EFFECT OF VITAMIN E ON IgE ANTIBODY FORMATION IN MICE

NAOKI INAGAKI, HIROICHI NAGAI and AKIhide KODA

Department of Pharmacology Gifu College of Pharmacy, 6-1, Mitahora-higashi 5 chome, Gifu, 502, Japan

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Effect of vitamin E (α-tocopheryl acetate and α-tocopheryl nicotinate) on IgE antibody formation in mice was investigated. Female BALB/c mice were immunized with dinitrophenylated ascaris protein (DNPA-As) and aluminium hydroxide gel (alum). Supplementation of vitamin E in diets or oral administration of vitamin E mixed with sesame oil resulted in a suppression of IgE antibody formation. On the contrary to IgE antibody formation, IgM or IgG (hemagglutinin; HA) formation was significantly enhanced. These results indicate that vitamin E is capable of suppressing IgE antibody formation and enhancing non-IgE antibody formation.

Keywords — vitamin E; α-tocopheryl acetate; α-tocopheryl nicotinate; IgE antibody; hemagglutinin; antibody formation

INTRODUCTION

Vitamin E is well known as a fertility factor and a powerful antioxidant. Therefore, it shows various biological activities such as inhibition of lipid peroxidation, normalization of microcirculation, stabilization of biological membranes, increase of resistance against hypoxia and inhibition of human platelet aggregation.

As for the effect on immunological system, the enhancement of humoral immune response and protection against micro-organism infection were reported. According to Tengerdy et al., vitamin E enhances the humoral immune response mainly due to increasing the number of antibody forming cells but not increasing antibody secretion. In addition, they have pointed out that IgG antibody response is more sensitive than IgM antibody response. These evidences were confirmed by Tanaka et al.

Contrary to these investigations, the effect of vitamin E on IgE antibody formation has not yet been reported. It is, therefore, interesting to investigate the effect of vitamin E on IgE antibody formation.

MATERIALS AND METHODS

Animals — Female BALB/c mice (4 or 8 weeks old) and male Wistar rats (8 weeks old) were purchased from Charles River Japan, Inc., Kanagawa, Japan and Shizuoka Laboratory Animal Center, Shizuoka, Japan, respectively.

Treatment with Vitamin E — Vitamin E supplemented diets and a vitamin E deficient diet were kindly donated by Dr. M. Torisu, Division of Clinical Immunology, Department of First Surgery, Kyushu University School of Medicine. One kg of vitamin E supplemented diet contains 20 or 200 mg of α-tocopheryl acetate (EA) or 226 mg of α-tocopheryl nicotinate (EN). Two hundred mg of EA is equivalent to 226 mg of EN. The animals were divided into 5 groups. Three groups were given three different kinds of vitamin E supplemented food from 4 weeks before the 1st immunization to the end of experiment. One group was given a vitamin E deficient food for the same period as above. The last group was given normal diet (CE-2, containing 41 mg of vitamin E per kg of food, Clea Japan, Inc., Tokyo).

In another experiment, EA and EN (Eisai Pharmaceutical Corp., Tokyo) were adminis-
Vitamin E and IgE Antibody Formation

tered orally. EA in doses of 0.1, 1.0 and 10 mg/head, and EN in doses of 0.113, 1.13 and 11.3 mg/head were given orally to the mice every other day from the 1st immunization to the end of experiment.

**Immunization** — Dinitrophenylated ascaris protein (DNP-As), prepared from an extract from *Ascaris suum* by dinitrophenylation with sodium 2,4-dinitrobenzene sulfonate was used as an antigen.\(^{18,19}\) Protein content of the antigen measured by the micro-buret method\(^{20}\) was 30.1% and the amount of antigen employed in this experiment was expressed as the amount of protein. As an adjuvant, aluminium hydroxide gel (alum) prepared by the method of Levine and Vaz\(^{21}\) with slight modification was used.

Mice were immunized with 10 μg of DNP-As and 2 mg of alum by intraperitoneal injections. The day of the 1st immunization was appointed day 0. The 2nd and the 3rd immunizations were carried out on days 30 and 60, respectively. Blood samples were collected from tail veins of mice at 10-day intervals and 7 d after the 2nd and the 3rd immunizations. On days 30 and 60, mice were bled and then immunized.

**Evaluation of IgE Antibody** — Serum IgE antibody levels were evaluated by passive cutaneous anaphylaxis (PCA) reactions according to the technique described by Ovary.\(^{22}\) Briefly, serum sample obtained from each mouse was two-fold serially diluted with saline and injected intradermally into the shaved back of male Wistar rats in duplicate. Twenty four hours after the sensitization, PCA reaction was elicited by an intravenous injection of 1 ml of saline containing 1 mg of DNP-As and 5 mg of Evans blue dye. The resultant bluing reactions were read 30 min after the challenge. PCA titer recorded is the greatest dilution that gave skin reactions with a diameter of greater than 5 mm and presented as logarithm to the base 2.

**Evaluation of Hemagglutinin (HA)** — To investigate the levels of non-IgE antibodies in sera, passive hemagglutination test was carried out by the method of Avrameas *et al.*\(^{23}\) Briefly, serum samples were two-fold serially diluted in microtiter plates and each portion of the diluted serum (50 μl) was mixed with equal volume of DNP-As coated sheep red blood cell suspension. After incubation for 2 h at 37°C, the agglutination at the bottom of the plates were evaluated. HA titer recorded is the greatest dilution that gave complete agglutination and presented as PCA titer.

**Statistical Analysis** — Data were evaluated statistically by Student’s t-test.

**RESULTS**

**Effect of Dietary Supplemented Vitamin E on Antibody Formations**

As shown in Table I, each PCA titer of serum from groups I, III and IV was not significantly different from that of group II. In group V, PCA titer on day 10 was significantly high but it was significantly low at days 37 and 60 as compared to those of group II.

Results of passive hemagglutination tests are shown in Table II. HA titers of groups I and II were not significantly different. In group III, a significant decrease of HA titer was seen on day 20 and a significant increase was seen on day 60 as compared to those of group II. In groups IV and V, HA titers were higher than those of group II at every examined days. Statistical significance was observed at day 10 of groups IV and V and at day 60 of group IV.

**Effect of Orally Administered Vitamin E on Antibody Formations**

To confirm the effect of vitamin E on antibody formations, EA and EN were administered orally. As shown in Table III, PCA titers of sera from vitamin E administered groups were lower than that of control at day 20. On days 10 and 37, there was no significant difference of PCA titer between vitamin E administered groups and control. EA at a high dose showed more potent tendency of inhibition of IgE antibody formation than EN.

In HA titers, shown in Table IV no reduction of HA titer against control group was seen except for those of group II on day 10 and groups V and VII on day 37. Especially, in
### TABLE I. Effect of Dietary Supplemented Vitamin E on IgE Antibody Formation

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet $^b$</th>
<th>Day 10</th>
<th>Day 20</th>
<th>Day 30</th>
<th>Day 37</th>
<th>Day 50</th>
<th>Day 60</th>
<th>Day 67</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>8.0 ± 0.42</td>
<td>6.8 ± 0.40</td>
<td>6.7 ± 0.79</td>
<td>10.8 ± 0.64</td>
<td>9.5 ± 0.56</td>
<td>9.4 ± 0.47</td>
<td>11.4 ± 0.25</td>
</tr>
<tr>
<td>II</td>
<td>E deficient</td>
<td>7.4 ± 0.23</td>
<td>7.2 ± 0.31</td>
<td>6.2 ± 0.31</td>
<td>11.5 ± 0.34</td>
<td>9.9 ± 0.24</td>
<td>9.6 ± 0.24</td>
<td>11.4 ± 0.24</td>
</tr>
<tr>
<td>III</td>
<td>EA 20</td>
<td>7.3 ± 0.17</td>
<td>6.4 ± 0.35</td>
<td>5.5 ± 0.43</td>
<td>10.6 ± 0.42</td>
<td>9.6 ± 0.47</td>
<td>9.7 ± 0.25</td>
<td>11.2 ± 0.38</td>
</tr>
<tr>
<td>IV</td>
<td>EA 200</td>
<td>7.1 ± 0.44</td>
<td>6.6 ± 0.35</td>
<td>6.8 ± 0.56</td>
<td>11.0 ± 0.28</td>
<td>10.3 ± 0.38</td>
<td>10.2 ± 0.33</td>
<td>11.4 ± 0.40</td>
</tr>
<tr>
<td>V</td>
<td>EN 226</td>
<td>8.1 ± 0.31 $^c$</td>
<td>6.8 ± 0.34</td>
<td>6.6 ± 0.52</td>
<td>10.3 ± 0.21 $^d$</td>
<td>9.4 ± 0.20</td>
<td>8.7 ± 0.25 $^c$</td>
<td>11.0 ± 0.04</td>
</tr>
</tbody>
</table>

$a)$ Mean ± S.E. of 6 mice immunized with DNP-As and alum on days 0, 30 and 60.  
$b)$ Mice were fed 5 kinds of diets starting 4 weeks before the 1st immunization.  
$c)$ $p < 0.05$ against group II.  
$d)$ $p < 0.01$ against group II.

### TABLE II. Effect of Dietary Supplemented Vitamin E on Hemagglutinin Formation

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet $^b$</th>
<th>Day 10</th>
<th>Day 20</th>
<th>Day 30</th>
<th>Day 37</th>
<th>Day 50</th>
<th>Day 60</th>
<th>Day 67</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>4.5 ± 0.22</td>
<td>4.0 ± 0.26</td>
<td>5.5 ± 0.22</td>
<td>9.2 ± 0.31</td>
<td>7.0 ± 0.26</td>
<td>5.7 ± 0.21</td>
<td>8.2 ± 0.17</td>
</tr>
<tr>
<td>II</td>
<td>E deficient</td>
<td>4.3 ± 0.21</td>
<td>4.0 ± 0.26</td>
<td>5.2 ± 0.40</td>
<td>9.3 ± 0.21</td>
<td>6.8 ± 0.60</td>
<td>6.0 ± 0.26</td>
<td>7.3 ± 0.21</td>
</tr>
<tr>
<td>III</td>
<td>EA 20</td>
<td>4.7 ± 0.21</td>
<td>3.2 ± 0.17 $^d$</td>
<td>4.8 ± 0.17</td>
<td>9.3 ± 0.33</td>
<td>6.5 ± 0.50</td>
<td>7.0 ± 0.45 $^d$</td>
<td>6.8 ± 0.31</td>
</tr>
<tr>
<td>IV</td>
<td>EA 200</td>
<td>5.0 ± 0.26 $^c$</td>
<td>4.0 ± 0.00</td>
<td>5.5 ± 0.22</td>
<td>9.8 ± 0.31</td>
<td>6.8 ± 0.17</td>
<td>6.8 ± 0.37</td>
<td>7.3 ± 0.21</td>
</tr>
<tr>
<td>V</td>
<td>EN 226</td>
<td>5.2 ± 0.31 $^c$</td>
<td>4.2 ± 0.17</td>
<td>5.8 ± 0.17</td>
<td>9.5 ± 0.22</td>
<td>6.8 ± 0.40</td>
<td>7.3 ± 0.21 $^d$</td>
<td>7.7 ± 0.21</td>
</tr>
</tbody>
</table>

$a)$ Mean ± S.E. of 6 mice immunized with DNP-As and alum on days 0, 30 and 60.  
$b)$ Mice were fed 5 kinds of diets starting 4 weeks before the 1st immunization.  
$c)$ $p < 0.05$ against group II.  
$d)$ $p < 0.01$ against group II.

### TABLE III. Effect of Orally Administered Vitamin E on IgE Antibody Formation

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment $^b$</th>
<th>Day 10</th>
<th>Day 20</th>
<th>Day 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Sesame oil</td>
<td>9.3 ± 0.26</td>
<td>9.8 ± 0.32</td>
<td>11.4 ± 0.19</td>
</tr>
<tr>
<td>II</td>
<td>EA 0.1 mg</td>
<td>9.5 ± 0.38</td>
<td>9.0 ± 0.16 $^c$</td>
<td>12.0 ± 0.35</td>
</tr>
<tr>
<td>III</td>
<td>EA 1.0 mg</td>
<td>9.8 ± 0.34</td>
<td>9.4 ± 0.40</td>
<td>11.0 ± 1.04</td>
</tr>
<tr>
<td>IV</td>
<td>EA 10 mg</td>
<td>9.5 ± 0.24</td>
<td>8.6 ± 0.20 $^d$</td>
<td>11.4 ± 0.29</td>
</tr>
<tr>
<td>V</td>
<td>EN 0.113 mg</td>
<td>9.4 ± 0.33</td>
<td>9.0 ± 0.39</td>
<td>10.5 ± 0.92</td>
</tr>
<tr>
<td>VI</td>
<td>EN 1.13 mg</td>
<td>8.7 ± 0.35</td>
<td>9.2 ± 0.24</td>
<td>11.4 ± 0.86</td>
</tr>
<tr>
<td>VII</td>
<td>EN 11.3 mg</td>
<td>8.6 ± 0.41</td>
<td>9.0 ± 0.29</td>
<td>11.2 ± 1.08</td>
</tr>
</tbody>
</table>

$a)$ Mean ± S.E. of 5 mice immunized with DNP-As and alum on days 0 and 30.  
$b)$ EA and EN mixed with sesame oil were administered orally every other day from day 0.  
$c)$ $p < 0.05$ against group I.  
$d)$ $p < 0.01$ against group I.
TABLE IV.  Effect of Orally Administered Vitamin E on Hemagglutinin Formation

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment b)</th>
<th>HA titer (log₂) a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 10</td>
</tr>
<tr>
<td>I</td>
<td>Sesame oil</td>
<td>6.8 ± 0.20</td>
</tr>
<tr>
<td>II</td>
<td>EA 0.1 mg</td>
<td>6.6 ± 0.24</td>
</tr>
<tr>
<td>III</td>
<td>EA 1.0 mg</td>
<td>7.6 ± 0.24 c)</td>
</tr>
<tr>
<td>IV</td>
<td>EA 10 mg</td>
<td>7.4 ± 0.24 c)</td>
</tr>
<tr>
<td>V</td>
<td>EN 0.113 mg</td>
<td>7.2 ± 0.20</td>
</tr>
<tr>
<td>VI</td>
<td>EN 1.13 mg</td>
<td>7.8 ± 0.20 d)</td>
</tr>
<tr>
<td>VII</td>
<td>EN 11.3 mg</td>
<td>6.8 ± 0.20</td>
</tr>
</tbody>
</table>

a) Mean ± S.E. of 5 mice immunized with DNP-As and alum on days 0 and 30.
b) EA and EN mixed with sesame oil were administered orally every other day from day 0.
c) p< 0.05 against group I.
d) p< 0.01 against group I.

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DISCUSSION

It is well established that allergic disorders such as bronchial asthma and hay fever are caused by IgE antibody. Therefore, in order to cure these disorders, many attempts have been made to regulate the IgE antibody formation. One of the effective treatments is the desensitization with specific antigen. This therapy makes a decrease of serum IgE antibody level and an increase of blocking antibody level.24) These phenomena suggest that the suppression of IgE antibody formation and the enhancement of non-IgE antibody formation are good for the treatment of allergic disorders.

As shown in the present results, vitamin E displays the inhibitory effect on IgE antibody formation and the enhancing effect on non-IgE antibody formation at the same time. When the potency is compared between EA and EN, EN is slightly better than EA. However, the potency is not so strong and the effects of vitamin E are not dose-dependent. It is very frequently observed that the inhibitory or enhancing effect of drugs on IgE antibody formation does not appeared dose-dependently. In order to characterize the nature of vitamin E in IgE antibody formation, further investigation will be necessary by using many kinds of EN derivatives.

Furthermore, the mechanism on the suppression of IgE antibody formation by vitamin E is interesting. Tada and Okumura25) reported a negative feed-back mechanism of IgE antibody formation by passively administered IgG antibody. To investigate whether or not the suppression of IgE antibody formation caused by vitamin E is resulted from enhancing non-IgE antibody formation is important. The relationship between IgE and IgG antibody formations is now unclear.

Since vitamin E possesses both activities, vitamin E may be a useful tool to investigate the mechanisms of IgE antibody formation.

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