase class II expression. The timing in Dr. Sanderson's studies is similar to that in your studies of mice, with regard to when oral antigens produce sensitization versus tolerance. Dr. Sanderson can delay class II antigen expression if an infant is weaned onto an elemental rather than a regular diet. The implication is that there are some parallels between infants and the mouse, and I urge you to follow up on those observations.

DR. KLEINMAN: Dr. Strobel, do you believe that the site of presentation in the gut makes a difference? For example, would it make a difference if the antigen were sampled mostly in the area of the "M" cells and the underlying immunoreactor cells?

DR. STROBEL: I am uncertain about the role of the "M" cell and how it actually affects soluble antigen presentation, but I do believe that "M" cells may affect antigen presentation, because this effect is absent if the antigen is injected intravenously.

DR. KLEINMAN: Have you digested ovalbumin in vitro and fed the hydrolyzed products?

DR. STROBEL: We have started to do that and the results are ambiguous.

DR. ARNAUD-BATTANDIER: Is tolerance a lifelong phenomenon?

DR. STROBEL: Yes, the suppression of delayed-type hypersensitivity lasts at least 17 months without continuous antigen exposure and may last longer. Antibody suppression lasts 3 to 6 months. However, if tolerant animals receive the antigen continuously, the tolerance might be extended. Our experiments were single-feed experiments to find out how

9/0/42378

long the effect lasts. In other experiments we showed that if the tolerance is transferred, the antigen must be given simultaneously. If the antigen is not given, or if it is given too late, tolerance is not induced. It might be that if a tolerant animal is exposed to the antigen continuously, the tolerance in that animal will be "eternal".

DR. LEARY: We have fed guinea pigs for up to 8 weeks and not observed tolerance.

DR. FERGUSON: It is remarkable that you can show oral tolerance in the rat, because we know that the timing is not related to classic gut closure. Gut closure occurs 2 weeks after birth in the mouse. At birth the rat small intestine is as mature as that of a mouse 3 or 4 days of age. The human species goes through that stage of development at about week 12 or 14 of gestation, with respect to villi and enterocyte development and the appearance of lymphocytes in the gut. For some species, that stage of development may be complete before birth. The changes occur at different times in different species.

DR. STROBEL: I believe that one can also induce tolerance in human subjects.

DR. LEARY: The guinea pig gut is essentially closed at birth; closure does not play a role.

DR. STROBEL: We also have done those experiments, and gut closure does not play a major role.

An exciting prospect is the possibility of reinducing tolerance in sensitized human beings via the gut. One hypothesis suggests that one can actually suppress autoimmunity through oral feeding, as has been shown in rheumatoid arthritis in animals. The gut has been rediscovered as a major source of suppression of the immune system, but we do not understand how the suppression is being achieved.

ULRICH WAHN, MD: Comparison of the residual allergenic activity of six different hydrolyzed protein formulas (see pp. S80-S4)

DR. STROBEL: In the clinical setting, what tests would you recommend before feeding a hydrolyzed product to a child who has a cow milk allergy?

DR. WAHN: No test is 100% predictive except the challenge test.

DR. FERGUSON: Two children in your study had greater mean wheal diameters than the rest when tested with the casein hydrolysates. Were these the same two children with positive results on the radioallergosorbent inhibition test (RAST)? Did the same two children have positive results for all the products?

DR. WAHN: There was a tendency for the same child to have high positive values for the different materials, but the correlation was not as good as we had hoped. None of the tests correlated with the clinical outcome so that we could predict the clinical reaction.

DR. BUSINCO: The negative predictive accuracy of the skin test is fairly good. If an infant has a negative response to skin testing, the product could be given. If the skin test result is positive, the response is not as reliable.

You showed that many of the children had positive RAST reactions to the soy and collagen hydrolysate. Was the immune response to the collagen part of the hydrolysate?

DR. WAHN: We believe so.

DR. BUSINCO: When you challenged these infants, only one had a positive response. This indicates, again, that the RAST, especially in this population of children, is not reliable.

DR. WAHN: I agree completely.

DR. STROBEL: You said that the IgE tests or RAST inhibition did not correlate with the clinical response to your challenge. Is it worthwhile to do RASTs for any child?

DR. WAHN: For this study, it was important to focus on a defined subgroup with IgE antibodies.

DR. STROBEL: In clinical settings, I think performing some IgE tests is not sufficient for a diagnosis of food allergy.

DR. SCHMITZ: You presented casein hydrolysate I and II as though they are different, but I thought they were the same.

DR. WAHN: The hydrolysate was the same but the formulas differed in the other components.

DR. SCHMITZ: Wouldn't you expect the results to be the same?

DR. WAHN: It was a kind of control.

DR. FERGUSON: Presumably, your challenges were done in random order. I have always been a bit concerned about the restimulating effect of challenges.

DR. WAHN: The hydrolysates were challenged in a random order, but cow milk was always last.

DR. STROBEL: Have you actually observed priming for subsequent IgE responses in the skin after skin testing?

DR. WAHN: No, we have not studied that.

DR. BURKS: I know that Hugh Sampson challenged the same patient with the same materials for several days in a row, and the same response continued.

DR. FERGUSON: The standard way of reporting RAST results is very insensitive. If one titrates accurately, one can certainly show variations of titer.

DR. ESTEBAN: Would the continuous administration of the hydrolysates increase the IgE responses?

DR. WAHN: We have never observed that, but we have not thoroughly followed up on that question.

JACQUES SCHMITZ, MD: Effects of brief early exposure to partially hydrolyzed and whole cow milk proteins (see pp. S85-S9)

DR. WALKER: Were the IgE levels in your study significantly greater than those previously reported as the physiologic norm for any child exposed to proteins?

DR. SCHMITZ: These are physiologic levels. In this population, we had very few infants with high levels of cord blood IgE who were high responders, so we saw mostly a modulation of the response in normal infants. The suspected clinical manifestations of atopy were not modified.

DR. WALKER: Are you showing that, with more antigen exposure, there are higher levels of specific α-lactalbumin IgE?

DR. SCHMITZ: Yes.

DR. ESTEBAN: Normally, we cannot detect specific IgE antibodies to cow milk in the general population. It is striking that you detected it in all the children.

DR. SCHMITZ: No, it was not detected in all the infants.

DR. ESTEBAN: How many infants had a positive test for a specific IgE antibody?

DR. SCHMITZ: There were about 20 or 30 in each group. It was less than one fourth or one third of each group.

DR. WAHN: Every infant in your study received the partially hydrolyzed formula as a supplement until the third month. Do you believe that the specific IgE and IgG differences would have been more apparent if you had fed the adapted formula, instead of the hydrolyzed formula, during the weaning period?

DR. SCHMITZ: With that protocol, the infant would have been completely inundated with whole cow milk proteins during the first 3 months.

DR. WAHN: But you minimized the immunologic responses by giving both groups the partially hydrolyzed formula.

DR. SCHMITZ: Initially, we wanted to prove that it is possible to modulate the immune reaction by modifying the antigenic load during the first days of life. We planned to use a group of infants who were exclusively breast fed for 3 months, but the number of breast-fed infants was much less than expected. If a comparison had been made between a few days of hydrolyzed formula and a few days of the adapted formula, in infants fed an adapted formula for the first 90 days, there probably would have been no difference.

DR. STROBEL: Obviously, the best design would have been a Latin square design, but that was not possible at that stage.

DR. BUSINCO: I agree with Dr. Wahn. Maybe it would have been better to feed the partially hydrolyzed formula for just a few days and then supplement the human milk with an adapted formula. If you want to check the capacity of a given type of feeding during the first few days of life, the protocol should be different. The decreased IgG response that you found was probably not due just to the short period of feeding the partially hydrolyzed formula early in life. Feeding this formula later possibly could have caused the significant decrease in IgG response to cow milk proteins. Did you check the IgE antibodies to other food antigens, such as those in egg or wheat, at 6 months or 1 year of age?

DR. SCHMITZ: No, we did not. For example, we did not check gluten.

DR. FERGUSON: Your formula was not extensively hydrolyzed. Did it contain milk antigens?

DR. SCHMITZ: Yes.

DR. FERGUSON: Even if the formula contained 100 times fewer milk antigens, the infants were still adequately exposed to enough milk antigens for immunization. You did find some differences in IgG antibodies to a particular protein. I suggest that the formula given to your infants during the first few days of life might have influenced the nature of the gut flora that were becoming established. We know that the gut flora have an important background effect in creating, modulating, and enhancing immunity at all levels. It is possible that you might have enhanced general IgG responses. If you had tested for antibodies to an unrelated antigen such as tetanus toxoid, you also might have found differences between the groups. I fully appreciate that because only a limited amount of blood can be drawn from an infant, it is not feasible to test a whole range of other proteins, but it should be considered. If you examined total IgG levels in subclasses, you would almost certainly find differences between the groups.

DR. SCHMITZ: For that reason, Dr. Strobel asked us to include ovalbumin as a control. Because the antibodies to ovalbumin did not differ, the phenomenon is not a general one.

DR. FERGUSON: The capacity to make an IgG response to an antigen such as tetanus could be measured at 1 year of age because most of the infants would have been vaccinated against it.

DR. STROBEL: All the infants were fed human milk, and they would not be receiving ovalbumin except through the mother's milk. We therefore wanted to check the mother's diet. Because all the infants had been breast fed, one would also not expect a major difference in the gut flora.

J. ROBERTO MORAN, MD: Effects of prolonged exposure to partially hydrolyzed milk protein (see pp. S90-S4)

DR. WALKER-SMITH: Did any infant in the three groups have a clinical illness, such as gastroenteritis, requiring a visit to a physician during the period of evaluation?

DR. MORAN: This cohort of infants was large. If the infants had acute gastroenteritis, the protocol required that they be given an oral electrolyte solution and returned to their formula within 5 days if they were to continue in the study.

DR. WALKER-SMITH: During your study, were there different patterns of significant illnesses between the groups?

DR. MORAN: None was identified.

DR. WALKER: Each of the three groups appear to have the same number of children with an allergy history. Have you studied the antibody data within each of these subgroups?

DR. MORAN: No, I have not, but such a study might be interesting.

DR. WALKER: That question is important. Do you plan to study it?

DR. MORAN: We might not have enough infants to separate out those with a family history and relate that to the IgG and IgE levels. We measured IgG and IgE in about 20 babies per group. To measure growth, we needed a much larger group. We are studying that issue in infants who are considered at high risk by family allergic history or because of elevated cord blood levels of IgE. That is the only population that we believe is suitable for that kind of study.

DR. STROBEL: Was there a slight bias in the selection of the groups in your study, in that you chose the breast-fed group according to the mother's intention to breast-feed and only randomly selected the others?

DR. MORAN: That is correct.

DR. SCHMITZ: In our partially hydrolyzed formula, 50% of the peptides were made up of 15 or fewer amino acids. What was the peptide size in the partially hydrolyzed product used in your study?

DR. LEE: The size of peptides in the hydrolysate used in this study was similar.

DR. KLEINMAN: When were these infants started on solid feedings?

DR. MORAN: They were started on solid feedings at 4 months of age, which is standard practice.

DR. KLEINMAN: When do they start getting whole cow milk? Will you be following these infants?

DR. MORAN: No, this study was completed. Most infants were followed until 4 months of age and the others until 8 months of age.

DR. KLEINMAN: Did any receive whole cow milk during that time?

DR. MORAN: No.

DR. KLEINMAN: Did any of the breast-fed babies receive supplemental feedings?

DR. MORAN: Yes, the feedings were supplemented with the partially hydrolyzed formula.

DR. STROBEL: That is a major problem with studies of breast-feeding. How often are breast-fed infants given only human milk? Most methods sections in published papers do not comment on that.

DR. BUSINCO: I have some clinical evidence that the hydrolyzed product available in Italy is immunogenic. Recently I saw two infants who had received this product during the first 2 days of life; they then were totally breast fed. Their mothers were strongly motivated, and they totally avoided cow milk during the period of lactation. One infant was fed the same formula at 1 to 2 months of age, and the other in the third month. Both went into severe anaphylactic shock. They had, of course, strongly positive RAST and skin test responses to this product. This is evidence that this product can be not only allergenic but also immunogenic in a sensitized individual.

DR. STROBEL: That is an important point, but it is not surprising from an immunologic point of view. One must be careful to obtain an accurate family history.

DR. SCHMITZ: Partially hydrolyzed products are immunogenic, but they are less immunogenic than formulas with complete proteins.

DR. BUSINCO: Yes, but the term "hypoallergenic" should be eliminated. It is not correct to define a product as "hypoallergenic" when it induces positive reactions to challenge tests in at least 50% of the children with cow milk allergies.

ERIC MALLET, MD, PhD: Long-term prevention of allergic diseases by using protein hydrolysate in at-risk infants (see pp. S95-S100)

DR. ESTEBAN: In your study, was the children's eczema caused by milk allergy? If not, is there an explanation for how the feeding of cow milk hydrolyzed formula can diminish the incidence of non-milk-related atopic eczema?

DR. MALLET: I have no explanation. We measured total IgE levels in every infant, and only three infants had positive RAST reactions against cow milk protein. These children did not have eczema.

DR. STROBEL: Did you show that it was the severity of eczema, instead of the number of children who had eczema, that was affected by early diet?

DR. MALLET: We observed both a lower incidence and a lower severity.

DR. FERGUSON: During the study, did you actually reduce the severity or the incidence of eczema in the town?

DR. MALLET: Our data cannot answer the question of eczema in the overall population.

DR. BURKS: You described your study sample as being "at risk," but your table showed that only a certain number had a family history of atopic disease—is that correct?

DR. MALLET: The high-risk group was selected according to a modified scale based on the work of Kjellmann (Allergy 1982;37:463). All infants in this category had scores greater than 1, and they were only those with family histories of major allergies such as eczema, severe rhinitis, and proven cow milk allergy. We selected only about 4% of about 4500 infants born in the maternity hospital during a period of 1½ years.

DR. WAHN: I still do not understand the criteria for a

positive family history. What are high allergy scores? Their cord blood IgE levels seemed like those of a low-risk group.

DR. MALLET: All had a score greater or equal to 1, and about half had a score greater than 3. Family histories did not correlate with cord blood IgE levels. We were surprised to find that only 10 subjects, or about 6% of the entire population studied, had a cord blood IgE level greater than 1 IU/ml.

DR. WAHN: That is more than in our general population.

DR. MALLET: It is the same as in the general population, as published by Bousquet et al. (Ann Allergy 1984;53:692-5) and others. We measured cord blood IgE levels by radioimmunoassay, and 27% had IgE levels greater than 0.5 IU/ml. We also measured IgA.

DR. WAHN: Being involved in a similar study, I realize how important it is to define the populations for these studies. If one uses just parental allergy history for the definition, which I think is the crucial factor, one should validate the criteria. If you just ask parents whether they are allergic, every second answer will be wrong because they classify themselves as they feel they are. What were your criteria for a positive family allergic history?

DR. MALLET: We selected major manifestations, such as eczema, asthma, severe rhinitis, and proven cow milk allergy by using official medical documents with evidence of treatment provided by the practitioner. By our criteria, we selected only 4% of the population, although about 10% of the general population has a positive family history.

DR. STROBEL: You asked for the diagnoses of parents and first-degree relatives?

DR. MALLET: Yes, we asked the parents for proof of treatment by a dermatologist, immunologist, or other appropriate medical specialist. We know two of the families with cow milk allergy very well because the infants were hospitalized in our unit.

DR. STROBEL: The criteria were very strict in this study. This low percentage was also found in other studies in France, such as Dr. Schmitz's study. We also have shown that family history of atopy and cord blood IgE do not correlate well.

DR. CRONER: The proper way to determine whether the parents are actually atopic and have IgE-mediated allergies is, of course, to do prick tests with some standardized allergens.

DR. STROBEL: Do you mean atopy without clinical symptoms of atopy?

DR. CRONER: These tests would be conducted to find out whether the parents still retain the allergies.

DR. WAHN: Another method of validation would be the phadiatop test, a multiallergen, specific-IgE screening test. If one is able to validate certain questions that one asks the mother or father in the obstetric ward, one finds that certain questions are better than others in terms of sensitivity and specificity. It becomes more and more evident that family history is most important in defining the high-risk population, so we must be very critical of the criteria.

DR. WALKER: It is difficult to do human studies such as these, and I commend you for your effort. However, your findings contrast with the results of Zeiger et al. (J Allergy Clin Immunol 1989;84:72-89); they reported that differences occurred only during the first year of life. Dr. Mallet, in your study, it appears that the differences occur after 2 years of age. Have you further analyzed the data to separate those children within groups whose families were more severely allergic from those who had a less severe allergic history?

DR. MALLET: We observed no correlation between the severity of the family allergic history and the occurrence of allergic manifestations in the infant.

SESSION IV: GENERAL DISCUSSION

DR. STROBEL: Does anyone have information about the effects of early weaning on IgE responses and the development of atopic eczema? Some articles suggest that the protective effects of breast-feeding are associated with the delayed introduction of solid foods.

DR. SCHMITZ: We controlled that more or less in our study. No solid foods were given to the infants before 3 months of age. There were no differences between groups in the way *beikost* was given between 3 and 5 months of age. After 5 months of age, there were no food limitations.

DR. MALLET: In France the duration of breast-feeding is a problem because most mothers work. Most infants are breast fed for less than 8 weeks after birth. In our region, only 20% of the mothers are breast-feeding their infants at the end of the first month of life, and only 12% after 6 weeks (Arch Fr Pediatr 1991;48:391-5).

DR. WAHN: In the German multicenter study, which is a nonintervention study and is still ongoing, we observed that the number of *beikost* sources is extremely important for the clinical manifestation of allergies. According to our data on children who are now less than 1 or 2 years of age, the introduction of *beikost* is an important risk factor for the highest-risk population.

DR. CRONER: The effects of *beikost* have not been studied in Scandinavia and Finland since Katosaari's study (Acta Paediatr Scand 1983;72:411-4), because people generally say that solid foods should be postponed until at least 6 months of age in a high-risk population.

DR. ESTEBAN: In our children who have cow milk allergy, we have found a strong association between the time of introduction of these foods and the initiation of the symptoms of the food allergy related to them.

DR. BUSINCO: The diet of the nursing mothers is another

important factor that may influence the results of preventive studies. Dr. Mallet, were the mothers in your study on a standardized diet during the period of lactation?

DR. MALLET: They were not.

DR. BUSINCO: The Swedish group led by Linkping (EAACI meeting, Zurich, Switzerland, 1991) showed that the avoidance of cow milk and fish by mothers is significantly associated with a decreased incidence of atopic dermatitis in their children, even at 4 years of age.

DR. MALLET: I agree with that, but in France it is difficult to get mothers to comply with these recommendations.

DR. STROBEL: We must be careful when evaluating the effects of recommending a blanket diet to mothers of high-risk children, because one is also probably decreasing the antigens in the household when eliminating dietary milk and eggs. I believe that nobody has studied that.

DR. FERGUSON: In severe adult eczema related to foods, egg is very important. Sometimes a very strict elimination of egg, even if previously eaten only in trace amounts, further improves eczema in someone who is clinically aware of egg-related symptoms. One can become obsessed with cow milk IgE-mediated reactions and forget that egg is an important weaning food and a highly potent allergen. Perhaps eliminating egg, plus or minus peanuts or fish as a second phase, is what is needed to clarify the process. We will never completely eliminate eczema, but one could improve what has already been achieved, which is significant.

DR. STROBEL: Would you recommend that pediatricians advise mothers of children coming from high-risk backgrounds to breast-feed with or without hydrolyzed formulas and advise the mother to use a special diet? Alternatively, would you advise that highly allergenic weaning foods be delayed until, for example, 9 months or 1 year of age?

DR. CRONER: As yet we have no general agreement about that in Scandinavia. Our studies show that eliminating egg, milk, and fish during the first months of lactation reduces atopic dermatitis in children up to 2 years of age—the duration of these studies. The studies are only preliminary and must be broadened before we can make recommendations.

DR. SCHMITZ: Dr. Mallet's study is the only one that shows such a clear-cut difference in the incidence of eczema after such a long time. The other studies of atopy prevention usually do not last as long and usually show an effect that is the highest at 1 year of age, and then the effects decrease progressively. In Dr. Mallet's study, there is no effect at 1 year and a striking effect at 2 and 4 years of age. What might explain this striking difference?

DR. SAVILAHTI: The extrapolation of results from studies of high-risk children to the general population is very dangerous. Dietary manipulation for healthy infants in the general population with no allergic background has little effect. For example, when we followed 200 healthy children, we observed more allergic symptoms in those who were exclusively breast fed for 9 months than in those who were breast fed for shorter periods (Arch Dis Child 1987;62:269-73). The same might apply to hypoallergenic formulas. One should be careful about recommending these dietary measures, because these recommendations tend to spread to the general population when there is no indication for their use.

DR. STROBEL: That point is important. In the normal population, there is very little indication for these kinds of measures because 80% of the population lives very happily with cow milk.

DR. WALKER: Dr. Strobel, how would you devise a study of oral tolerance in human subjects, including its induction and what factors affect it?

DR. STROBEL: One would give to the child antigens that the mother, father, and child had not previously had. Further, the antigens would be those that are not common in the surroundings, so that the infants would not have been inadvertently exposed to them. For example, keyhole limpet hemocyanin is a very rare antigen in children. Alternatively, one could use artificially made proteins or large peptides and then observe T-cell responsiveness. One could isolate antigen-specific precursor frequencies in the blood and analyze their response to a particular antigen. In coculture experiments, for example, one could determine whether it is suppressive. Such a human study would have more interfering variables than a study using an in-bred mouse strain, but such a study is possible.

DR. FERGUSON: You would not need to study the phenomenon of oral tolerance in infants; you could study it perfectly well in adults. Nearly all the work with the mouse has been done in adult mice.

DR. WALKER: How do you dissect out the mechanisms to show a cause-and-effect relationship?

DR. STROBEL: One must determine the timing and development of tolerance. First, one must examine the induction phase, which is very early—between 1 and 7 days of age, at least in the mouse—and, second, the maintenance phase. That is when the antigen is given again, and there is still no response. It is the most difficult phase to investigate. At the beginning, one would not want to tackle that because there may be total energy. We currently cannot investigate silenced cells, which have lost their antigen responsiveness. One must look early to see whether those cells become silent after antigen exposure.

DR. BURKS: A reason for doing that study in the infant is that one expects to observe the eventual development of tolerance in the infant. Maybe the mechanisms could be differentiated more easily in an infant than in an adult, in whom they are an established phenomenon.

DR. STROBEL: By choosing a new antigen, however, it will

be possible. Obviously, both adults and neonates must be studied.

DR. BURKS: If there is a period of priming in the human infant, followed by a period of tolerance, maybe the process could be observed in some way more easily in an infant.

DR. FERGUSON: We know from studies of premature infants that they do not have undue susceptibility to the development of allergy, including milk allergy.

DR. KLEINMAN: One could use bacterial antigens to which we have not been exposed. Could another animal model be used, such as the pig, which has a gastrointestinal system closer to that of the human than does the rat or the mouse?

DR. STROBEL: The problem with the pig is that not enough of its antibodies and cell surface markers have been identified.

Most of the data have been presented as means. I wonder whether that is a good way to present population data; one might erroneously increase the means with high outliers. Wouldn't it be better to present medians?

DR. MORAN: That is especially true for IgEs. We examined the individual points to find out whether we were just pulling the results up or down. We also counted the number of patients who had positive or negative responses. That did not affect the results either. I suppose that one could use medians. I am not sure about the advantage of medians for IgG values.

DR. SCHMITZ: Clearly the distribution is not normal, so we must use medians.

DR. ESTEBAN: One can use geometric means or logarithms for IgE values.

DR. STROBEL: Yes, but we must consider other things. Antibodies are measured as a continuum. Moreover, one might be measuring production and affinity. Nobody has addressed the question of affinity. If one is interested in immune regulation, affinity, or somatic mutations induced by antigen exposure, is an important point to consider.

Our study was not designed specifically for clinical responses. We wanted to find out whether immunologic memory of early antigen exposure can be identified later in life. Although many other antigens and things affect the infant, there is still an immunologic memory of what happened during the first few days in life. That was the main idea of our study presented by Dr. Schmitz. It mimicked in human subjects the animal experiments that I have done. The "best" study would have had a group that was breast fed only and a complete 2×2 design. That design would need about 1000 children—250 in each group—to reach statistical significance in the normal population. A normal population provides different results from those obtained by studying the high-risk population.

DR. PASCUAL: I believe that the first 3 days of life are very important for the future of IgE sensitization to milk or whatever antigen the infant takes in. In our experience, about 50% to 60% of our children with an IgE-mediated milk allergy had what we call a "pirate" bottle. In our country, even the children who will be breast fed receive a formula supplement in the nursery during the night if they cry. The IgE-mediated allergy of the infants who have received this supplement behaves differently from the allergy in the other infants. They have symptoms, especially cutaneous ones, with their first contact with a bottle at 3 or 4 months of age, when they are starting to be weaned. In the other group of infants, 4 to 7 days of feeding are needed before symptoms develop. I believe that an infant who will be breast fed should not receive any kind of bottle feeding—even hydrolyzed formulas—during the first 3 days of life.

DR. STROBEL: Is treating cow milk and food allergies with cromolyn sodium a standardized practice? Does anyone have any good evidence that it works? We all know that it works occasionally. When cromolyn sodium is given to some children with multiple food allergies, the children can tolerate the foods without having clinical symptoms.

DR. FERGUSON: I think it is now licensed in Australia for food allergies, but therapy is usually combined with elimination diets.

DR. MALLET: We use cromolyn sodium in children more than 2 years of age with a proven cow milk intolerance. We give cromolyn sodium and reintroduce cow milk by drop, increasing the milk quantities for 6 months. Afterward, we decrease the cromolyn. We have 10 patients who became tolerant after this period of cromolyn administration.

DR. STROBEL: Everybody has anecdotal reports that the drough does work well, but I have not found out which children will respond to it, except by trying it.

DR. WALKER-SMITH: Syme (Pepy SJ, Edwaos AM, eds. *Mast Cell: Its Role in Health and Disease.* Tunbridge Wells, United Kingdom: Pitman Medical, 1979:438-42) originally described a few children in Edinburgh who responded to it. I see a group of children who have multiple food allergies and very high serum levels of IgE, and often eosinophilia, who improve symptomatically during treatment with cromolyn sodium.

DR. BUSINCO: In 1986, we published a double-blind, placebo-controlled study in a highly selected population of children with food allergy and atopic dermatitis. All the children were on an elimination diet and received the placebo or cromolyn sodium. We demonstrated a significant effect of the drug. However, Hugh Sampson did a double-blind study in 10 children with atopic dermatitis. The protocol was not the same because he gave the children lower doses for a short period. He was not able to confirm our results. Thus the use of cromolyn sodium remains controversial.