Effect of Rose Bengal on Immunoglobulin Production by Mouse B Lymphoma, WEHI-279 Cells

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The effect of Rose Bengal (RB) on immunoglobulin (Ig) production by mouse B lymphoma WEHI-279 cells was examined. At 100 μM, RB increased IgE concentration in the culture medium but decreased the levels of IgG and IgM. To examine whether RB affects to the secretion of IgE from WEHI-279 cells or its synthesis, intracellular, and extracellular Ig levels were compared. The cytoplasmic IgE level in the cells treated with 100 μM RB was higher than in the cells cultured without RB. Reverse transcriptase-polymerase chain reaction (RT-PCR) experiment showed that the level of productive ϵ transcripts of the RB-added group was higher than that of the control but RB did not affect the germ-line ϵ transcripts. These results suggested that RB enhancement of IgE was due to the increase of the Ig synthesis by WEHI-279 cells and not to the increase of secretion activity of Ig from the cells nor to the enhancement of class-switching to IgE producing cells.

Keywords: *Rose Bengal; IgE; WEHI-279 cell; productive ϵ transcripts*

INTRODUCTION

Our lifestyle and dietary habits have changed during the last 4 decades. It is suggested that following environmental changes have resulted in increasing allergic symptoms. Especially in our daily food, we are subjected to many food additives and chemicals. Before food consumption, many kinds of pesticide are used to prevent vermin or spoilage during transportation. Food additives are used for coloring, preserving, flavoring, and so on for our benefit. But many adverse reactions are reported when ingested. For instance, sulfites can cause asthmatic attacks, Tartrazine can trigger urticaria (Tarlo and Sussman, 1993; Weber, 1993; Wüthrich, 1993), annato dye has been reported to cause anaphylaxis (Nish et al., 1991). However, only a few of these agents are currently known to play a role in promoting allergic-like reactions, little information about the mechanism of allergic reaction to the additives is available. Therefore, we focused on the effect of food additives on immunoglobulin production. Allergies to food or environmental allergens are mainly induced by the reaction classified as type I allergy, in which the induction of allergen-specific IgE plays an essential role (Metcalfe, 1991). Thus, first, we examined the physiological effect of common commercial food additives and reported that water-soluble and water-insoluble natural colorings inhibited IgE production by rat spleen lymphocytes at 10 and 1 μM, respectively (Kuramoto et al., 1996). With regard to artificial food colorings, Rose Bengal, which is one of the xanthene colorings, stimulated IgE production at 100 μM (Kuramoto et al., 1997), on the other hand Cu-chlorophyllin suppressed IgE production by rat spleen lymphocytes (Kuramoto et al., 1998). In this paper, we report the effect of Rose Bengal on Ig production by WEHI-279, mouse B lymphoma cells.

EXPERIMENTAL PROCEDURES

Reagents. Rose Bengal as a food additive and Tween 20 as the washing reagent of ELISA were purchased from Wako Pure Chemicals (Osaka, Japan). RPMI1640 medium was provided by Nissui Pharmaceutical Co. (Tokyo, Japan). Block Ace purchased from Dainippon Pharmaceutical Co. (Osaka, Japan) was used for blocking.

Mouse IgG, M antibody, goat anti-mouse IgG, and HRP-conjugated goat anti-mouse IgG were provided by Zymed Lab (San Francisco, CA), and mouse IgE was provided by Seikagaku Co. (Tokyo, Japan). Rat anti-mouse IgE was purchased from Experimental Immunology (Brussels, Belgium), and goat anti-mouse IgM and HRP-conjugated goat anti-mouse IgM were purchased from Cappel (West Chester, PA). Biotin-conjugated rat anti-mouse IgE was provided from Experimental Immunology and HRP-conjugated avidin from Dakopatts (Glostrap, Denmark).

Cells and Cell Culture. Mouse B lymphoma WEHI-279 cells were kindly donated by the Medical Institute of Bioregulation, Kyushu University (Fukuoka, Japan). They were maintained in RPMI1640 medium supplemented with 10% fetal bovine serum (GIBCO BRL, Grand Island, NY) at 37 °C in an incubator under 5% CO_2 atmosphere. The different concentrations of RB were dissolved in PBS. Then, the cells (1×10^5 cells/mL) were cultured with RB or PBS in 24-well flat-bottomed culture plates (Becton Dickinson Labware, Lincoln Park, NJ), and culture supernatants were collected for Ig measurement. To examine the cytoplasmic Ig level, cells were washed in PBS three times and homogenated (sonicated) using a Handy Sonic (model UR-20P; Tomy Seiko Co., Ltd.;

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Table 1. Primer Sequences Used in the Experiment

amplified RNA	primer sequence (position and ref)	size of amplified product
Pϵ	J_{H4}: 5′-TGGACTACTGGGGTCAAGG-3′ (no numbered; Gough et al., 1981) Cϵ2: 5′-AGCGATGAATGGAGTAGC-3′ (991-1008; Ishida et al., 1982)	365 bp
Gϵ	Iϵ: 5′-ACTAGAGATTCACAACG-3′ (771-778; Gerondakis, 1990) Cϵ2: 5′-AGCGATGAATGGAGTAGC-3′ (991-1008; Ishida et al., 1982)	423 bp
Gγ1	Iγ1: 5′-CGAGAAGCCTGAGGAATGTG-3′ (351-370; Honjo et al., 1979) Cγ1: 5′-GACAGATGATACTTCTGGGA-3′ (991-1008; Ishida et al., 1982)	418 bp
β-actin	sense: 5′-TGGAATCCTGTGGCATCCATGAAAC-3′ antisense: 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′	348 bp

Tokyo, Japan) for 10 s. The homogenate was centrifuged at 2000*g* for 25 min, and the supernatants were recovered. Ig concentration in the culture supernatants, and the cell lysates was measured by ELISA, as reported previously (Yamada et al., 1993). Data were analyzed by a one-way analysis of variance followed by Duncan's new multiple-range test to evaluate significant differences (Duncan, 1955).

RNA Purification and cDNA Preparation. WEHI-279 cells were lysed using TRIzol reagent (GIBCO BRL), and RNA was extracted according to the manufacturer's directions. Purified RNA was precipitated and washed in 75% ethanol, and then the precipitate was dissolved in H_2O. Ten-micrograms of RNA was reverse-transcribed using reverse transcriptase (United States Biochemical Inc., Cleveland, OH).

RT-PCR and Agarose Gel Electrophoresis. mRNA were amplified using RT-PCR. To amplify productive ϵ transcripts (Pϵ), we used a 5′ primer (donated by the Medical Institute of Bioregulation, Kyushu University) derived from the mouse J_{H4} exon that has 74, 89, and 79% homology with nucleotide sequences within the mouse J_{H1}, J_{H2}, and J_{H3} exons, respectively (Gough and Bernard, 1981), and a 3′ primer that was identical to an 18-bp sequence within the Cϵ_2 exon (Ishida et al., 1982). For amplification of germ-line ϵ transcripts (Gϵ), the 5′ primer (donated by the Medical Institute of Bioregulation, Kyushu University) was derived from the Iϵ exon (Gerondakis, 1990), and the 3′ primer was the same as the one used for amplification of Pϵ cDNA. For amplification of Gγ1 (Biosynthesis Inc., Louisville, TX), the 5′ primer was derived from the Iγ1 exon (Honjo et al., 1979), and the 3′ primer was from the Cγ1 exon (Ishida et al., 1982). To amplify the mRNA for β-actin, the primers used in this experiment are shown in Table 1.

RT-PCR amplification was separately performed with regard to Pϵ, Gϵ, or β-actin with a cocktail that contained 0.4 units of Taq polymerase (Fermentas Ltd., Vilnius, Lithuania), 2.5 mM each of the four deoxynucleotide triphosphates, 200 μM each primer, and 10 μg of each cDNA. A supplement of the Taq polymerase was used for the buffer and $MgCl_2$ solution for RT-PCR and added to the cocktail according to the manufacturer's directions. The temperatures and reaction periods used during amplification cycles were 94 °C for 30 s for DNA denaturation, 55 °C for 1 min for primer annealing, and 72 °C for 1 min for primer extension. The amplification of RT-PCR was performed for 35 cycles.

The PCR products were separated on a 2% agarose gel (Sawady Technology Co., Ltd., Tokyo Japan) and visualized by staining ethidium bromide (Wako Pure Chemicals, Osaka, Japan) to take electrical pictures with Kodak digital science devices (Eastman Kodak Company, Rochester, NY).

RESULTS

Expression and Production of Immunoglobulins by WEHI-279 Cells. Prior to the investigation of the RB effect on Ig production, we examine the ability of Ig production by mouse B lymphoma, WEHI-279 cells. We first examined the time course of Ig accumulation in the culture supernatant of WEHI-279 cells. As shown in Figure 1, IgE or IgG as well as IgM was detected in the supernatant. IgG and IgM concentration peaked on day 4 from the onset of culture. On the contrary, IgE concentration increased to almost maximum level in 1 day, and the level was maintained to day 8. B cells producing IgG or IgE express germ-line γ or ϵ transcripts before class-switching to IgG or IgE producing cells (Ichiki et al., 1993; Li et al., 1994). Thus, Gγ1 and Gϵ expression by WEHI-279 cells was investigated. mRNA of the cells was isolated and amplified using RT-PCR. The primer sequences used in this experiment are summarized in Table 1. As shown in Figure 3A, both Gγ1 expression and Gϵ expression by the cells were detected. These results suggested that WEHI-279 cells used in this experiments were able to switch to IgG or IgE producing cells, and part of them accomplished class-switching to IgG and IgE producers.

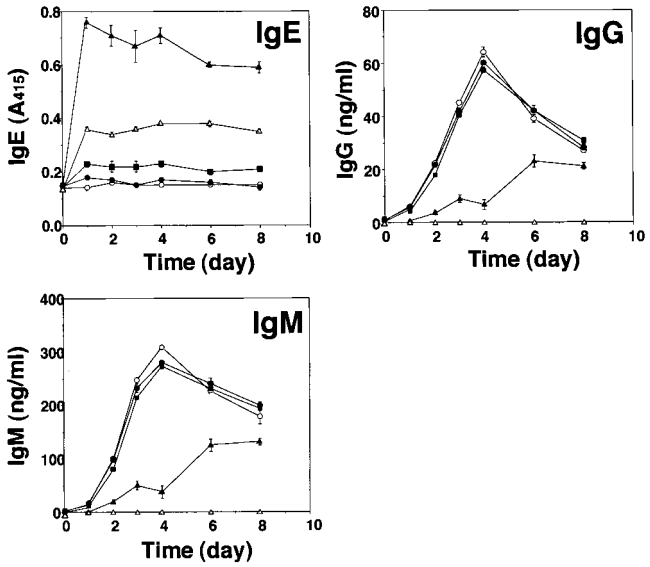

Figure 1. Time courses of Ig production by WEHI-279 cells. Cells were cultured with PBS (as vehicle of RB; ○), 1 μM RB (●), 10 μM RB (■),100 μM RB (▲), 1 mM RB (△) for various times, and the culture supernatant was collected to measure the Ig concentrations by ELISA (n = 3). Both IgG and IgM concentrations were indicated as ng/mL and IgE as optical density (A_{415}). The results indicated here were the mean ± SE.

Effect of Rose Bengal on Immunoglobulin Production by WEHI-279 Cells. We have already reported that RB increased IgE production and inhibited IgG and IgM production by rat spleen lymphocytes (Kuramoto et al., 1997). However, the mechanism of

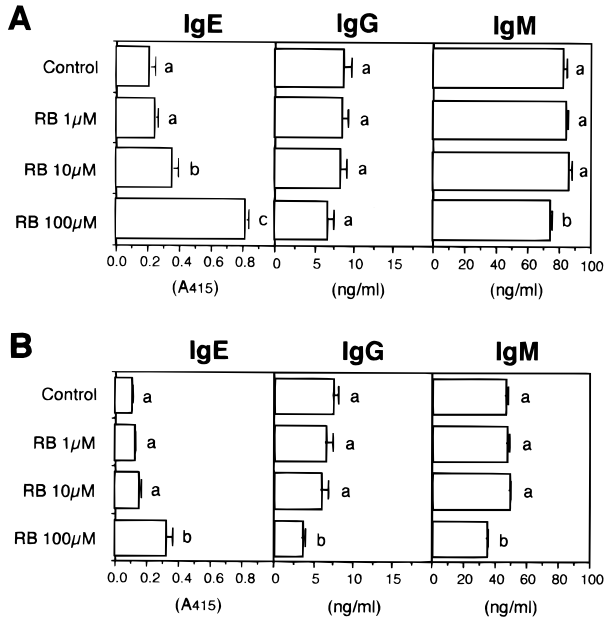

Figure 2. (A) Effect of RB on Ig production by WEHI-279 cells and (B) effect of RB on cytoplasmic Ig concentration in WEHI-279 cells. Cells were cultured for 24 h in the absence or presence of different concentrations of RB, and the Ig concentrations in the culture supernatant were determined (for result of A) or were lysed by sonication to determine the Ig concentration in the cells (for result of B) by ELISA ($n = 5$). Both IgG and IgM concentrations were indicated as ng/mL and IgE as optical density at 415 nm. Footnotes *a*−*c*: Values in the same group without a common letter are significantly different at $p < 0.05$.

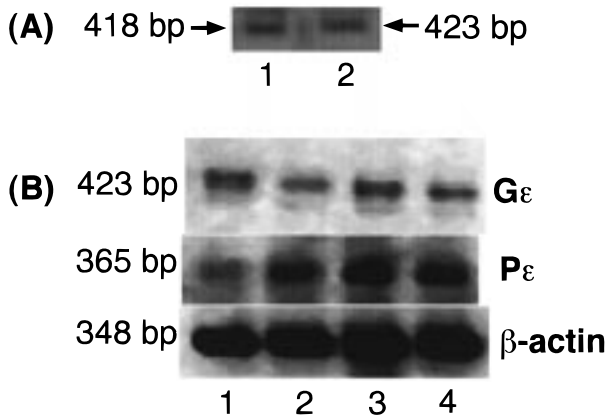

Figure 3. (A) Expression of germ-line mRNAs in WEHI-279 cells. Cells were cultured for 72 h, and each isolated mRNA from WEHI-279 cells was amplified by RT-PCR. Each lane was indicated as lane 1, germ-line $\gamma 1$ (Gγ1) mRNA; lane 2, Gϵ mRNA. (B) Effect of RB on the expression of Pϵ and Gϵ of WEHI-279 cells. Cells were cultured for 72 h in the presence or absence of RB at each concentration, and RNA was extracted from the cells to apply to RT-PCR. Lane 1: Control (PBS as a vehicle of RB); lane 2: 1 μM RB; lane 3: 10 μM RB; lane 4: 100 μM RB.

the Ig production regulating activities of RB has not been clarified. To investigate this mechanism, we used WEHI-279 and excluded the possibility of T cell commitment.

First, we examined the dose-dependent effect of RB on Ig production by WEHI-279 cells. As shown in Figure 1, Ig concentration in the culture supernatant increased to 60 ng/mL for IgG and to 300 ng/mL for IgM when cultured for 4 days with 0−10 μM RB, and then these Ig levels decreased. When cells were cultured with 100 μM RB, these Ig levels increased gradually until day 8. In both cases, the cell viability was over 90% (data not shown). However, 1 mM RB completely suppressed both IgG and IgM production by the cells, and the cell viability was almost 0%. On the other hand, IgE concentration in the culture supernatant increased to 0.8 in o.d. when cultured for 1 day with 100 μM RB, and then the level decreased gradually. When cells were cultured with 10 μM RB, the IgE level increased more gradually than that with 100 μM RB, while 0−1 μM RB did not affect the IgE level markedly.

Effect of Rose Bengal on Intracellular and Extracellular Immunoglobulin Levels. To clarify whether RB affects the production or secretion of these Igs, WEHI-279 cells were cultured for 24 h in the presence of RB, and intracellular and extracellular Ig levels were determined. WEHI-279 cells were cultured with or without various concentrations of RB for 24 h, and culture supernatant was collected to apply to ELISA. At the same time, the cells were homogenized to measure cytoplasmic Ig concentration.

As shown in Figure 2, panels A and B, RB elevated both intra- and extracellular IgE levels at 100 μM and weakly at 10 μM. On the contrary, RB did not affect either intra- or extracellular IgG levels at 1−10 μM; however, 100 μM RB suppressed intracellular IgG levels weakly. The effects were also seen in the case of both intra- and extracellular IgM levels. These results suggest that RB enhancement of IgE at 100 μM was due to the increase of the Ig production by WEHI-279 cells and not to the increase of secretion activity of Ig from the cells.

Expression of ϵ Heavy Chain mRNA from WEHI-279 Cells Cultured with Rose Bengal. To clarify the effect of RB on the transcription of the IgE gene, WEHI-279 cells were cultured for 72 h with or without RB, and total RNA was isolated from the cells to determine the IgE mRNA level. In addition to the Pϵ, the level of Gϵ, which is expressed in the course of class-switching to the IgE producer (Thyphronitis et al., 1993; Snapper et al., 1997), is also determined. The primer sequences used in this experiment are summarized in Table 1. As shown in Figure 3B, the Pϵ level of WEHI-279 cells was enhanced in the presence of 1−10 μM RB. On the other hand, Gϵ was expressed in the cells, irrespective of the presence of RB. Thus, it is suggested that RB enhances IgE production of WEHI-279 cells through the increase of Pϵ expression and not through the stimulation of class-switching to the IgE producer.

DISCUSSION

As a reason for increasing allergic symptoms, we first focused on the relationship between the environmental factor and allergy. Takafuji et al. (1987, 1989) and Takenaka et al. (1995) reported that diesel-exhaust particulates (DEP) have an adjuvant effect on Ig production when a mixture of DEP and ovalbumin (OVA) is injected intraperitoneally or intranasally. However, this factor alone cannot explain the increase of allergy; thus, we next examined the relationship between food components and allergy. We chose food additives, especially food dyes, as presumed allergy-potentiating factors. Although we previously reported that RB increased IgE production and inhibited IgG and IgM production by rat spleen lymphocytes (Kuramoto et al., 1997), the mechanism of the Ig production regulating activities of RB has not been clarified. To investigate

this mechanism, we selected WEHI-279, mouse B-lymphoma cell line and excluded the possibility of T cell commitment. Although expression of high-density membrane IgM of WEHI-279 cells was observed, but Ig production by the cells was not detected by Warner et al. (1979) and Gutman et al. (1981), we showed production of Ig by the cells. WEHI-279 cells used in this experiment constantly expressed $G\gamma 1$ or $G\epsilon$. It is suggested that the expression of these mRNAs is one of the reasons why the cell established as membrane IgM-positive lymphoma produces IgG or IgE. The WEHI-279 subline WEHI-279.1 has been used as a model to study Ig synthesis in B lymphocytes as well as their activation to terminal maturation and Ig secretion in vitro (Sibley et al., 1980, 1981; Paige et al., 1982), suggesting that during the passage of the cells the property of the cells was changed to secrete Ig.

As shown in Figure 3B, RB enhanced IgE levels and suppressed IgG and IgM levels in the culture supernatant of WEHI-279 cells. Such an Ig production regulatory effect of RB was observed in rats (Kuramoto et al., 1997), suggesting that RB directly affects B cell and enhances IgE levels. When RB was added to WEHI-279 cells at 100 μM, the level of both cytoplasmic and extracellular (e.g., secreted) IgE was enhanced. This tendency was obtained in our previous data, which indicates the enhancement of IgE production by mouse spleen lymphocytes stimulated by RB (unpublished data). In consideration of these results, it is suggested that RB directly enhances IgE synthesis because of the increase of cytoplasmic IgE levels. To confirm this hypothesis, the expression of $P\epsilon$ and $G\epsilon$ was investigated by means of RT-PCR. RT-PCR revealed that RB enhanced $P\epsilon$ expression by WEHI-279 cells but did not affect $G\epsilon$ expression. These results suggest that RB only enhances the transcription of $P\epsilon$ and induces the synthesis of IgE; however, RB does not induce class-switching because RB did not enhance the expression of $G\epsilon$, which is induced prior to class-switch recombination (Alt et al., 1982). The enhancement of $P\epsilon$, not $G\epsilon$, expression was also observed when 2C4/F3, a human Epstein−Barr virus-transformed B cell line, was cultured with DEP (especially phenanthrene, one of the polycyclic aromatic hydrocarbons) that binds to the cytoplasmic aryl hydrocarbon (Ah) receptor. The binding results in transducing signals to express $P\epsilon$ (Tsien et al., 1997). With regard to the relationship between the structure of RB and Ig production regulating effect, we examined the effect of xanthene dyes, which include RB, suggesting that the halogen atoms (especially iodine atoms) play an important role in the regulation of immunoglobulin production by xanthene dyes; however, iodine atom(s) on tyrosine derivatives did not affect IgE production by either spleen or mesenteric lymph node lymphocytes (Kuramoto et al., 1996). While phenanthrene is highly hydrophobic and is able to enter cells easily, RB also has a hydrophobic region of both iodine and chlorine atoms (Luxon and King, 1995; Forker and Luxon, 1983; Fleischer et al., 1985). Thus RB can pass through the cell membrane and may affect the Ig production system to transduce the $P\epsilon$-expressing signals in WEHI-279 cells.

ABBREVIATIONS USED

Ig(s), immunoglobulin(s); RB, Rose Bengal; HRP, horseradish peroxidase; $P\epsilon$, productive ϵ transcripts; $G\epsilon$, germ-line ϵ transcripts; $G\gamma$, germ-line γ transcripts; RT-PCR, reverse transcriptase-polymerase chain reaction; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.

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