Effect of Synthetic Food Colorings on Immunoglobulin Production by Rat Lymphocytes

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The effect of 4 azo dyes and 5 non-azo dyes used for food coloring on immunoglobulin (Ig) production by rat spleen and mesenteric lymph node (MLN) lymphocytes was examined. The amounts of IgG and IgM produced by MLN lymphocytes after 72 hr cultivation were the same as those of control culture in the presence of 10⁻⁷ and 10⁻⁶ M azo dyes. On the other hand, amaranth inhibited IgE to control level at 10⁻³ M while IgE level was 1.5 times higher than control for new coccine and sunset yellow FCF. With regard to non-azo colorings, sodium Cu-chlorophyllin inhibited IgE production by both lymphocytes at the concentrations between 10⁻⁶ and 10⁻⁵ M. Among chlorophyll derivatives, Cu- and Fe-derivatives inhibited IgE production by both lymphocytes. Among Mg-derivatives, chlorophyll-b, having aldehyde group as the constituent, enhanced it by both lymphocytes. These results suggest that minerals and aldehyde in porphyllin ring have an important role in the regulation of IgE production.

INTRODUCTION

Our life style, especially dietary life style, has changed in the last four decades. It is suggested that following environmental changes has resulted in increase of allergic patients. It is reported that diesel exhaust particulates (DEP) have an adjuvant effect on IgE production (Muranaka et al., 1986; Takafuji et al., 1987, 1989; Takenaka et al., 1995). With regard to our life style, chronic exposure of rats to cigarette smoke has resulted in inhibition the antibody-forming cell response to both T cell-dependent and -independent antigens and reflect B cell dysfunction (Savage et al., 1991).

Focused on our daily food, we are surrounded by many food additives or chemicals. Though food additives are used for coloring, preservation, flavoring, and so on for our benefit, many adverse reactions have also been reported. For instance, sulfites causes asthmatic attack, Tartrazine reveals urticaria (Tarlo et al., 1993; Weber, 1993; Wüthrich, 1993), and annatto dye causes anaphylaxis (Nish et al., 1991). However, only a few of these agents are currently known to play a role in promoting allergic reactions and there was few information about the mechanism by which food additives enhance allergic reaction. Therefore, we focused on the effect of food additives on Ig production, which

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Abbreviation(s): ELISA, enzyme-linked immunosorbent assay; Ig(s), immunoglobulin(s); MLN, mesenteric lymph node.
Effect of Non-azo Food Colorings on Ig Production by Rat Spleen and MLN Lymphocytes.

We also investigated the effect of non-azo food colorings on Ig production by rat lymphocytes. The colorings used here were water-soluble annatto, brilliant blue FCF (food blue No. 1), sodium Cu-chlorophyllin, fast green FCF (food green No. 3) and indigocarmine (food blue No. 2). As shown in Fig. 3, sodium Cu-chlorophyllin inhibited IgG and IgM production at the concentrations above $10^{4} \mu M$ and strongly inhibited IgE production at the concentrations above $10^{2} \mu M$. In addition, water-soluble annatto, fast green FCF and brilliant blue FCF also inhibited IgE production at $10^{4}$ and $10^{3} \mu M$. On the other hand, water-soluble annatto, brilliant blue FCF, fast green FCF and indigocarmine showed no stimulatory or inhibitory effect on IgG and IgM production. These results
Ig regulating effect of synthetic food colorings.

Fig. 1. Effect of azo colorings on Ig produced by rat spleen lymphocytes. Spleen lymphocytes were cultured with various azo dyes at around $10^1$ to $10^3 \mu M$ for 72 hr and the culture supernatant was collected to evaluate Ig concentration.

coccine, tartrazine and sunset yellow FCF did not affect to the IgE level at the concentrations between $10^1$ and $10^3 \mu M$, while amaranth decreased IgE level to half of the control level at $10^3 \mu M$.

Then, the effect of azo dyes on Ig production by rat MLN lymphocytes was examined. Since MLN plays an important role in the gut immune system and induction of food allergy, the effect of food colorings on Ig production of MLN lymphocytes was compared with that of spleen lymphocytes which are involved in the systemic immune system. As shown in Fig. 2, these azo dyes did not affect to the level of IgG and IgM at the concentrations used here as well as spleen lymphocytes. However, IgE level was 1.5 times higher than the control level in all azo dyes. On the other hand, amaranth decreased IgE level to control level at $10^3 \mu M$. 
MLN lymphocytes was 10 times lower than that seen in spleen lymphocytes. This suggests that MLN lymphocytes are more sensitive to sodium Cu-chlorophyllin than spleen lymphocytes. On the other hand, the dyes other than sodium Cu-chlorophyllin showed no stimulatory or inhibitory effect on IgG and IgM production. However, water-soluble annatto and fast green FCF inhibited IgE production at 10 and 10^2 μM.
suggest that sodium Cu-chlorophyllin has an Ig production-inhibitory effect at the concentrations above $10^{-1}$ M and water-soluble annatto, fast green FCF and brilliant blue FCF have a selective inhibitory effect of IgE at 10 and $10^2$ M.

In the case of MLN lymphocytes, sodium Cu-chlorophyllin strongly inhibited IgE production at the concentrations above $1 \mu$M, and the production of IgG and IgM at $10 \mu$M (Fig. 4). The concentration, at which the inhibitory effect of this coloring was seen in
xanthene dyes, enhanced IgE production and inhibited IgG and IgM production by rat spleen lymphocytes (Kuramoto et al., 1997). In the present study, we examined the effect of synthetic food colorings such as 4 azo and 5 non-azo dyes on Ig production by rat lymphocytes.

In the case of azo dyes, the effect on Ig production by spleen lymphocytes was negligible, but they exerted IgE production-enhancing activity in MLN lymphocytes. Lim et al. (1994b and 1995) reported that bile acid cultured with LPS in the presence of IL-4 or IL-5 inhibited IgE production by spleen lymphocytes, while it enhanced IgE production in the presence of lectins by MLN lymphocytes. These results suggest that the responses of spleen and MLN lymphocytes to food components are different from each other. In addition, as reported in the experiment which was made to establish the etiologic role of foodstuffs and/or food additives and the possible associated immunological alterations, the relationship between food additives (including tartrazine) and allergic symptoms were not correlated (Morales et al., 1995). Thus, the effect of azo dyes to the systemic immune system might be more modulate that to the gut immune system.

As for non-azo food colorings, the level of IgG and IgM were the same as that of control at the concentration selected in this experiment, while brilliant blue FCF and fast green FCF (categorized to triphenylmethane dyes) or sodium Cu-chlorophyllin strongly inhibited IgE produced by both spleen and MLN lymphocytes at the concentrations above 1 and 10μM. In addition, the inhibition of IgE production was induced by Cu- and Fe-chlorophyllin, but not Mg-chlorophyllin. It had been reported that the toxicity of sodium Cu-chlorophyllin was derived from free Cu²⁺ (Worden et al., 1955). Steffensen et al. (1994) reported that cell membrane of human T and B lymphocytes and monocytes were more strongly damaged by Cu²⁺ than by Pb²⁺ or Zn²⁺ by scanning electron microscopy. The inhibitory effect of Cu ion on Ig production or immune response has also been reported in guinea pig (Boroskova et al., 1993) or in human (Bumgardner et al., 1993; Mehanna et al., 1994; Smith et al., 1996). These results suggest that Cu ion plays an important role in Ig production by human and other animals. As for Fe²⁺ ion, Schwartzer et al. (1992) reported that iron liberated from malarial pigment resulted in the lipid peroxidation. Cu²⁺ have also been reported to enhance peroxidation of unsaturated fatty acids (Albertini et al., 1996). These results suggest that lipid peroxidation enhanced by Fe²⁺ or Cu²⁺ ions plays an important role on the inhibition of IgE production.

REFERENCES
Kuramoto, Y., K. Yamada, B. O. Lim, and M. Sugano 1997 Stimulating effect of xanthene dyes on
Effect of Chlorophyll Derivatives on Ig Production by Rat Spleen and MLN Lymphocytes.

Since sodium Cu-chlorophyllin showed an inhibitory effect of Ig production by both spleen and MLN lymphocytes, we investigated the effect of chlorophyll derivatives on Ig production by rat spleen and MLN lymphocytes. As chlorophyll derivatives, we selected sodium Cu-chlorophyllin, sodium Fe-chlorophyllin and two Mg-chlorophyllins (chlorophyll-a having methyl and phytyl groups and chlorophyll-b having aldehyde and phytyl groups). As shown in Fig. 5, both sodium Cu- and Fe-chlorophyllin inhibited IgE production by spleen lymphocytes at 100 μM. Among Mg-derivatives, chlorophyll-b enhanced IgE production by both lymphocytes, while chlorophyll-a had no effect on IgE production by these lymphocytes. These results suggest that minerals and aldehyde group in the porphyllin ring have an important role in the regulation of Ig production.

DISCUSSION

As a reason of the increase in allergic patients observed during the last decade in Japan, many researchers have been studied on the relationship between environmental factors and allergy incidence. Takafuji et al. (1987 and 1989) and Takenaka et al. (1995) reported that DEP had an adjuvant effect on Ig production when the mixture of DEP and ovalbumin (OVA) is injected intraperitoneally or intranasally. However, only this factor can not explain the increase in allergy patients. Thus, we also studied on the relationship between food components and allergy incidence and found that Rose Bengal, a member of


