

Feeding of protein antigens before systemic immunization with the same antigen results in profound immune unresponsiveness characterized by suppressed T cell proliferation and cytokine production with concomitant suppressed antibody production, and this phenomenon is termed oral tolerance. The feeding treatment by itself is also ineffective in generating humoral immune responses. Oral tolerance has been nominated for many decades as a prophylactic strategy of food allergy. In this sense, milk proteins in infant formulas can be potential tolerogens since they are probably the first foreign proteins for most of infants. But this is not the case. In addition, there is scanty evidence that has proved the feasibility even in animal studies. This is because it is difficult in inducing immune responses by oral immunization and thus in confirming the hyporesponsiveness against the priming challenge with the antigen. In order to examine the feasibility of oral tolerance as a prophylactic strategy of food allergy, the development of animal model of oral immunization is required.

It has been of concern with food allergic diseases as to what determines whether to activate or to suppress the immune response to dietary allergens that were exposed to. In several studies, mice and guinea pigs have been demonstrated to generate specific serum antibodies to protein antigens given orally. It can be of note that these animals were given protein antigens as a constituent of their diet or drinking milk, whereas silent animals were intragastrically given a single or multiple doses of purified antigens dissolved in saline or water. Taking account of different regimen accompanied with inconsistent immune responses to protein antigens fed, it might be relevant to consider that the response to a single purified food component can be quite different from the response to the whole food. I hypothesized that food components other than proteins might play a critical role in determining immunological consequences to the protein antigens in the food, and among various food components the influence of dietary oil was evaluated.

BALB/c mice were intragastrically given a daily dose of 25 mg bovine  $\beta$ -lactoglobulin ( $\beta$ -Lg) dissolved in distilled water or after emulsifying 50  $\mu$ l of refined soybean oil for 12 days. A remarkable increase of anti- $\beta$ -Lg IgG antibody was generated in sera from mice who received the emulsion whereas no increase at all in mice receiving the aqueous solution. The adjuvant-like effect of soybean oil in emulsion was also demonstrated for the different proteins and in mice with different genetic background, suggesting that oil in food might play an important role in the sensitization

to protein antigens therein.

The feature of adjuvant-like activity of oil was then investigated in BALB/c strain. When mice were fed emulsions containing different amount of either soybean oil or  $\beta$ -Lg, the magnitude of the response was found to be related to the amount of soybean oil rather than that of  $\beta$ -Lg. It was also found that the extent of anti- $\beta$ -Lg IgG antibody response was closely related to the particle size of emulsion and that unemulsified oil liquid was incapable of generating any serum antibody responses. Furthermore, aqueous  $\beta$ -Lg became capable of eliciting the specific IgG antibody response when emulsified oil was simultaneously given, but the response was much lower when compared with that induced by  $\beta$ -Lg-stabilized emulsions.

These results strongly suggest that although  $\beta$ -Lg is usually a T cell-dependent antigen, the molecules adsorbed onto and arrayed over the surface of oil particles may behave like a polyvalent hapten of T cell-independent type-2 (TI-2) antigen. Different from a monomeric form, protein antigens covalently conjugated to microspheres are highly immunogenic by supporting the cross-linking of surface antibodies in the resting B cells, a prerequisite in the T cell independent step of the B cell activation and humoral immune response. Microspheres having similarly small diameter are shown to disseminate within macrophages to mesenteric lymph nodes and spleen. The particle size of  $\beta$ -Lg-stabilized emulsions is small enough to be absorbed in such as through M cells. Contrary to this hypothesis, however, no meaningful antibody response was evoked when T cell-deficient nude mice were fed  $\beta$ -Lg-stabilized emulsions. It was also found that the i.p. administration of anti-CD40L mAb resulted in a marked suppression on the emulsion-driven anti- $\beta$ -Lg IgG1 antibody synthesis. The provision of CD40L costimulatory signal by T cells through CD40 molecules on B cells following recognition of protein antigen plays a pivotal role in B cells proliferation, immunoglobulin secretion, and class switching to IgG1. Therefore, these results strongly suggest that the presence of T cells is required for the emulsion-driven antibody response; accordingly, it is unlikely that most of  $\beta$ -Lg molecules are not taken up as emulsion and that the protein antigens do not act in a fashion of TI-2 antigen. Eventually, there was no antibody synthesis of IgM class in serum of mice following a 2-week feeding of  $\beta$ -Lg-stabilized emulsions which is a characteristic isotype in response to TI-2 antigen. Probably, most of antigen-stabilized emulsions are disrupted by bile in the digestive tract as indicated on liposome.

Oral tolerance has been expected to be a safe, painless, and useful tool for curing and preventing immunological adverse reaction such as autoimmune diseases and food allergies. As to its application to food allergies, however, it is necessary to see if oral

tolerance can work following antigen challenge by oral route and not parenteral route. It was shown by using this oral immunization model that an unresponsive immune state that had been once developed by feeding aqueous  $\beta$ -Lg was maintained after oral challenges with the same antigen with oil-emulsions. The usefulness of oral tolerance was also observed for IgE antibody responses in DBA/2 mice fed diet of bovine milk casein.

On the basis of results as discussed above, I expect that the principle of oral tolerance can be applicable to the prophylaxis of food allergy, and a key tactics for inducing naive infants a tolerant immune state against dietary antigens is to minimize such an oil-born immunological help as support of B cell response at the first introduction of tolerogens. As indicated in a separate feeding study, this could be between two successive practices of breast milk feeding during the complete breast feeding period of time, because these practices are separated by an interval of certain hours which may minimize the oil-derived immunomodulatory effect. When breast milk feeding is not available, hypoallergenic formulas can be used as a substitute for breast milk. However, taking into account many reports indicating a possible in utero sensitization and an immune complexity of infants, further studies should be conducted before clinical evaluation.