IMMUNOMODULATION OF OVALBUMIN-SPECIFIC IgG AND OTHER CLASSES OF ANTIBODY RESPONSE BY HONEY IN MICE

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ABSTRACT

It was reported earlier that intraperitoneal administration of honey had immunosuppressive activity on elicitation of allergen-specific murine antibody response as evaluated by passive cutaneous anaphylaxis and double immunodiffusion methods. In this study, the immunomodulatory effect of honey is evaluated by enzyme linked immunosorbent assay (ELISA) using ovalbumin as model allergen. It was found that ovalbumin (OVA)-specific IgG antibody responses elicited with various doses of OVA were significantly suppressed by rock bee honey ($p < 0.01$). Honey was also found to have inhibited the production of OVA-specific IgM, IgA, IgG₁, and IgG_{2b}, whereas that of IgG_{2a} and IgG₃ were not affected. Furthermore, honey also suppressed the OVA-specific total IgG antibody response in various inbred mice with different genetic background. In addition, the suppressive activity of honey was examined in different groups of mice by injecting honey at different time intervals, before and after immunization with OVA. The anti-OVA IgG antibody response was suppressed significantly when honey was injected 12 hours prior/ latter to OVA injection. These results confirm the suppressive activity of honey on antibody response and suggest possible clinical application.

KEYWORDS : honey, immunosuppression, humoral antibody response, ELISA

INTRODUCTION

The regulation of either humoral antibody response and/or cell mediated immune response, by using external agents such as the natural products or their derivatives has been generating lot of interest in clinical research. Several immunomodulatory compounds have been identified from natural sources and some of them have been used either in upregulation or downregulation of the immune response (1,2). Immunosuppressive agents have been mainly used in control of the immune response in conditions like transplantation, autoimmune disorders and in alleviation of allergic diseases. In addition to the widely used immunosuppressants like cyclosporin A, rapamycin and FK 506, several natural and derivative

pharmaceuticals have been evaluated for their effects on antibody response. For example, glucocorticoids like dexamethasone alter the levels of IgA and IgG antibodies in serum (3). Deoxyspergualin, a synthetic analogue of the antitumor antibiotic, spergualin produced by *Bacillus laterosporus* inhibits the primary and anamnestic responses, particularly IgG and IgM antibody response in mice (4). Immunomodulator, LZ8, prevents the production of antibodies in mice (5). The immunosuppressant 3-(2-ethylphenyl)-5-(3-methoxyphenyl)-1H-1,2,4-triazole (DL111-IT) exhibited a significant downregulation of antibody response (6) and a synthetic muramyl peptide derivative, SDZ 280.636 inhibited the induction of polyclonal IgE response without affecting the levels of other immunoglobulin classes (6). Sulfonamide reactive metabolites suppressed the antibody production by human cells stimulated with pokeweed mitogen (7). A naphthalene derivative, TEI-6472 could preferentially suppress IgE response both *in vitro* and *in vivo* (9).

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The oral administration of Hochu-ekki-to, a traditional Chinese medicine has shown to suppress IgE antibody response without influencing other isotypic antibodies, IgG₁ and IgG_{2a} (10).

Honey has been reported to possess antibacterial (11), antiseptic (12) and preservative (13) properties, in addition to its traditional attribution of enhancing resistance. In our laboratory, honey has been examined for its possible immunomodulatory activity and it was found that the murine anti-OVA IgE and total antibody responses were completely suppressed by intraperitoneal administration of honey as evaluated by passive cutaneous anaphylaxis and Ouchterlony double immunodiffusion methods (14). This effect was further confirmed by inhibition of murine T cell proliferation stimulated by antigens and mitogens (communicated). In this study, the effect of honey on induction of OVA-specific IgG subclasses and other classes of murine antibody responses was evaluated by enzyme linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Chemicals and reagents

Ovalbumin (OVA) used for inducing anti-OVA antibody response in mice was obtained from Sigma Chemical Co., USA. Rock bee honey (*Apis dorsata*) was obtained from Andhra Pradesh Girijan Cooperative Corporation, Visakhapatnam.

Animals

Eight weeks old BALB/c (H-2^d), C57 BL/6 (H-2^b) and SWR/J (H-2^d) were obtained from National Center for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad. Four mice were used in each group for determination of antibody response.

Immunization protocol

Different groups of mice were intraperitoneally injected with 10 µg OVA or OVA plus 100 µl honey on days 0, 21 and 35 and were bled on days 14, 28 and 42. The antisera was separated and used for estimation

of individual immunoglobulin classes/IgG subclasses. The suppressive effect of honey on OVA-specific IgG antibody response was further studied by administering honey at different time intervals (0, 2h, 6h, 12h, 24h) prior to and after immunization with OVA.

Determination of immunoglobulin levels by ELISA

Total IgG and IgG subclasses, IgM and IgA levels in the sera from both control and test groups of mice were assayed by ELISA. Briefly, the 96-well microtiter plates (Nunc, Denmark) were coated with 100 µl of OVA at a concentration of 100 ng/ml in carbonate buffer (pH 9.6) and incubated overnight at 4°C. After incubation, the wells were washed thrice with PBS containing 0.05% Tween-20 (PBS-T). 3% skimmed milk powder (Anikspray, Hindusthan Lever Limited, India) in PBS was used to block the nonspecific binding sites for 10-12 hr at room temperature. After washing the plates, the wells were further incubated with 100 µl of diluted sera (1:400 in PBS) in triplicates for one hr at 37°C. The unbound serum constituents were washed off and the levels of total bound IgG were measured by incubating with 100 µl of horseradish peroxidase (HRPO) conjugates of goat antimouse IgG (Sigma Chemical Co., USA) at a dilution of 1:1000 for one hr at 37°C. Finally, the unbound conjugates were washed with PBS-T and 100 µl of freshly prepared substrate solution [10 ml of 1.5 M citrate phosphate buffer, pH 5.0 containing 4.0 mg of orthophenylene diamine (OPD) and 10 µl H₂O₂] was added per well. The reaction was stopped after 5 min. by adding 50 µl of 8 N H₂SO₄. The color developed was read at 490 nm using automatic ELISA reader (BioRad model-550). The data expressed was the mean of the optical density (OD) of the triplicates after subtracting the absorbance for the preimmune sera.

For determination of IgM and IgA classes and IgG subclass antibody responses, the antigen coated wells were incubated successively for one hr each at 37°C with mice antisera at fixed dilution (1:200 in PBS) and subsequently with diluted goat antimouse immunoglobulins against IgM, IgA, IgG₁, IgG_{2a}, IgG_{2b} and IgG₃ (1:1000 in PBS) were added individually for one hr. at 37°C. Then diluted HRPO-conjugates of

antigoat-IgG (gift from NII, New Delhi) (1:1000 in PBS) was added and incubated for one hr at 37°C. Thorough washings with PBS-T were done after each incubation step. Finally, the substrate solution was added and the color developed was measured as described above.

RESULTS

Effect of honey on the induction of OVA-specific IgG subclasses and other classes of antibody responses.

In order to examine the possible immunomodulatory activity of honey, the antibody response was induced in mice by intraperitoneal injection of different doses of OVA (0.1, 1.0, 10, 100 µg) in absence or presence of honey. As seen in Fig. 1, 100 µl honey was found to exhibit a significant suppression of anti-OVA IgG antibody response against different doses of OVA tested. For further studies, the moderate dose 10 µg OVA has been chosen since it induce a significant suppression of anti-OVA IgG levels.

The effect of honey was examined on the

Fig. 1. Effect of honey on dose-dependent OVA-specific secondary IgG antibody response. BALB/c mice were injected with different doses i.e. 0.1, 1.0, 10, 100 µg of OVA alone or OVA plus 100 µl honey on days 0, 21 and 35. Mice were bled on days 14, 28 and 42, sera separated and used to measure the total IgG antibody responses by ELISA (*p<0.01).

induction of OVA-specific primary, secondary and tertiary IgG antibody responses and it was found that

Fig. 2. Downregulatory effect of antigen-specific total IgG antibody response. BALB/c mice were immunized with 10 µg OVA alone or OVA plus 100 µl honey on day 0, second and third booster doses were given on days 21 and 35 respectively. The mice were bled on days 14, 28 and 42. The separated sera was used for measurement of the total IgG antibody responses by ELISA (*p<0.01).

Fig. 3. Downregulatory effect of honey on OVA-specific IgG antibody response in different inbred mice. BALB/c (H-2d), C57BL/6 (H-2b) and SWR/J (H-2a) haplotypes of mice were injected with 10 µg OVA alone or OVA plus 100 µl honey on days 0 and 21 and were bled on day 28. Sera was separated and used to measure the total IgG antibody responses by ELISA (*p<0.01).

Fig 4. Immunomodulatory effect of honey on OVA-specific antibody class/IgG subclass response. BALB/c mice were injected with 10 µg OVA alone or OVA plus 100 µl honey on days 0 and 21 and were bled on day 28. Sera was separated and used to measure the antibody response by ELISA (* $p < 0.01$).

they are significantly suppressed by honey (Fig.2). Dose effect of honey was studied on the induction of OVA-specific IgG antibody response by administering honey (50, 100, 150 and 200 µl) and OVA (10 µg) simultaneously. Complete suppression was observed with all the doses of honey tested and hence 100 µl dose was used in further studies (data not shown). The results shown in Fig. 3, indicate that a significant suppressive effect was observed in different haplotypes of mice namely BALB/c (H-2^a), C57BL/6 (H-2^b) and SWR/J (H-2^a). Furthermore, the results in Fig. 4 demonstrate the influence of honey on induction of OVA-specific IgM, IgA classes and IgG subclasses of antibody response. Very significant suppression of IgG₁ and a moderate suppression of IgA, IgM and IgG_{2b} antibody responses ($p < 0.01$) was observed whereas IgG_{2a} and IgG₃ isotype levels were unaffected.

The kinetic effect of honey administration on OVA-specific IgG antibody responses

To find whether honey could exert its suppressive effect when injected separately from OVA, different groups of mice were administered with 100 µl honey at different time intervals viz. 24, 12, 6, 2, hr before and 2, 6, 12 and 24 hr after immunization with OVA. Similarly, the second immunization was carried out on day 21 and the mice were bled on day 28. The results in Fig. 5 show that the administration of honey

Fig 5. The kinetic effect of honey administration to that of OVA on IgG antibody response. Different groups of mice were treated with honey at different time intervals (in hours) prior to and after immunization with OVA on days 0 and 21. The sera collected on day 28 was evaluated for IgG antibody responses (* $p < 0.01$).

within 12 hours before or after OVA injection significantly ($p < 0.01$) suppresses the anti-OVA IgG antibody response whereas in groups that received honey beyond 12 hours before or after OVA immunization there was only partial suppression of the antibody response.

DISCUSSION

In India, honey has been traditionally associated with Ayurveda and Unani systems of medicine. Most of the Ayurvedic drugs are taken orally along with honey. The reason could be attributed to its sweetness, known or unknown medicinal properties. Previously, honey has been shown to have antibacterial (11), antiseptic (12) and preservative (13) properties. Earlier from our laboratory, it has been reported that honey could suppress the murine antibody response as evaluated by passive cutaneous anaphylaxis and Ouchterlony double immunodiffusion methods (14).

In this study, it is found that the OVA-specific IgG antibody responses against different doses of OVA i.e. 0.1, 1.0, 10.0 and 100 µg were suppressed by the simultaneous intraperitoneal administration of honey (100 µl) as measured by ELISA, indicating the effect is independent of the allergen doses tested (Fig. 1). Furthermore, the suppressive effect is found to be

consistent in primary, secondary and tertiary responses (Fig.2) as well as in different inbred strains of mice namely, H-2^d, H-2^b and H-2^k (Fig. 3). As seen in Fig. 4 the IgA and IgM classes of antibodies responses were also found to be inhibited significantly by honey ($p < 0.01$). The suppressive influence of honey on IgG, IgA and IgM class antibody responses, is in accordance with that of some immunosuppressive agents like dexamethasone (3) and 15-deoxyspergualin (4). It was also observed that the IgG₁ isotype which is otherwise predominant amongst the IgG subclasses was significantly suppressed by honey. However, honey did not show its suppressive effect on IgG_{2a} and IgG₃ antibody responses. These results are in contrast to the effect of the chinese medicine Hochu-ekki-to which suppresses IgE antibody response without affecting IgG₁ and IgG_{2a} or IgG_{2b} levels (10) and also that of dexamethasone that selectively inhibits the IgE and IgA but did not influence IgG and IgG₁ levels (15). Furthermore, as shown in

Fig. 5, honey was found to have inhibited the IgG antibody response significantly when administered within 12 hr prior to and after immunization while honey injected beyond 12 hr. before or after OVA immunization, showed no significant inhibition.

In addition, the suppressive effect of honey was also observed on ongoing IgG antibody response (data not shown). From these results it could be concluded that honey induces suppression of antiovalbumin antibody responses and therefore might prove useful as an antiallergic agent after further investigations. Presently, we are purifying the immunosuppressive principle(s) from honey.

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