オバルブミン感作したBALB／cマウスに対するγ－アミノ酪酸（GABA）の血清IgEレベル抑制作用

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Influence of γ-aminobutyric acid (GABA) on IgE Production in Ovalbumin-immunized BALB/c Mice

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Summary

γ-aminobutyric acid (GABA) is a kind of amino acid and has been received much attention for their biological activities. In the present study, we examined the influence of GABA on immunomodulatory activities and serum IgE levels, employing ovalbumin (OVA)-immunized BALB/c mice. In cytokine production assay in vitro, GABA showed increased IFN-γ production concomitant with decreased IL-4 production by splenocytes from OVA-immunized BALB/c mice. When administrated orally, GABA was effective for decreasing both total and OVA-specific IgE levels in serum. In addition, these decreased IgE levels by administrated GABA were paralleled with increased IFN-γ production and decreased IL-4 production in splenocytes ex vivo. It seems that GABA can potentially suppress Th2 responses including IL-4 production via promoting Th1-skewed response in vivo, leading to down-regulation of IgE synthesis. These results suggest that GABA might be useful for preventing IgE-mediated allergic diseases.

Key words : γ-aminobutyric acid (GABA), IgE, IL-4, IFN-γ, Th1/Th2

Many allergic diseases associated with immediate hypersensitivity are closely related to elevated production of serum immunoglobulin E (IgE). It is well known that Type-I allergic reactions are triggered by mast cell degranulation subsequent to antigen cross-linking of IgE molecules that are bound to high affinity IgE receptor FceRI on the cell surface. IgE is produced by plasma B cells, which is mainly regulated by type 2 helper T cells (Th2) via producing interleukin (IL)-4. IL-4 induces IgE isotype class switching (Coffman et al. 1986) and itself promotes Th2 differentiation. It is realized that Th2-biased state is participate in clinical condition of allergic diseases (Romagnani 1994, Cross et al. 2004). On the contrary, Th1 produce IFN-γ that plays the opposite role to IL-4, and itself promotes Th1 differentiation. Both Th1 and Th2 subsets are produced from naïve T cells, a non-committed population of precursor T cells. Th1 and Th2 can cross-inhibit each other. The Th1/Th2 balance is considered to be critical for IgE production. Therefore, agents with an ability to induce Th1-biased state, whereby Th2 responses are weakened, are expected to prevent IgE-mediated allergic deceases. Recently, it has been reported that several dietary ingredients including lactic acid bacteria (Matsuzaki et al. 1998, Shida et al. 2002), saccharides (Nagura et al. 2002), polyphenols (Yano et al. 2002) and saponins (Katayama et al. 2006) are effective for lowering serum IgE levels through modulating Th1/Th2 balance.

γ-amino butyric acid (GABA), which is synthesized by glutamic acid decarboxylase from glutamic acid, is the major inhibitory neurotransmitter on the mammalian central nervous system. GABA is also occurred in various kinds of foods including fermented food products. Administrated GABA has been shown to exert some biological activity including anti-hypertensive (Hayakawa et al. 2002, Matsubara et al. 2002). However, almost no studies dealing with the immunomodulatory properties of GABA have been reported.

In this study, we investigated the effect of GABA on immunomodulatory activities to induce IFN-γ and IL-4 production by cultured splenocytes from ovalbumin (OVA)-immunized BALB/c mice. We further examined the effect of orally administrated GABA on total and antigen-specific IgE levels in serum and ex vivo cytokine production levels in splenocytes.

MATERIALS AND METHODS

Mice

Female BALB/c mice at 5 to 6 weeks of age were purchased from Charles River Japan (Yokohama, Japan) and were maintained conventionally in plastic cages at about 22 °C under a 12-h light-dark cycle. The mice were provided with a standard CRF-1 diet (Oriental Yeast, Tokyo, Japan) and allowed ad libitum access to autoclaved water throughout the experimental period. This experiment was carried out according to the guidelines laid out by The Ethical Committee for Animal Experiments of Niigata University.

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In Vitro Cytokine Production Assay

To obtain ovalbumin-sensitized splenocytes, female BALB/c mice at 6 weeks of age were intraperitoneally immunized on day 0, 14 and 28 with 100 μg ovalbumin (OVA; Wako, Osaka, Japan) and 1 mg of Al(OH)₃ gel (Wako). After checking that the OVA-specific IgE level was rising, mice were sacrificed and their spleens were obtained at day 35 to 42. Single splenocyte suspensions were prepared by crushing and pressing the organs through a nylon mesh and removing red blood cells with lysis buffer followed by washing twice with phosphate buffered saline (PBS). Splenocytes were suspended at 2 x 10⁶ cells/mL in RPMI-1640 (Sigma-Aldrich, St. Louis, MO) containing 10 % (vol/vol) heat-inactivated fetal bovine serum (FBS; Roche, Mannheim, Germany), 100 U/mL penicillin and 100 μg/mL streptomycin and, in the presence or absence of GABA (Wako), cultivated with 100 μg/mL OVA using 96-well flat-bottomed culture plates (Nunc, Roskilde, Denmark) in a humidified atmosphere of 5 % CO₂ at 37 °C. Following cultivation for 7 days, culture supernatants were collected to measure the amount of cytokines by ELISA.

Ex Vivo Cytokine Production Assay

Splenocytes obtained from mice given GABA orally were seeded at 2 x 10⁶ cells/mL in 96-well flat-bottomed culture plates and cultivated with 100 μg/mL OVA in RPMI-1640 containing 10 % (vol/vol) heat-inactivated FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, under a humidified atmosphere of 5 % CO₂ at 37 °C. Following cultivation for 7 days, culture supernatants were collected to measure the amount of IgE and cytokines by ELISA.

ELISA

The concentration of IFN-γ and IL-4 were measured using murine Opt EIA ELISA set (BD Biosciences, San Diego, CA) in accordance with the instructions from the manufacture. For determination of total IgE, sandwich ELISA was employed using anti-mouse IgE antibody (LO-ME-2) (ZYMED, San Francisco, CA) as a primary antibody and biotinylated anti-mouse IgE antibody (LO-ME-3) (Acris, Hiddenhausen, Germany) as a secondary antibody in combination with streptavidin-horseradish peroxidase (HRP) conjugate. OVA-specific IgE levels were also assessed by almost same sandwich ELISA system using OVA for coating the ELISA plates instead of primary antibody (LO-ME-2). As HRP substrate, 3,3',5,5'-tetramethylbenzidine (Sigma-Aldrich) were used and absorbance at 450 nm was measured using a microplate reader (Model 680, BIO-RAD, Hercules, CA).

Statistical Analysis

Data are represented as the mean ± standard deviation (SD). Statistical analyses were performed by using Student’s t-test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Effect of GABA on in vitro production of IFN-γ and IL-4 by splenocytes

To evaluate the potential of GABA to induce IFN-γ production and reduce IL-4 production, splenocytes from OVA-immunized BALB/c mice were cultivated with OVA in the presence of GABA. The amounts of IFN-γ and IL-4 in supernatants of splenocytes cultures were shown in Fig. 2. After cultivation without GABA for 7 days, the amounts of IFN-γ and IL-4 were 4.57 ± 0.23 ng/mL and 844 ± 20 pg/mL respectively. These two cytokines in supernatants without OVA were below the limits of detection (data not shown). At a concentration of 1 to 100 μg/mL, GABA caused the increase in the amount of IFN-γ with the concomitant decrease in the amount of IL-4 (Fig. 2a), with a significantly higher IFN-γ/IL-4 ratio compared to control (P < 0.05) (Fig. 2b). Cell viability during the culture period was evaluated by employing MTT assay and no decreased percentages of cell viability were observed in this culture condition with 100 μM
Effect of orally administrated GABA on serum IgE levels

To evaluate whether GABA has the ability to lower serum IgE levels, GABA was administered orally to OVA-immunized mice at 0.5 and 1 mg/day/mouse, and the sera were collected every 7 days during the experiment. The concentrations of total IgE and the levels of OVA-specific IgE in serum were shown in Fig. 3. OVA-immunization without administration of GABA resulted in gradual elevation of both total and OVA-specific IgE levels in serum during the experimental period. On day 35, oral administration of GABA suppressed the elevation of total IgE level in serum, with statistical significance at 1 mg/day/mouse (P < 0.05) (Fig. 3a). Administered GABA was also effective for decreasing OVA-specific IgE level in serum with significance at 1 mg/day/mouse (P < 0.05) (Fig. 3b). The suppressive efficacy of GABA was observed in a dose-dependent fashion. Non-immunized mice showed almost no changes in total and OVA-specific IgE levels during the experimental period (data not shown).
Ex vivo cytokine production by splenocytes from mice given GABA orally

To evaluate the immunomodulatory activities of GABA in vivo, antigen-induced IFN-γ and IL-4 production of splenocytes from mice administrated GABA orally were measured. After confirming decreased levels of total and OVA-specific IgE in serum by GABA administration on day 35, splenocytes were collected from the mice and stimulated with OVA in culture. Fig. 4 shows the concentration of IFN-γ and IL-4 in culture supernatants of splenocytes. Levels of IFN-γ were significantly higher in splenocytes from GABA-administrated groups than those in control group (P < 0.05) (Fig. 4a). Conversely, levels of IL-4 were lower in splenocytes from GABA-administrated groups than those in control group, with statistical significance at 1 mg/day/mouse (P < 0.05) (Fig. 4b). Both IFN-γ and IL-4 in supernatants without OVA were below the limits of detection (data not shown). In accordance with in vitro cytokine production assay described above, the IFN-γ/IL-4 ratio was significantly higher in the GABA-administrated groups compared to that in the control group (P < 0.05) (Fig. 4c).

DISCUSSION

Currently, it is generally accepted that homeostasis between Th1 and Th2 activity is critical for immune regulation. While Th2 is believed to emphasize protection against extracellular pathogens, the Th2 pathway is seen as underlying allergy and IgE-based diseases (Parris 2003). Type-1 allergic symptoms are generally caused by antigen cross-linking of IgE on mast cells. It is well known that the Th2 cytokines such as IL-4 and IL-5 are essentially associated with IgE production and IgE-mediated allergy because of their actions to help recruit B cells, mast cells and eosinophils involved in allergic inflammatory reaction (Djukanovic et al. 1990, Leung et al. 1995, Hamelmann and Gelfand 1999), while the Th1 cytokine, IFN-γ, has an inhibitory effect on IgE production (Pene et al. 1988) and Th2 differentiation (Gajewski et al. 1988). The balance between Th1 and Th2 cytokines is considered to be critical for IgE production. Since the crucial role of Th2-dominated immune responses in the pathogenesis of allergic diseases is well established, therapeutic interventions which suppress Th2 responses by inducing Th1-polarization might be effective in prevention and treatment of allergic diseases (Durham et al. 1998, Bohle 2002).

This study focused on the suppressive effect of GABA on IgE production through its immunomodulatory activity on Th1/Th2 balance. It now seems that Th1 and Th2 responses are heavily related to IFN-γ or IL-4, respectively. At first, GABA was examined with respect to their ability to modulate in vitro production of IFN-γ and IL-4 by splenocytes from OVA-immunized BALB/c mice, in order to elucidate its potential to induce Th1-skewed response. After 7 days...
cultivation, GABA showed increased IFN-γ production concomitant with decreased IL-4 production by splenocytes in response to OVA (Fig. 2). Next, we evaluated the effect of oral administration of GABA on serum IgE levels in vivo. In this trial using OVA-immunized BALB/c mice, GABA was effective for decreasing both total and OVA-specific IgE levels in serum (Fig. 3). In parallel with the suppression of serum IgE levels, orally administered GABA showed increased IFN-γ production concomitant with decreased IL-4 production by splenocytes upon stimulation with OVA in culture (Fig. 4). This suppression of serum IgE levels could be explained by the down-regulation of IL-4 production since serum IgE levels remained to be elucidated at cellular and molecular levels.

Although the mechanisms by which GABA suppresses serum IgE levels remain to be elucidated at cellular and molecular levels, the amino acid is expected as a dietary ingredient with a potential to modulate immune responses. This is the first demonstration that orally administered GABA can lower serum IgE levels. Recently, GABA showed increased IFN-γ production and suppression of IgE production.

Further examination concerning anti-allergic effects of GABA on symptoms in Nc/Nga mice which are accepted as a suitable model for human atopic dermatitis, are now on going in our research group.

REFERENCES


オバルブミン感作した BALB/c マウスに対する γ-アミノ酪酸（GABA）の血清 IgE レベル抑制作用

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要 約

γ-アミノ酪酸（GABA）は、食べ物に含まれる機能性成分として注目されている。本研究では、オバルブミン（OVA）感作した BALB/c マウスに対する GABA の免疫制御活性について調べ、経口投与による血清 IgE レベルの抑制作用について検討した。

in vitro 培養実験において、GABA はマウス脾臓細胞のインターフェロン-γ（IFN-γ）の産生を促進すると同時にインターロキン-4（IL-4）の産生を抑制した。GABA を経口投与した結果、OVA 感作 BALB/c マウスにおける総 IgE 及び OVA 特異的 IgE の血清レベルが有意に低下した。さらに、ex vivo 培養実験において、GABA を経口投与したマウスの脾臓細胞では OVA 誘導性的 IFN-γ 産生促進と IL-4 産生抑制が認められた。GABA は、Th1 の誘導を介して IL-4 産生を始めとした Th2応答を抑制し、IgE 産生を抑制する可能性がある。以上の結果から、GABA は IgE が関与するアレルギー疾患の予防に有用であることが示唆される。

キーワード：γ-アミノ酪酸（GABA）、IgE、ヒスタミン、IL-4、IFN-γ、Th1/Th2

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