Immunosuppressive Effects of 3,4-Dideoxyglucosone-3-ene, an Intermediate in the Maillard Reaction

Fuminori Kato,* Sadanori Mizukoshi, Yoshiyuki Aoyama, Hiroyoshi Matsuoka, Hisaki Tanaka, Kyoko Nakamura, and Masamitsu Tsukamoto

Medicinal Research Laboratory, Central Research Institute, Ishihara Sangyo Kaisha, Ltd., 2-3-1 Nishi-Shibukawa, Kusatsu, Shiga 525, Japan

3,4-Dideoxyglucosone-3-ene (DGE), an intermediate in the Maillard reaction, was found in *Sargassum kjellmanianum* Yendo, a kind of brown seaweed, which has been reported to have immunosuppressive activities. DGE was isolated from the seaweed and studied its biological activity, focusing on immune functions. As a result, DGE had marked suppressive effects on T cell proliferation, antibody production from B cells, and IL1 production from Mϕ, but not on the proliferationn of bone marrow cells, L929 fibroblastoma, and B16 melanoma. The suppressive activity seems to be selective for the immune cells. Furthermore, the treatment with DGE was effective on not only the delayed-type hypersensitivity model of mouse but also the collagen-induced mouse arthritis model. These findings suggest that DGE acts on immune responses in vivo.

Keywords: *Seaweed extract; Maillard reaction; sugar derivative; immunosuppressive effect; anti-rheumatic effect*

INTRODUCTION

The Maillard reaction is a universal natural phenomenon, and the browning reaction nonenzymatically advances in the coexistence of glucose or other reducing sugars and amino acids, proteins, or nucleic acids. Various kinds of substances are produced in various steps of this reaction (Angrick and Rewicki, 1980; Nursten, 1981). Although this chemistry has been studied mainly by food chemists for many years, it was only in the past decade that the products have been studied with physiological interest (Brownlee et al., 1984; Arai et al., 1987). A considerable amount of Maillard reaction products are contained in foodstuffs ordinarily taken (Reynolds, 1965), and also in vivo this reaction is usually advancing (Kato et al., 1970; Hayase et al., 1989). These facts have much interest bearing on the physiology and pathology of the products. Much attention has recently been paid to advanced glycosylation endoproducts (AGEs) relating to aging or chronic complications of diabetes (Brownlee et al., 1984, 1988; Monnier et al., 1984). Nevertheless, many biological studies remain to be done yet for most other products.

Here, we show the biological activities of 3,4-dideoxyglucosone-3-ene (DGE), one of the Maillard products. This compound is a known intermediate in a major pathway from Amadori compounds to (5-hydroxymethyl)furfural (HMF) or melanoidins (Figure 1) (Angrick and Rewicki, 1980; Nursten, 1981), but its biological activity is not known. We found that *Sargassum kjellmanianum* Yendo, a kind of brown seaweed, contained a considerable amount of DGE. Interestingly, the seaweed has been reported to have immunosuppressive activities (Mizukoshi et al., 1992, 1993). Herein, we isolated DGE from the seaweed and studied the biological activity, focusing on immune functions. Furthermore, we examined the possibility of medical treatment with DGE for autoimmune diseases using animal models of physiological interest.

* Author to whom correspondence should be addressed (telephone 0775-64-7533; fax 0775-64-2284).

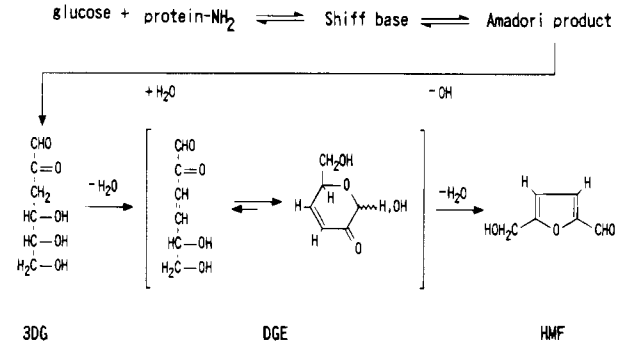

Figure 1. Maillard reaction: a major pathway from glucose to (5-hydroxymethyl)furfural (HMF) [based on the work of Nursten (1981)]. 3DG, 3-deoxyglucosone; DGE, 3,4-dideoxyglucosone-3-ene.

MATERIALS AND METHODS

Isolation of DGE. Bunches of *S. kjellmanianum* Yendo collected at the beach in Kuzaki, Mie, Japan, were frozen and stored at −40 °C until usage. The seaweed (200 g) was cut into small pieces and immersed in cold water (1600 mL) at 4 °C. After being stirred for 3 days, the water extract was filtered and lyophilized. The obtained powder (10 g) was added to methanol (500 mL), and after stirring for 30 min, the mixture was filtered again. The solution was concentrated to 10% volume (50 mL). Dichloromethane (200 mL) and ethyl acetate (250 mL) were added to the solution, which was then allowed to stand at −20 °C. After 30 min, the precipitation thereby appeared was discarded. The filtrate was evaporated to obtain a gummy extract, which was washed with the mixed solvent of dichloromethane and ethyl acetate (8:2) to remove pigments. The obtained oily substance was purified by column chromatography on silica gel.

Each fraction eluted sequentially with methanol−dichloromethane (0:100, 1:100, 2:100, 3:100) was collected and the 3:100 fraction evaporated in vacuo to yield DGE (69.5 mg) as liquid. The purity of natural DGE in the sample was more than 95%, and HMF and 3-deoxyglucosone (3DG) were not detected. The chemical structure was identified by comparison with the authentic compound chemically synthesized in accordance with previously described methods (Anet, 1960, 1962b) (Figure 2). These ^{1}H NMR data showed that naturally occurring and synthetic DGE had the same chemical structure

0021-8561/94/1442-2068$04.50/0

and existed as two isomers of hemiacetal. In our preliminary study, we confirmed that the immunosuppressive activitty of natural DGE was the same as that of synthetic DGE. To avoid complicated synthetic procedure, we used naturally occurring DGE for the following examinations.

Chemical Structure Analysis. The following spectroscopic and analytic instruments were used: ^1H and ^{13}C NMR spectra were obtained at 400 (^1H NMR) and 100 MHz (^{13}C NMR), respectively, using a JEOL GSX 400 spectrometer. Chemical shifts were expressed in δ downfield from tetramethylsilanne as an internal standard.

Animals. Male BALB/cAnN Crj, C57BL/6N Crj, and DBA/1JN Crj mice (4 weeks old) were supplied from Charles River Japan Inc. (Kanagawa, Japan). Male C3H/HeJ Jcl mice (4 weeks old) were supplied by Japan CLEA (Tokyo, Japan). After a resting period of 2–3 weeks, they were subjected to the experimental protocols.

Cell Lines. L929 mouse fibroblastoma and B16 mouse melanoma were kindly provided from Chiba University in Japan.

Culture Conditions. RPMI1640 (Flow Laboratories; No. 10-601-20) medium containing 10% of a fetal calf serum (10% FCS medium) was used for cell culture. Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2–95% air, if not mentioned otherwise.

Effects of DGE on the Mitogenic Response of Murine Thymocytes. The thymus obtained from a BALB/c mouse was teased into a single-cell suspension on a steel mesh and filtered through the mesh. The cell suspension (4×10^5 cells) was cultured with ConA (5 μg/mL, Vector Laboratories Inc., Burlingame, CA; No. L-1000) and DGE (0.03–3 μg/mL) in 0.2 mL of 10% FCS medium on a 96 well tissue plate for 52 h. The cultures were pulsed with 0.5 μCi of [*methyl*-^{3}H]thymidine (^{3}H-TdR) (specific activity, 74 Ci/mmol; Amersham International Plc, Buckinghamshire, England; TRK·758) during the final 4 h and harvested onto glass fiber paper (Flow Laboratories; No. 78-115-05). The filter paper was dried and processed for liquid scintillation counting.

Effects of DGE on IL1 Production from Murine Peritoneal Mϕ. Peritoneal cells were collected from BALB/c mouse 3 days after the injection of 1 mL of 3% thioglycolate solution (Wako Pure Chemical Industries, Osaka, Japan; No. 399-00143) into the peritoneal cavity. The cells were filtered through a steel mesh and washed with 10% FCS medium twice. After the cell suspension (5×10^5 cells) was incubated on a 48 well tissue plate for 24 h, nonadherent cells were removed by gentle rinsing. The adherent cells were cultured with LPS (Wako Pure Chemical Industries; No. 520-02051) (2 μg/mL) and DGE (0.03–3 μg/mL) for 2 h and were washed with 0.5 mL of RPMI medium twice. The stimulated cells were further cultured with 0.25 mL of 10% FCS medium for 24 h. IL1 bioactivity in the culture supernatant was measured by the mouse thymocyte comitogen assay. The thymus obtained from an endotoxin-resistant C3H/Hej mouse was used, and the cell suspension (1×10^6 cells) was cultured with 2 μg/mL PHA (Sigma, St. Louis, MO; No. L-8754) in 0.2 mL of 10% FCS medium in a 96 well tissue plate for 52 h. The cultures were pulsed with 0.5 μCi of ^{3}H-TdR during the final 4 h.

Effects of DGE on the Antibody Production from Murine Spleen Cells. The effects of DGE on the IgG, IgE, and IgM antibody production from murine spleen cells induced by LPS and recombinant murine IL4 (Genzyme Co., Cambridge, MA; No. MIL-4-C) were studied. The spleen obtained from a BALB/c mouse was teased into a single-cell suspension on a steel mesh and filtered through the mesh. The cell suspension was treated with a murine anti-thy-1 antibody (obtained from Chiba University) and a rabbit complement (Cederlane Laboratories Ltd., Ontario, Canada; No. CL3051) to remove T cells. The spleen B cells (3×10^5 cells) were cultured with LPS (10 μg/mL), murine IL4 (100 units/mL), and DGE (0.03–3 μg/mL) in 0.2 mL of 10% FCS medium on a 96 well tissue plate for 7 days. The amount of each antibody in the culture was measured by the following ELISA. First, on a microplate with 96 wells, 1 μg/mL of a rabbit anti-mouse IgG1 antibody (Organon Teknika Co., West Chester, PA; No. 36243), 1 μg/mL of a goat anti-mouse IgM antibody (Organon Teknika; No. 0611-0201), or 10 μg/mL of a rat anti-mouse IgE monoclonal antibody (Experimental Immunology Unit, Belgium; No. LO-ME-2) was coated in an amount of 50 μL/well at room temperature for 60 min, and then nonspecific bonds were blocked with a 0.1% bovine serum albumin (BSA)-containing 10 mM sodium phosphate physiological saline buffer (PBS; pH 7.2) at room temperature for 60 min. Then, the culture supernatant or its diluted solution was added in an amount of 50 μL/well and allowed to react at room temperature for 60 min. Furthermore, an alkaline phosphatase-labeled rabbit anti-mouse IgG1 antibody (Zymed Laboratories, Inc., San Francisco, CA; No. 61-0122) diluted 1000 times, an alkaline phosphatase-labeled rabbit anti-mouse IgM antibody (Zymed; No. 61-6822) diluted 2000 times, or an alkaline phosphatase-labeled goat anti-mouse IgE antibody (The Binding Site Ltd., Birmingham, England; PA-284) diluted 500 times was added in an amount of 50 μL/well and allowed to react at room temperature for 60 min. The wells were washed with PBS three times, then a 10% of diethanolamine buffer solution (pH 9.8) containing 1 mg/mL of *p*-nitrophenyl phosphate (Sigma; No. 104-105) was added in an amount of 100 μL/well and allowed to react at room temperature, and the absorbance was measured at 405 nm using a microplate reader (Bio-Rad, Richmond, CA; No. 2550). The amount of each antibody was calculated from a calibration curve of each standard antibody.

Effects of DGE on the Murine Bone Marrow Cells. The bone marrow plugs were flushed out of femurs of BALB/c mice and dissociated by repeated aspiration. The single-cell suspension passed through a steel mesh was put into a plastic Petri dish (diameter of 10 cm) and incubated. Two hours later, only floating cells were recovered and adhesion cells were discarded. The floating cells (1×10^5) were cultured with DGE (0.03–3 μg/mL) and 10% L929 culture supernatants as colony-stimulating factor in 0.2 mL of 15% FCS medium on a 96 well tissue plate for 52 h. The cultures were pulsed with 0.5 μCi of ^{3}H-TdR during the final 4 h.

L929 Culture Supernatant. L929 cells were grown as adherent monolayers in 20 mL of 10% FCS medium or plastic Petri dishes (diameter of 10 cm) and cultured for several days. The confluent cell supernatants were collected and filtered through a 0.22 μm filter. No significant difference in activity was noted after storage of the supernatants for 6 months at −80 °C.

Effects of DGE on the Growth of Mouse Tumor Cell Lines. The effect of DGE on the growth of L929 mouse fibroblastoma and B16 mouse melanoma was studied. The cell suspension (3×10^3 L929 cells or 6×10^3 B16 cells) was cultured with DGE (0.03–3 μg/mL) in 0.2 mL of 10% FCS medium on a 96 well tissue plate for 26 h. The cultures were pulsed with 0.5 μCi of ^{3}H-TdR during the final 2 h.

Delayed-Type Hypersensitivity (DTH) in Mice. Male BALB/c mice were used in groups each consisting of five mice. Each animal received intradermally at the left footpad a suspension of SRBC (5×10^5/50 μL; Shimizu Jikken Zairyo, Shizuoka, Japan). The second booster of 2×10^8/50 μL SRBC was given at the right foot-pad by the same way on day 4. The thickness of the footpad on both sides was measured on day 5, and the difference in the thickness between the right and left footpads was taken as the degree of swelling.

Collagen-Induced Arthritis (CIA) in Mice. The DBA/1J mice were divided in several groups with three or four animals per group. Each animal received at the base of the tail an emulsion of bovine type II collagen (150 μg/0.1 mL) (Collagen Gijutsu-kenshukai, Japan; No. K41) in an equal volume of complete Freund's adjuvant (ICN ImmunoBiologicals, Costa Mesa, CA; No. 64-285-1). On day 21, a second booster was conducted in the same way to induce arthritis. The degree of arthritis was evaluated under the following standards and represented by scores (from 0 to 3 points per foot, the maximum of 12 points as the total score of four feet): 0 points, no change; 1 point, detectable swelling in one or more digital joints; 2 points, mild swelling in one or more joints; 3 points, severe swelling of the entire paw and/or ankylosis.

Serum IgG anti type II collagen antibody levels were measured by the following ELISA. First, a 96 well microplate

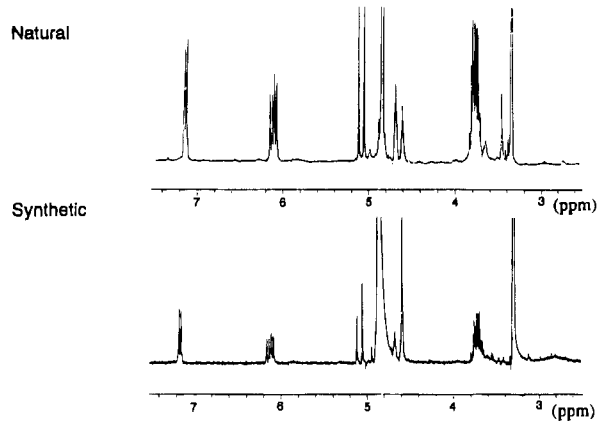

Figure 2. ^{1}H NMR (400 MHz) data for natural and synthetic DGE in CD_3OD.

was coated with 50 μL/well of a 10 μg/mL solution of bovine type II collagen at room temperature for 60 min and then blocked with a 0.1% BSA-containing PBS at room temperature for 60 min. Then the diluted serum (1:2500) was added, 50 μL/well, and allowed to react at room temperature for 60 min. Furthermore, an alkaline phosphatase-labeled rabbit anti-mouse IgG diluted 1000 times was added, 50 μL/well, and allowed to react at room temperature for 60 min. The wells were washed with PBS three times. The above-mentioned substrate buffer was added in an amount of 100 μL/well and allowed to react at room temperature. The absorbance was measured at 405 nm. The amount of each antibody was calculated from a calibration curve of the standard antibody.

Statistics. Differences between values were evaluated for statistical significance using the multiple comparison test of Williams after the test for homogeneity of variance of Bartlett.

RESULTS AND DISCUSSION

In present study, we isolated DGE from the water extract of *S. kjellmanianum* Yendo, a kind of brown seaweed. The extracted DGE existed as a two-isomer mixture of hemiacetal and had the same bioactivity and chemical characteristics as the DGE synthesized chemically (Figure 2). DGE is also known as an intermediate in a major pathway from Amadori compounds to (5-hydroxymethyl)furfural or melanoidins (Figure 1) (Angrick and Rewicki, 1980; Nursten, 1981) and to be so unstable especially in acidic conditions as to be liable to change to 5-(hydroxymethyl)-2-furaldehyde (HMF). Although its chemistry has been well studied (Angrick and Rewicki, 1980; Nursten, 1981; Anet, 1960, 1962a,b), little biological significance has been understood. Here, we show DGE having biologically interesting activities. As shown in Figures 3 and 4, DGE had marked suppressive effects on the T cell proliferation, IL1 production from Mϕ, and antibody production from B cells. The IC_{50} values on the T cells and the Mϕ were calculated to be 0.17 and 0.52 μg/mL, respectively, from the dose–response curves. The IC_{50} values on the IgG1, IgE, and IgM antibody production were calculated to be 0.52, 0.50, and 0.50 μg/mL, respectively. On the other hand, no or slight effect was observed on the proliferations of bone marrow, L929 fibroblastoma, and B16 melanoma cells. These tumor cell lines were not more resistant to a toxic agent such as adriamycin than the primary thymus cells (data not shown). These findings show that DGE suppressed more selectively the immune cells rather than the bone mallow or the tumor cells.

These immunosuppressive activities suggest that DGE acts on immune responses even in vivo. First, we

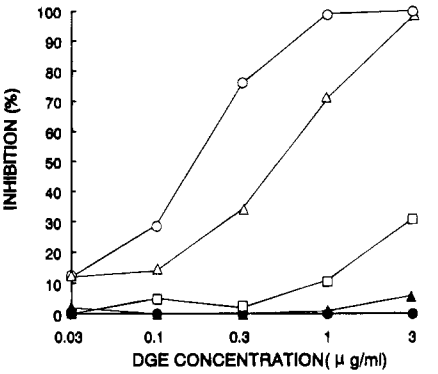

Figure 3. Effects of DGE on the cultured cells: effect on the mitogenic response of murine thymocytes (○), the IL1 production from murine peritoneal Mϕ (△), the murine bone mallow cells (□), the growth of L929 fibroblastoma (●), and the growth of B16 melanoma (▲). The values show the percent inhibition compared with the positive controls.

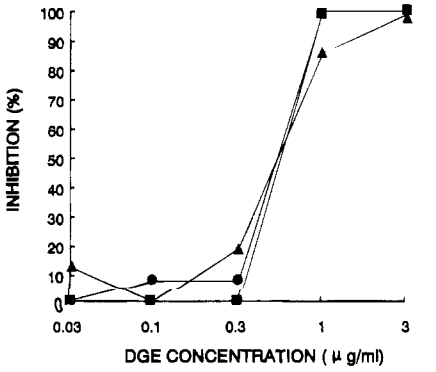

Figure 4. Effect of DGE on the antibody production from B cells. The values IgE (●), IgM (▲), and IgG1 (■) show the percent inhibition compared with the positive controls.

Table 1. Effect of Treatment with DGE on DTH in Mice

	swelling (mean ± SD),[a] mm		swelling (mean ± SD),[a] mm
positive control	0.37 ± 0.09	DGE 20 mg/kg	0.22 ± 0.09*
negative control	0.02 ± 0.02	10 mg/kg	0.33 ± 0.09
		5 mg/kg	0.37 ± 0.10

[a] Values are means ± SD of five mice. * Indicates significant difference from the positive control at $p < 0.05$.

obtained evidence supporting this view on the murine DTH model. The administration of DGE was conducted intravenously into the tail in amounts of 5, 10, or 20 mg/kg (of body weight) 30 min prior to the second booster. To the control group, physiological saline was administered. The footpad swelling was measured after 24 h. As shown in Table 1, DGE dose-dependently suppressed the swelling and there was a significant difference between the administration of 20 mg/kg and the positive control. This suggests that DGE has a suppressive activity on the cell-mediated immune response, which plays an important role in the incidence of DTH. This possibility is supported from the suppressive effects of DGE on the T cells and IL1 production from Mϕ (Figure 3).

Second, we attempted to examine DGE for the treatment of murine CIA. The development of CIA is associated with humoral and cell-mediated immune responses specific for type II collagen (Trentham et al., 1978; Wooley et al., 1981; Stuart et al., 1982). IL1 is potentially capable of triggering the various inflammatory events of murine CIA (Hom et al., 1988). The susceptibility to CIA in mice is H-2 restricted and

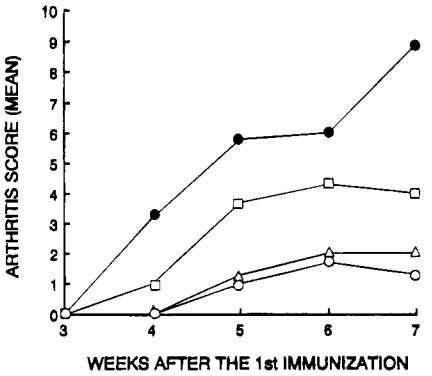

Figure 5. Effect of the treatment with DGE on the development of the CIA murine model. The DBA/1J mice were immunized with type II collagen in CFA on days 0 and 21. The drugs were administrated intraperitoneally for 4 weeks once a day, starting on the day after the second booster, in a dose of 50 (○), 10 (△), or 2 mg/kg (□). As a control, physiological saline was administered in the same way (●). The development of arthritis was followed for 7 weeks. The degree of arthritis was represented by scores.

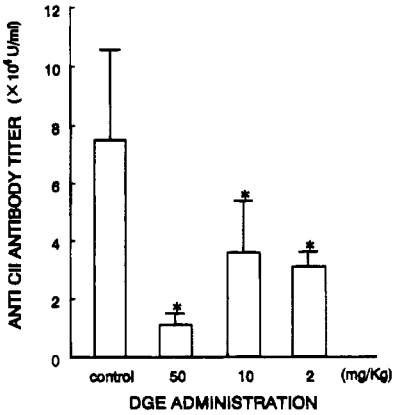

Figure 6. Effect of the treatment with DGE on the anti-collagen antibody level in serum from CIA mice. At the end of the experiment, all mice were bled from the tail and the sera were measured at a dilution of 1:2500 for IgG anti-type II collagen antibody levels with ELISA. The amount of each antibody was calculated from the calibration curve of the standard antibody and represented in terms of units per milliliter. Values are means ± SD of three or four mice. (*) and (**) indicate significant difference from the positive control at $p < 0.05$ and $p < 0.01$, respectively.

limited to the mouse strain H-2q (Wooley et al., 1981) or H-2r haplotype (Wooley et al., 1985). Histologically, the lesions of murine CIA are similar to those of human rheumatoid arthritis (RA) (Trentham et al., 1977; Courtenay et al., 1980; Terato et al., 1982). Thus, the murine CIA model has some resemblances to human RA and has been proposed to be a good model to study the immune phenomena involved in the development of the disease and to detect a new immunosuppressive drug (Phadke et al., 1985). From the suppressive effects of DGE on T, B, and Mϕ cells in vitro (Figures 3 and 4), DGE seemed to be useful for treatment of a disease caused by abnormal acceleration of the immune function such as the CIA model. The administration of DGE was repeated for 4 weeks once a day from the day of the second inoculation. The degree of swelling in the foot joints was observed every week until the final administration. As shown in Figure 5, DGE dose-dependently inhibited the progression of CIA in the range 2–50 mg/kg, and marked suppression was observed in a dose of more than 10 mg/kg. On the next day of the final administration of DGE, the blood was collected, and then the titer of anti-type II collagen antibody in serum was measured by ELISA. As shown in Figure 6, DGE significantly reduced the serum antitype II collagen antibody level in a dose of 2–50 mg/kg. These findings show that DGE suppressed the clinical inflammatory signs and also the humoral response to type II collagen. The total results containing the examinations of DTH and CIA models suggest the physiological significance of DGE suppressively acting on the immune responses such as the cell-mediated and humoral immunity even in vivo. Also, DGE may have a potential activity as an immunosuppressive drug.

There are some important findings described before this study for the physiology and pathology of Maillard reaction products. Advanced glycosylation endoproducts (AGEs) are a group of Maillard reaction products, and recently much attention has been paid to them with physiological and pathological interest (Brownlee et al., 1984; Arai et al., 1987). AGEs are implicated in many chronic complications associated with diabetes and aging (Monnier et al., 1984; Brownlee et al., 1988). Several types of human cells, e.g., monocytes/Mϕ (Gmelig-Meyling and Waldmann, 1980; Vlassara et al., 1984, 1985), endothelial cells (Esposito et al., 1989), mesangial cells (Skolnik et al., 1991), and fibroblasts (Kirstein et al., 1990a) have been shown to express membrane proteins that specifically bind AGE-modified proteins. In Mϕ, AGE protein uptake is accompanied by the release of a variety of cytokines (Vlassara et al., 1988; Kirstein et al., 1990b). Furthermore, 3-deoxyglucosone (3DG), another intermediate of the Maillard reaction, has also been well studied. In the early step of the reaction, the Amadori rearrangement compound is degraded into 3DG (Kato et al., 1987a), which can attack lysyl, arginyl, and tryptophanyl residues of proteins (Cho et al., 1984; Saito et al., 1986). 3DG has been reported to be a cross-linker responsible for the polymerization of proteins by glucose under physiological conditions (Kato et al., 1987b). 3DG was detected not only in some foodstuffs (Reynolds, 1965) but also in animal tissues such as calf and rabbit liver (Kato et al., 1970). In the formation of pyrraline, which may contribute to a pathological effect, such as carcinogenesis, 3DG has been postulated to be an intermediate (Hayase et al., 1989). As mentioned above, the physiological and pathological importance of these products has been well studied. However, much work remains to be done yet for most other Maillard products. Especially with regard to low molecular weight Maillard products, there has been no study focusing on immunological function. The present study is the first to provide new information on the physiological function. There are various kinds of substances produced in various steps of the Maillard reaction (Angrick and Rewicki, 1980; Nursten, 1981), which is generally advancing in foodstuffs and also in vivo. Therefore, more attention should be given to not only the products mentioned above but also the other Maillard products. From this viewpoint, the present study serves as a good demonstration.

The following points must be resolved. The first is to elucidate the biological significance for the seaweed including the large amount of DGE, for which we currently have little information. Interestingly, this seaweed is classified as a brown seaweed, and the brown color may derive from the advancement of the Maillard reaction in it. As all brown seaweeds examined at our

laboratory are not immunosuppressive (Mizukoshi et al., 1992, 1993), the browning reaction may moderately occur in it. The second point is to determine the mechanism of action of DGE at the biochemical level. In the chemical structure, DGE has not only an aldehyde group as a reducing sugar but also an enone structure active for conjugate addition of nucleophile such as sulfur, nitrogen, and oxygen. Therefore, DGE is liable to change to HMF, which has no effect on the immune cells (IC_{50} > 100 μg/mL), especially in acidic condition. The characteristic structure may be a key point to resolve this question. Finally, the third point is to demonstrate the existence of DGE in vivo and determine the physiological and pathological contributions, especially the influences in aging and hyperglycemia such as diabetes from the immunological viewpoint. It may be also interesting to explain the physiological relationship between DGE and AGE proteins, because AGE proteins are promotive for Mϕ to induce the release of IL1 (Vlassara et al., 1988), but on the contrary DGE is suppressive for the IL1 production from Mϕ (Figure 3).

In conclusion, DGE had suppressive effects on the cultured immune cells and the murine immune disease models of DTH and CIA. These findings show that DGE functionally acts on the immune responses and has a potential activity for an immunosuppressive drug. Studies on modified compounds for a new rheumatic medicine are underway. Although there are several points to be resolved, the importance of further investigations is emphasized.

LITERATURE CITED

Anet, E. F. L. J. Degradation of carbohydrates. *Aust. J. Chem.* **1960**, *13*, 396–403.

Anet, E. F. L. J. Degradation of carbohydrates. II. Formation of cis-unsaturated hexosones. *Aust. J. Chem.* **1962a**, *16*, 270–277.

Anet, E. F. L. J. Degradation of carbohydrates. III. Unsaturated hexosones. *Aust. J. Chem.* **1962b**, *15*, 503–509.

Angrick, M.; Rewicki, D. The Maillard-reaction. *Chem. Unserer Zeit.* **1980**, *5*, 149–157.

Arai, K.; Maguchi, S.; Fujii, S.; Ishibashi, H.; Oikawa, K.; Taniguchi, N. Glycation and inactivation of human Cu-Zn-superoxide dismutase. Identification of the in vitro glycated sites. *J. Biol. Chem.* **1987**, *262*, 16969–16972.

Brownlee, M.; Vlassara, H.; Cerami, A. Nonenzymatic glycosylation and the pathogenesis of diabetic complications. *Ann. Intern. Med.* **1984**, *101*, 527–537.

Brownlee, M.; Cerami, A.; Vlassara, H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N. Engl. J. Med.* **1988**, *318*, 1315–1321.

Cho, R. K.; Okitani, A.; Kato, H. Chemical properties and polymerizing ability of the lysozyme monomer isolated after storage with glucose. *Agric. Biol. Chem.* **1984**, *48*, 3081–3089.

Courtenay, J. S.; Dallman, M. J.; Dayan, A. D.; Marten, A.; Mosedale, B. Immunization against heterologous typeII collagen-induced arthritis in mice. *Nature* **1980**, *283*, 666–668.

Esposito, C.; Gerlach, H.; Brett, J.; Stern, D.; Vlassara, H. Endothelial receptor-mediated binding of glucose-modified albumin is associated with increased monolayer permeability and modulation of cell surface coagulant properties. *J. Exp. Med.* **1989**, *170*, 1387–1407.

Gmelig-Meyling, F.; Waldmann, T. A. Separation of human blood monocytes and lymphocytes on a continuous Percoll-gradient. *J. Immunol. Methods* **1980**, *33*, 1–9.

Hayase, F.; Nagaraj, R. H.; Miyata, S.; Njoroge, F. G.; Monnier, V. M. Aging of proteins: Immunological detection of a glucose-derived pyrrole formed during Maillard reaction in vivo. *J. Biol. Chem.* **1989**, *263*, 3758–3764.

Hom, J. T. H.; Bendele, A. M.; Carson, D. G. In vitro administration with IL-1 accelerates the development of collagen-induced arthritis in mice. *J. Immunol.* **1988**, *141*, 834–841.

Kato, H.; Tsusaka, N.; Fujimaki, M. Isolation and identification of α-Ketoaldehydes in calf and rabbit livers. *Agric. Biol. Chem.* **1970**, *34*, 1541–1548.

Kato, H.; Cho, R. K.; Okitani, A.; Hayase, F. Responsibility of 3-deoxyglucosone for the glucose-induced polymerization of proteins. *Agric. Biol. Chem.* **1987a**, *51*, 683–689.

Kato, H.; Shin, D. B.; Hayase, F. 3-Deoxyglucosone crosslinks proteins under physiological conditions. *Agric. Biol. Chem.* **1987b**, *51*, 2009–2011.

Kirstein, M.; Deventer, S.; Vlassara, H. Advanced glycosylation endoproduct (AGE) binding to its specific receptor stimulates increase in EGF and EGF receptor mRNAs: role in tissue remodeling. *J. Cell. Biochem.* **1990a**, *14E* (Suppl.), 0224.

Kirstein, M.; Brett, J.; Radoff, S.; Ogawa, S.; Stern, D.; Vlassara, H. Advanced protein glycosylation induces selective transendothelial human monocyte chemotaxis and secretion of PDGF: Role in vascular disease of diabetes and aging. *Proc. Natl. Acad. Sci. U.S.A.* **1990b**, *87*, 9010–9014.

Mizukoshi, S.; Matsuoka, H.; Kato, F.; Noda, H. Search for bioactive substances from marine algae. *Bull. Fac. Bioresour., Mie Univ.* **1992**, *No. 8*, 27–34.

Mizukoshi, S.; Mastuoka, H.; Nakamura, K.; Kato, F. Effect of seaweed preparations on murine immunocytes. *J. Appl. Phycol.* **1993**, *5*, 629–637.

Monnier, V. M.; Kohn, R. R.; Cerami, A. Accelerated age-related browning of human collagen in diabetes mellitus. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 583–587.

Nursten, H. E. Recent developments in studies of the Maillard reaction. *Food Chem.* **1981**, *6*, 263–277.

Phadke, K.; Fouts, R. L.; Parrish, J. E.; Butler, L. D. Evaluation of the effects of various anti-arthritis drugs on type II collagen-induced mouse arthritis model. *Immunopharmacology* **1985**, *10*, 51–60.

Reynolds, T. M. Chemistry of nonenzymic browning. *Adv. Food Res.* **1965**, *14*, 167–283.

Saito, G.; Okitani, A.; Hayase, F.; Kato, H. Characterization of tryptophan derivative from the reaction of Na -acetyltryptophan with carbonyl compounds. *Agric. Biol. Chem.* **1986**, *50*, 2315–2323.

Skolnik, E. Y.; Yang, Z.; Makita, Z.; Radoff, S.; Kirstein, M.; Vlassara, H. Human and rat mesangial cell receptors for glucose-modified proteins: potential role in kidney tissue remodelling and diabetic nephropathy. *J. Exp. Med.* **1991**, *174*, 931–939.

Stuart, J. M.; Townes, A. S.; Kang, A. H. Nature and specificity of the immune response to collagen in type II collagen-induced arthritis in mice. *J. Clin. Invest.* **1982**, *69*, 673–683.

Terato, K.; Hashida, R.; Miyamoro, K.; Morimoto, T.; Kata, Y.; Kobayashi, S.; Tajima, T.; Otake, S.; Hori, H.; Nagai, Y. Histological, immunological, and biochemical studies on type II collagen-induced arthritis in rats. *Biomed. Res.* **1982**, *3*, 495–505.

Trentham, D. E.; Townes, A. S.; Kang, A. H. Autoimmunity to type II collagen: an experimental model of arthritis. *J. Exp. Med.* **1977**, *146*, 857–868.

Trentham, D. E.; Townes, A. S.; Kang, A. H. Humoral and cellular sensitivity to collagen in type II collagen-induced arthritis in rats. *J. Clin. Invest.* **1978**, *61*, 89–96.

Vlassara, H.; Brownlee, M.; Cerami, A. Accumulation of diabetic rat peripheral nerve myelin by macrophages increases with the presence of advanced glycosylation endproducts. *J. Exp. Med.* **1984**, *160*, 197–207.

Vlassara, H.; Brownlee, M.; Cerami, A. High-affinity receptor-mediated uptake and degradation of glucose-modified proteins: a potential mechanism for the removal of senescent macromolecules. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5588–5592.

Vlassara, H.; Brownlee, M.; Manogue, K. R.; Dinarello, C. A.; Pasagian, A. Cachectin/TNF and IL-1 induced by glucose-modified proteins: Role in normal tissue remodeling. *Science* **1988**, *240*, 1546–1548.

Wooley, P. H.; Luthra, H. S.; Stuart, J. M.; David, C. S. Type II collagen-induced arthritis in mice. 1. Major histocompatibility complex (I region) linkage and antibody correlates. *J. Exp. Med.* **1981**, *154*, 688–700.

Wooley, P. H.; Harvinder, S. L.; Griffiths, M. M.; Stuart, J. M.; Huse, A.; David, C. S. TypeII collagen-induced arthritis in mice. IV. Variation in immunogenetic reguration provides evidence for multiple arthritogenic epitopes on the collagen molecule. *J. Immunol.* **1985**, *135*, 2443–2451.

Received for review January 4, 1994. Revised manuscript received June 9, 1994. Accepted June 29, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1994.