

side the cytoplasm was thus concentrated on, the root hair was usually on the apical side of the cell in the still culture after centrifugation.

On the basis of the above experiments it seems, so far as the present material is concerned, that the apex of the root epidermal cell is determined as the site of root hair formation in the cell not by gravity but by the cellular polarity, and this polarity is related neither to localization of the vacuole nor to movable cytoplasm, but is

controlled by the stable cortex or cell membrane as reported in some other plants [4, 5].

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via the group(s) resulting in endocytosis of the antigens. Some of the conjugates which escape from digestion within the cells, may be released into lamina propria or systemic circulation. These conjugates may stimulate the immune systems, especially of the IgA-antibody response. For this purpose, we prepared conjugates of ovalbumin(OA) and lipopolysaccharide (LPS, from *E. coli* 055) or cholesterol (Chol), and the resulting conjugates were intubated into mice to examine the pattern of antibody responses.

OA-LPS conjugation was carried out by the oxidation method [2]. Briefly, LPS was oxidized by NaIO₄ followed by dialysis against acetate buffer (pH 4.5). The resulting oxidized LPS, 12 mg, was mixed with OA, 30 mg, adjusting the pH to 9.5 by carbonate buffer under stirring at R.T. for 4 h. The reaction was stopped by adding NaBH₄, 1.2 mg, to the mixture. Then the reaction mixture was chromatographed on Sephacryl S 200. The 1st peak fraction was used as OA-LPS conjugate. Groups of 5 mice each were intubated intragastrically (i.g.), twice a week for 2 weeks, with OA-LPS, 1 µg, 10 µg or 100 µg as OA dose. As controls, corresponding doses of OA alone, or 100 µg OA plus 60 µg LPS were given i.g. to mice. The mice were bled at several intervals after the intubations and after an i.p. challenge with 2 µg OA plus 4 mg alum to induce IgE-antibody response. Serum IgG and IgA anti-OA anti-

IgA- and IgG-enhancing but IgE-suppressing Characteristics of Lipopolysaccharide- or Cholesterol-Ovalbumin Conjugate Intubated to Mice

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It has been well studied that macromolecular substances pass through mucosal membrane and are released into submucosal tissue or systemic circulation to stimulate immune system in normal, especially in pathological conditions [1]. However, an

effective method of oral immunization has not been sufficiently established yet. We have investigated the method based on the following idea: Antigens conjugated with materials bearing lipophilic groups(s) may attach to the membrane of epithelial cells

Table 1. IgG-, IgA-enhancing and IgE-suppressive characteristics of OA-LPS conjugate intubated i.g. to mice

Immunogens [µg]	4 i.g. intubations ^a			6 i.g. intubations ^b			After challenge ^h		
	IgG ^c	IgA ^c	IgE ^d	IgG	IgA	IgE	IgG	IgA	IgE
OA-LPS									
1	1.0±0	1.6±0.3	nd ^e	2.8±1.7	2.7±0.4	nd	27.5±1.5	3.7±0.6	120
10	11.7±7.9	2.8±1.6	nd	14.0±9.5	4.0±2.4	nd	33.8±1.5	6.1±2.0	40
100	17.9±5.7	3.1±1.0	nd	24.8±4.6	6.1±1.4	nd	34.5±1.3	7.4±1.6	10
OA									
1	1.0	1.6±0.3	nd	1.0	2.0±0.9	nd	17.1±2.1	2.9±0.9	160
10	1.0	1.8±0.5	nd	3.0±2.9	2.2±1.2	24	22.2±1.4	2.5±0.5	320
100	1.0	1.8±0.3	nd	15.2±5.4	3.6±1.2	200	24.6±1.6	3.6±0.7	640
OA+LPS									
100 ^g	nt ^f	nt	nt	17.3±2.6	2.2±0.9	nt	20.9±1.6	2.3±0.6	640
OA in alum (control for challenge)							14.6±1.5	2.2±0.1	640

^a Serum was obtained 20 days after the 4 semiweekly intubations

^b Serum was obtained 10 days after the additional 2 semiweekly intubations

^c IgG- and IgA-antibody titers are expressed as enzyme activity (intensity of fluorescence) obtained with test serum divided by that obtained with normal serum

^d IgE-antibody titer is expressed as a reciprocal of the highest dilution giving a positive reaction in PCA using rat skin

^e Not detectable at 1:5 dilution ^f Not tested

^g A mixture of 100 µg OA and 60 µg LPS (corresponding to 100 µg OA-LPS conjugate)

^h 10 days after i.p. injection with 2 µg OA plus 4 mg alum

body titers were measured by enzyme-linked immunosorbent assay (ELISA) on microplate using rabbit anti-mouse γ or α chain and β -D-galactosidase-linked goat anti-rabbit IgG antibodies. The IgE-antibody titer was determined by PCA. A representative result of the experiments is shown in Table 1. IgG- and IgA-antibody responses were significantly higher in every group intubated with OA-LPS than in the corresponding control groups after the 4 and 6 intubations. Moreover, after the challenge, the OA-LPS-intubated groups showed pretty well IgG- and IgA-antibody responses compared to control groups, suggesting that the antibody responses are stimulated very strongly by intubation with OA-LPS, probably because of activation of submucosal immune system. In sharp contrast, however, IgE-antibody response was undetectable in OA-LPS-intubated groups after 4 and 6 intubations. After the challenge with an IgE antibody-inducing dose of OA, the IgE response in OA-LPS-intubated groups was suppressed dose-dependently, but, again in sharp contrast, the response in control groups increased dose-dependently up to the level of the response in unpretreated group (control for challenge). On the other hand, mixture of OA and LPS could not bring about the effect mentioned above. The findings indicate that intubation of OA-LPS exerts the enhancing effect on IgG- and IgA-antibody responses along with the suppressive effect on IgE response in mice. Additionally, antibody to LPS was detected in all of mice intubated with OA-LPS, suggesting that LPS together with OA reaches and stimulates immune systems.

For OA-Chol conjugation, OH group on C-3 position of Chol was esterified by succinyl-anhydride [3]. Then, the resulting material was conjugated to OA by the mixed-anhydride method described in [4]. OA-Chol 1 μ g, 100 μ g or 1000 μ g/mouse/day in water solution was used for i.g. intubation. Groups of 5 mice each were intubated i.g. once a week for 5 weeks, thereafter, 5 times a week at 7th and 10th week. As control, 1000 μ g OA was given i.g. to mice. The mice were bled and the sera were examined for anti-OA IgM- and IgG-antibody titer by passive hemagglutination (HA) using OA-coated sheep blood red cells, and for IgA antibody by the ELISA. A representative result of the experiments (2 weeks after the last intubation) is shown in Table 2. IgA-antibody response was significantly higher in every

Table 2. IgA- and IgG-enhancing characteristics of OA-Chol conjugate intubated i.g. to mice

Immunogens ^a [μ g]	Anti-OA antibody titer (2 weeks after the last intubation)	
	IgA ^b	IgM and IgG ^c
OA-Chol (Chol-suc _{1,0} -OA)		
1	3.5 $\pm$ 0.5	1.9 $\pm$ 0.5
100	2.7 $\pm$ 0.4	4.9 $\pm$ 1.7
1000	2.5 $\pm$ 0.2	6.8 $\pm$ 1.2
OA 1000	1.3 $\pm$ 0.4	2.8 $\pm$ 0.5

^a Immunogens were intubated i.g. once a week for 5 weeks, thereafter 5 times at 7th and 10th week

^b IgA-antibody titers determined by ELISA are expressed as indicated in Table 1

^c IgM- and IgG-antibody titers determined by HA are expressed as n of the highest dilution of 1:10 $\times$ 2ⁿ giving a positive reaction

group intubated with OA-Chol, especially with 1 μ g OA-Chol, than was in control group. HA-antibody response was elevated dose-dependently but IgE antibody was not detectable in the intubated groups. Although preliminary experiments showed that OA-Chol intubation exerted little influence on IgE response after the chal-

lenge, i.v. injection of the conjugate suppressed not only primary but also ongoing IgE responses to OA (manuscript to be submitted).

These results indicate that orally administered protein-lipophilic substance conjugates can prime immune response systems positively for IgA- and IgG- but negatively for IgE-antibody responses. From these results, following possibilities could be also considered: A life-long immunization can be achieved by oral administration of the toxin conjugates in young infants whose mucosal membranes allow easily the passage of macromolecules, followed by the boosters at appropriate ages, since we have data showing detoxification of bacterial toxins by the conjugations (manuscript in preparation). Prevention of allergic responses can be carried out by the similar treatment with the allergen conjugates in atopic infants to whom allergen is able to be estimated from genetical backgrounds.

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Why Twig-girdling Beetles Girdle Twigs

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Several ecologically and economically important species of cerambycid beetles (Coleoptera: Cerambycidae) girdle the stems of plants on which they subsequently feed and oviposit [1]. Advantages gained by beetles through stem girdling are essentially unknown (cf. [2]) and remain largely unexplored. At least in woody twigs, girdling interrupts phloem continuity; organic products normally exported from leaves to stems and roots would thereby be trapped in the girdled twigs distal to the girdle. I report here that beetle larvae, from eggs previously oviposited just beneath bark tissue by their girdling female parents, are provisioned with an abnormally concen-

trated, nutritious resource in girdled twigs, and their survival is enhanced accordingly.

In eastern Oklahoma, U.S.A., adult hickory twig-girdling beetles (*Oncideres cingulata* Say) abruptly emerge from their host twigs in late September, about one month prior to autumnal leaf drop. Upon emergence they immediately commence girdling twigs of several tree species, but primarily black hickory (*Carya texana* Buckl.; Juglandaceae) [3]. Twigs, averaging 9 mm diameter at base [4], are girdled to a depth of only 1–2 mm – completely severing bark and phloem tissues, but leaving the xylem more-or-less intact. Provided