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Immunology

Role of antigen structure in the regulation of IgE isotype expression

(tobacco glycoprotein/polyphenols/anaphylaxis/rutin)

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ABSTRACT **Tobacco glycoprotein (TGP) is a glycoprotein containing rutin-like polyphenol groups that is purified from cured tobacco leaves and can be detected in condensates of tobacco smoke. One-third of normal humans have been shown to manifest immediate, IgE-mediated, wheal and flare reactions to an intradermal injection of TGP. Rutin-like moieties are also found in a wide variety of vegetable foods. The possible importance of sensitivity to TGP in the pathogenesis of the vascular and pulmonary complications of tobacco smoking has stimulated us to study the immune response of mice to TGP and the role of rutin groups in influencing isotype expression. A series of three intradermal injections of TGP elicits a long-lasting IgE antibody response in mice. However, no hemagglutinating antibodies are produced. Similarly, immunization with a rutin derivative of bovine serum albumin stimulates IgE antibodies to bovine serum albumin but little hemagglutinating antibodies. In contrast, mice injected in the same manner with bovine serum albumin produce both IgE and hemagglutinating antibodies. Thus, the rutin moiety is implicated as exerting a regulatory effect on isotype expression by suppressing the production of serum antibodies of isotypes other than IgE. The immunization procedure employed (which involves an initial injection of 100 μg of antigen in phosphate-buffered saline, followed, at monthly intervals, by two intradermal injections of 100 μg of antigen precipitated on alum) apparently fails to stimulate the normal "down-regulation" of the IgE response so that a persisting high-titered response is obtained.**

The regulatory mechanisms involved in isotype expression in general, and in the IgE response in particular, are poorly understood. Genetic factors appear to influence the tendency to produce IgE antibodies (1–5). Antigens whose response is independent of helper T-cell activity, "T-independent antigens," generally do not elicit IgE antibodies (6, 7), whereas parasite infestation (8–10) and certain adjuvants seem to preferentially stimulate an IgE response (11–13). Animal models of the persisting IgE response that is seen in allergic humans have been achieved through various experimental manipulations that probably inactivate suppressor T cells (14–17).

It has been reported (18, 19) that one-third of normal human subjects give immediate, presumably IgE-mediated, wheal and flare skin reactions to tobacco glycoprotein (TGP), a glycoprotein containing rutin (R)-like polyphenol groups, which is purified from cured tobacco leaves. A similar incidence of positive skin tests was observed in smokers and nonsmokers. In addition, when neonate rabbits are immunized with TGP their anti-TGP response is restricted to the IgE class (20). Because of this unusual class preference in the response to TGP and because of its possible importance in the pathogenesis of pulmonary and cardiovascular diseases in human smokers we have studied the response of mice to TGP. We report here that the repeated intradermal injection of TGP into mice elicits a long-lasting specific IgE response but elicits little or no hemagglutinating (HA) antibodies. The hypothesis that the presence of R moieties on the antigen molecule depresses HA antibody production without effecting the IgE response was tested by measuring the IgE and HA antibody responses of mice immunized with bovine serum albumin (hereafter referred to as albumin) or with a R–albumin conjugate. It was observed that mice immunized with R–albumin produce IgE antibodies to albumin as do mice immunized with albumin, but the mice immunized with R–albumin produce significantly lower titers of HA antibodies to albumin than do mice immunized with albumin. In addition, an immunization protocol is described that elicits a long-lasting, high-titered response of IgE and HA antibodies.

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MATERIALS AND METHODS

Animals. Eleven-week-old male and female LAF_1 mice were obtained from The Jackson Laboratory. Sprague–Dawley male rats, 400–450 g, were obtained from Charles River Breeding Laboratories.

Antigens and Antibodies. TGP was isolated from cured tobacco leaves as described (21). Albumin was obtained from Sigma. R–albumin and R rabbit immunoglobulin (R–RIg) were prepared as described (22). Rabbit anti-TGP serum and rabbit anti-R–albumin serum were prepared as described (18, 22).

HA Assay. The HA assay used was previously described (22). Sera were heat-inactivated (56°C for 30 min) prior to assay.

Comparison of R–Albumin and Albumin by Immunoelectrophoretic and HA Inhibition Assays. The molecular weights of albumin and of R–albumin were estimated by $NaDodSO_4$/polyacrylamide gel electrophoresis (23) and were found to be M_rs 67,000 and 125,000, respectively. Therefore, it can be concluded that ≈95 mol of R (M_r 610) were coupled to 1 mol of albumin.

Immunoelectrophoresis was used to determine if R–albumin was bound by rabbit antibodies to albumin. Albumin and R–albumin at 1 mg/ml were placed in wells and were electrophoresed in 1.5% agarose containing barbital HCl buffer (pH 8.6; ionic strength = 0.05). Bromphenol blue was added as a tracing dye. After electrophoresis rabbit antiserum to albumin was added to the trough. Precipitation arcs of similar density were obtained with both antigens. The distances migrated from the origin by the proteins and the tracking dye were measured, in the case of the proteins from the midpoint of their precipitin arcs, and R_f values were calculated. The R_f value for R–albumin was

Abbreviations: ELISA, enzyme-linked immunoadsorbent assay; HA, hemagglutination or hemagglutinating; HRBC, human erythrocytes; P_i/NaCl, phosphate-buffered saline; PCA, passive cutaneous anaphylaxis; R, rutin; R–albumin, R conjugated to bovine serum albumin; R–RIg, R conjugated to rabbit immunoglobulin; TGP, tobacco glycoprotein.