

Adjuvant Effects of Sugar Cane Extracts (SCE) in Chickens

Moshira EL-ABASY^{1,2}, Maki MOTOBU², Toshiya SAMESHIMA³, Kenji KOGE⁴, Takashi ONODERA¹ and Yoshikazu HIROTA²*

¹Department of Molecular Immunology, Graduate School of Agricultural and Life Science, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, ²Departments of Production Diseases and ³Safety Research, National Institute of Animal Health, National Agricultural Research Organization, 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856 and ⁴Chigasaki Laboratory, Shin Mitsui Sugar Co., Ltd., 1-2-14 Honson, Chigasaki, Kanagawa 253-0042, Japan

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ABSTRACT. The effects of sugar cane extracts (SCE) on immune responses in chickens were studied. Two- or 10-month-old chickens orally administered SCE (500 mg/kg/day), for 3 consecutive days before immunized with sheep red blood cells, *Brucella abortus* and *Salmonella* Enteritidis organisms, showed significantly increased and prolonged antibody responses to these antigens, compared to control chickens without SCE. Furthermore, chickens orally administered SCE also revealed enhanced delayed type hypersensitivity responses to human gamma globulin. These results indicated that SCE has immunostimulating and adjuvant effects in chickens.

KEY WORDS: adjuvant effect, immunostimulation, sugar cane extract.

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Most of adjuvants are substances derived from mineral oil, aluminum, bacterial components, plants, host factors or synthetic products that can augment the immune responses to several antigens. Recent advances in molecular biology resulted in the production of many recombinant proteins and synthetic peptides that are candidate vaccine antigens and many traditional adjuvants have low immunogenicity and undesirable side effects such as local inflammations, granulomatous reactions, pyrogenesis and other potential risks [11]. Consequently, the development of novel adjuvants has gained wide interest to overcome hyporesponsiveness to particular antigens or to enhance the responses in immunocompromised animals. Recently, it is of importance to establish a novel production system of economically important food animals to produce the safe food of high quality. We already reported the immunostimulating and growth-promoting effects of sugar cane extract (SCE) as a native immunostimulant and/or immunomodulator in activation of antibody and cell-mediated immune responses in chickens. Therefore, the possibilities of the use of SCE as a natural adjuvant in augmenting the immune responses and in the prolongation of the reactivity to the antigens have been studied on the basis of the following two experiments; (1) effects of SCE on antibody responses to sheep red blood cells (SRBC) and *Brucella abortus* (BA) and delayed type hypersensitivity (DTH) responses to human gamma globulin (H γ G) and (2) adjuvant effects of SCE on immune response to *Salmonella* Enteritidis (SE) in chickens.

SCE prepared by Shin Mitsui Sugar Co., Ltd., Japan, as described previously [1], was used. The original concentration of SCE (10 mg/ml) was prepared in phosphate buffered saline. SCE was administered into the crop of 2-month- and

10-month-old inbred chickens (MHC; H.B15) at the dose of 500 mg/kg/day for 3 consecutive days which were referred to as SCE (3). Control chickens were administered 0.5 ml saline into the crop. After SCE or saline administration each chicken was injected intravenously with 0.1 ml of mixed antigens of SRBC (5×10^8 cells) and heat-inactivated BA (1×10^9 cells) [7]. Blood samples were collected 7 days after each immunization and serum agglutination titers against both antigens were determined as described previously [4]. The sera were also treated with 0.2 M 2-mercaptoethanol to evaluate 2-ME resistant titers. Agglutinin titers were expressed as the mean log₂ of the reciprocal of the highest dilution giving 50% agglutination [4]. Subsequently to evaluate the effects of SCE on DTH responses, chickens were sensitized intramuscularly with 1 ml of H γ G (400 μ g/ml) (Sigma, MO, U.S.A.) emulsified with complete Freund's adjuvant one week after the second immunization. The unsensitized control chickens were injected intramuscularly with 1 ml saline. Two weeks later, all chickens were challenged intradermally in the right wattle with 0.1 ml of saline solution of H γ G (400 μ g/ml) and the left wattle with 0.1 ml of saline [10]. The thickness of the wattles was measured with a Vernier caliper at 24, 48 and 72 hr after challenge. The net increased thickness more than 0.3 mm was considered to be positive [3]. Furthermore, 3-week-old commercial male chickens (Dekalb) and 10-month-old chicken (H.B15) orally administered SCE at the dose of 500 mg/kg/day for 3 or 6 consecutive days which were referred to as SCE (3) and SCE (6), respectively, and then injected subcutaneously with 0.5 ml of commercialized oil-emulsion formalin-inactivated *Salmonella* Enteritidis (SE) vaccine (LayermuneTM, Biomune Co., Lenexa, Kansas U.S.A.) [2]. Serum agglutinin titers against SE were weekly evaluated. The Student's *t* test was employed in the comparison of means. P values of less than 0.05 were considered to be statistically significant. All data were expressed as mean \pm

*CORRESPONDENCE TO: HIROTA, Y., Department of Production Diseases, National Institute of Animal Health, National Agricultural Research Organization, 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan.

Table 1. Antibody response to SRBC and BA in chickens orally administered SCE at the age of 2 or 10 months

Group	Age to be immunized	Immune response ^{a)}							
		SRBC				BA			
		1st response		2nd response		1st response		2nd response	
		No. of responders	Titer	No. of responders	Titer	No. of responders	Titer	No. of responders	Titer
Control	2-month-old	8/8	6.2 ± 0.8	8/8	11.0 ± 0.6	8/8	10.4 ± 0.5	8/8	9.0 ± 0.3
		8/8	(6.0 ± 0.9) [¶]	8/8	(9.5 ± 0.5)	7/8	(4.7 ± 0.6)	8/8	(6.6 ± 1.1)
SCE (3)	2-month-old	8/8	8.6 ± 0.5*	8/8	12.5 ± 0.9**	8/8	11.5 ± 0.3*	8/8	10.8 ± 0.4
		8/8	(8.2 ± 0.6)*	8/8	(11.7 ± 0.5)**	8/8	(4.7 ± 0.4)	8/8	(6.6 ± 0.3)
Control	10-month-old	5/5	8.0 ± 0.5	5/5	8.8 ± 0.3	5/5	12.0 ± 0.0	5/5	12.4 ± 0.4
		5/5	(5.6 ± 0.5)	5/5	(7.4 ± 0.6)	5/5	(7.4 ± 0.7)	5/5	(7.6 ± 0.4)
SCE (3)	10-month-old	5/5	10.0 ± 0.3*	5/5	10.4 ± 0.4**	5/5	12.0 ± 0.0	5/5	12.8 ± 0.4
		5/5	(6.8 ± 0.4)*	5/5	(8.7 ± 0.7)	5/5	(9.0 ± 0.3)*	5/5	(7.8 ± 0.5)

a) Mean ± standard error of log₂ of the reciprocal antibody titer.

* P<0.05, and ** p<0.01, when compared to each control.

¶ The parenthesis show 2-ME resistant titers.

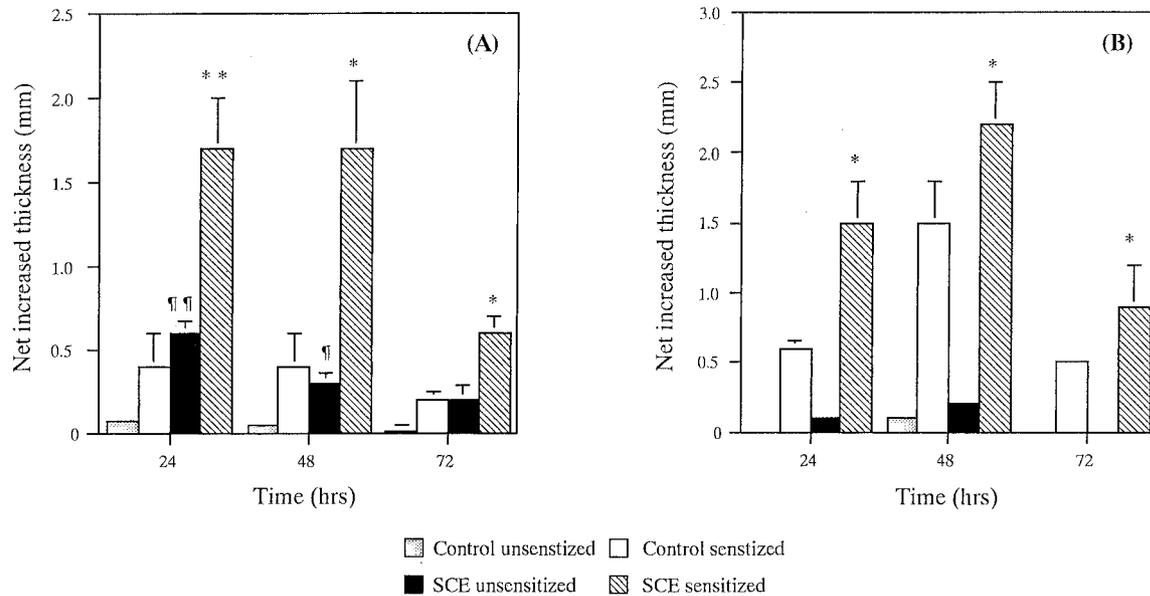


Fig. 1. DTH responses to H₇G. Chickens were orally administered SCE at the age of 2 (A) or 10 months (B). * p<0.05 and ** p<0.01, when compared to sensitized control. ¶ p<0.05 and ¶¶ p<0.01, when compared to unsensitized control.

standard error.

As shown in Table 1, chickens inoculated into the crop with SCE (500 mg/kg/day) for 3 consecutive days, SCE (3), showed significantly increased antibody responses to SRBC and BA, when compared to saline-administered control chickens. Moreover, the enhancing effects of SCE were shown in the titers of responding chickens producing 2-ME resistant antibodies to SRBC. In addition, chickens administered SCE into the crop for 3 consecutive days and sensitized intramuscularly with H₇G showed significantly higher DTH responses to H₇G than sensitized control chickens, when evaluated on the basis of the net increased wattle thickness at 24, 48 and 72 hr after challenge (Fig. 1, A; 2-

month-old and B; 10-month-old).

The adjuvant effects of SCE on antibody responses to formalin-inactivated SE vaccine are summarized in Fig. 2 (A; Dekalb and B; H.B15). SCE-administered chickens showed increased antibody titers against SE and maintained higher antibody titers than control chickens even when evaluated for 16 weeks after immunization.

The results of the present study are summarized as follows: (1) Chickens administered SCE into the crop showed significantly increased antibody responses to SRBC and BA and DTH responses to H₇G. (2) Oral administration of SCE also significantly increased antibody responses against SE and maintained higher antibody titers than those of control

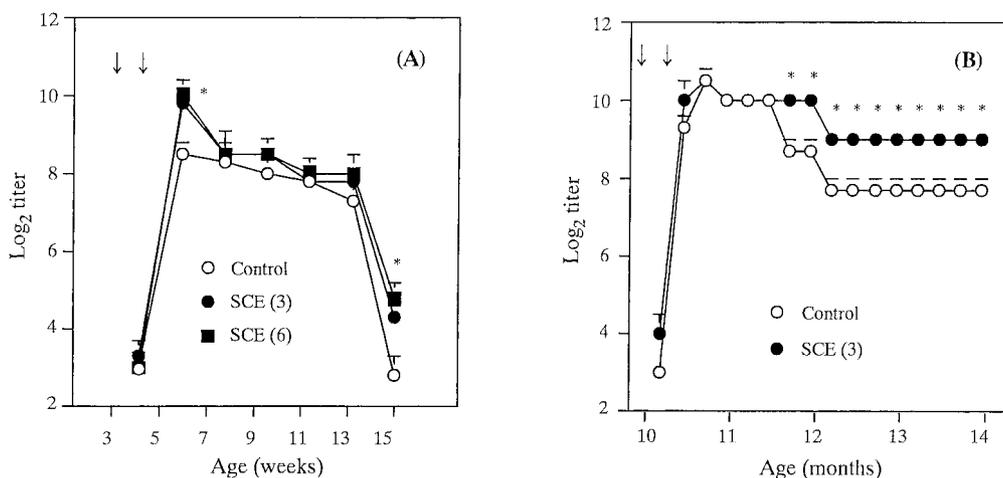


Fig. 2. Effects of SCE on antibody responses to *Salmonella* Enteritidis. Chickens were orally administered SCE for 3 or 6 consecutive days (SCE (3) and SCE (6), respectively), at the age of 3 weeks (A) or 10 months (B), and then injected subcutaneously with inactivated *Salmonella* Enteritidis vaccine (Layermune™). ↓; Immunization, and * $p < 0.05$, when compared to control.

chickens till 16 weeks after immunization, suggesting that SCE has stimulating effects on humoral and cell-mediated immune responses and also has adjuvant effects in chickens. These results also confirmed our previous findings [1] that SCE has stimulating effects on phagocytic activity, immune responses and growth in chickens. Pryce *et al.* [9] reported the enhanced immune responses by sugar cane factor and Li *et al.* [5, 6] also indicated that polysaccharide extracted from sugar cane has immunostimulating and activating effects on the classical-complement pathway in human serum by interacting with immunoglobulins. Nakamura *et al.* [8] reported the enhanced antibody response against SE in chickens by immunization with an oil-emulsion SE vaccine which showed the protective effects on challenge infection with this bacteria. Oral administration of SCE resulted in the prolongation of antibody responses to SE when evaluated for at least 16 weeks after immunization, and also an increase in DTH responses to H₇G indicating its effects on immune responses including cell-mediated immunity. The mechanism by which SCE is involved in the increasing and maintaining these responses remains to be open. Our preliminary results showed that chickens administered SCE into the crop for 3 consecutive days significantly increased the relative proportion of CD4⁺ cells in peripheral blood lymphocytes but not Bu-1a⁺ and CD8⁺ cells (data not shown), suggesting that the magnitude of immune response depends on the number and function of lymphocytes. The increase in the function and proportion of these cells may be responsible for the enhancement of the immune response.

SCE, the product of natural materials, may provide a possible promising candidate adjuvant. Further studies concerning the usefulness of this substance in practical fields are needed.

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