

## Oral probiotic protects against UV-induced immunosuppression of skin *in vivo*

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### ABSTRACT

The immunosuppressive effect of ultraviolet (UV) light is evidenced by the inhibition of contact hypersensitivity (CHS) reactions after allergen application on UV-exposed skin. Recent studies indicate that probiotics have immunomodulatory functions in skin. *Lactobacillus johnsonii* (La1, NCC533) is a probiotic that has shown potential in maintaining the skin's defense mechanisms, regulating the immune system and enhancing recovery from photo-induced damage. Our objective was to investigate the effect of dietary La1 supplementation on UV suppression of CHS response to dinitrochlorobenzene (DNCB). Caucasian males aged 20-40 years, with Fitzpatrick skin types (FST) II-III were enrolled in a double-blind randomized controlled trial. Subjects were randomized into two groups taking oral preparations of either placebo (maltodextrin) or La1 ( $1 \times 10^9$  CFU). After 8 weeks of oral intake, UV exposure to the upper buttock area was initiated on each subject, using a 1 kW solar simulator with bis81017/WG320 and UG11 filters, followed 2-3 days later by DNCB sensitization. DNCB challenge was performed 2 weeks later on the arm. Both skinfold thickness (SFT) measurement and visual evaluation of CHS (the North American Contact Dermatitis Group or NACDG scoring) were

performed. Ninety-Six men completed the study, (48 placebo, 48 La1). There was a significant difference in the CHS response between the two groups in the subjects who received 2 minimal erythema dose (MED), in favor of the La1 group, indicating the capacity of La1 to protect against UV suppression of skin immune responses. Our data suggest that the La1 oral probiotic decreased UV-induced immunosuppression of the skin.

**KEYWORDS:** probiotics, ultraviolet light, immunosuppression, contact hypersensitivity

### INTRODUCTION

Immunosuppression is one of the detrimental effects of UV exposure. A decrease in the skin's capacity to recognize and respond to antigens may allow proliferation of dysplastic cells, leading to skin cancer. Various mechanisms are known to play a role in UV immunosuppression, including DNA damage and oxidative stress that lead to alterations in gene expression, Langerhans cell (LC) depletion, changes in cytokines and soluble factors, and others [1]. Because of the complexity of the mechanisms involved, current sun protection methods, though helpful, may not be sufficient to address the problem completely. Other interventions, such as oral agents, may exert additional protection. Probiotics are living organisms which, when ingested in sufficient quantities, have beneficial health effects in humans

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beyond nutritional benefits. Aside from their well-recognized importance in the gastrointestinal system, studies indicate that probiotics exert immunomodulatory functions relevant to improving skin conditions [2]. The probiotic *Lactobacillus johnsonii* La1 (Nestlé Research Center) has been shown to maintain the number and function of LCs upon UV exposure to the skin. It has also been shown to regulate skin inflammation [3]. These facts suggest the immunoprotective potential of La1.

## METHODS

University Hospitals of Cleveland/Case Western Reserve University Institutional Review Board approved the study protocol prior to initiation of study procedures, obtaining written informed consent and data collection. This was a double-blind randomized controlled trial with two parallel groups.

### Subjects

Healthy Caucasian male volunteers between 20 and 40 years of age, with FST II-III, were recruited. Those with significant medical conditions, immunocompromised, and using photosensitive and/or phototoxic substances were excluded from study enrollment. Volunteers that followed vegetarian and vegan diets were also excluded. Subjects were required to keep a daily log monitoring for food restrictions, oral medications and sun exposure.

### MED determination

Baseline MED was calculated by exposing eight 1 cm areas of buttock skin to increasing doses of simulated solar radiation (SSR). This was delivered by a 1000-Watt xenon arc lamp, emitting ultraviolet wavelengths from 290-400 nm, closely resembling natural sunlight. Twenty-four hours later, skin erythema was clinically assessed by colorimetric measurement using a chromometer (CR-300 Minolta, Tokyo, Japan). The value of 1 MED was calculated according to The European Cosmetic, Toiletry and Perfumery Association (COLIPA) task force recommendations [4] as the dose of UV generating an increase in the redness parameter ( $\Delta a$ ) of +2.5.

### Study product

Each subject was provided with specific numbers of packets of test product in powder form and was instructed on how to reconstitute and drink the product daily for eight weeks. The active test product

consisted of  $1 \times 10^9$  CFU of La1, whereas the placebo consisted of 10 g of maltodextrin.

### SSR irradiation

Eight weeks after daily study product use, SSR (Oriel 1 kW with bis81017/WG320 and UG11 filters) was delivered to one-inch square area of buttock skin at a dose of 0.75 or 2.0 MED. This irradiation site was contralateral to the MED testing site described above. Twenty-four hours later, the irradiated skin was evaluated for erythema both visually and by colorimetry.

### DNCB sensitization

Forty-eight hours after SSR irradiation, a 0.0625% DNCB solution (30  $\mu$ g/48  $\mu$ L acetone) was applied to the irradiated buttock skin using standard patch test materials (filter paper-lined Finn chamber). This was kept in place for 48 hours.

### DNCB challenge

Two weeks after sensitization the irradiated and sensitized area of buttock skin was evaluated for erythema both visually and by colorimetry. DNCB challenge was performed on the upper inner arm contralateral to the irradiated site on the buttock. Twenty microliter solutions of 0, 3.125, 6.25, 8.75, and 12.5  $\mu$ g/20  $\mu$ L DNCB were applied using five 8-mm Finn chambers lined with filter paper. These were kept in place for 6 hours. The SFT of the five challenge sites was measured before the application of the patches and 48 hours later using a micrometer (Mitutoyo, Japan). The total increase in skin fold thickness (SFT) (in millimeters) from the five challenge sites was then determined per subject. In addition each site was given a clinical score using NACDG grading system: 1, no reaction; 2, macular erythema; 3, erythema with induration; 4, vesicular/blistering reaction. CHS was evaluated using these two parameters: SFT and NACDG score.

### Statistics

Study parameters were analyzed using a mixed-design analysis of variance model with repeated measures (SAS PROC MIXED). Threshold of significance was set at 5% in a bilateral approach.

## RESULTS

### Subject demographics

Data from 96 subjects (48 in each experimental arm) were analyzed; 6 failed screening and 20 were

withdrawn from the study. Seventy-three subjects were enrolled into the 0.75 MED UV exposure group and 23 into the 2 MED group (Table 1). There was no significant difference in the baseline MED between the groups. Both placebo and La1 were well tolerated without significant adverse events.

La1 enhances CHS response in 2 MED UV irradiation group. CHS response was assessed along with the change in the SFT and NACDG score at the five DNCB challenge sites (S1–S5) on the upper inner arm. These were measured before the application of the DNCB patches and 48 hours later. In both irradiation groups (0.75 and 2 MED) there was a significant difference in ΔSFT between S2,

S3, S4, and S5 before and after DNCB challenge tests; however there was no difference in ΔSFT between La1 and placebo groups (Tables 2 and 3). Using the NACDG grading system a CHS response was given a score of 1 for no reaction, 2 for erythema only, 3 for erythema with induration or edema or papule formation, 4 for vesicle formation and 5 for ulceration. There was a significant difference in NACDG score between sites S2, S3, S4, and S5 before and after DNCB challenge tests; however a difference between La1 and placebo was only seen in the 2 MED irradiation group (Figure 1). Among subjects who were exposed to 2 MED of UV, those who received La1 demonstrated stronger

**Table 1.** Demographic data of enrolled subjects.

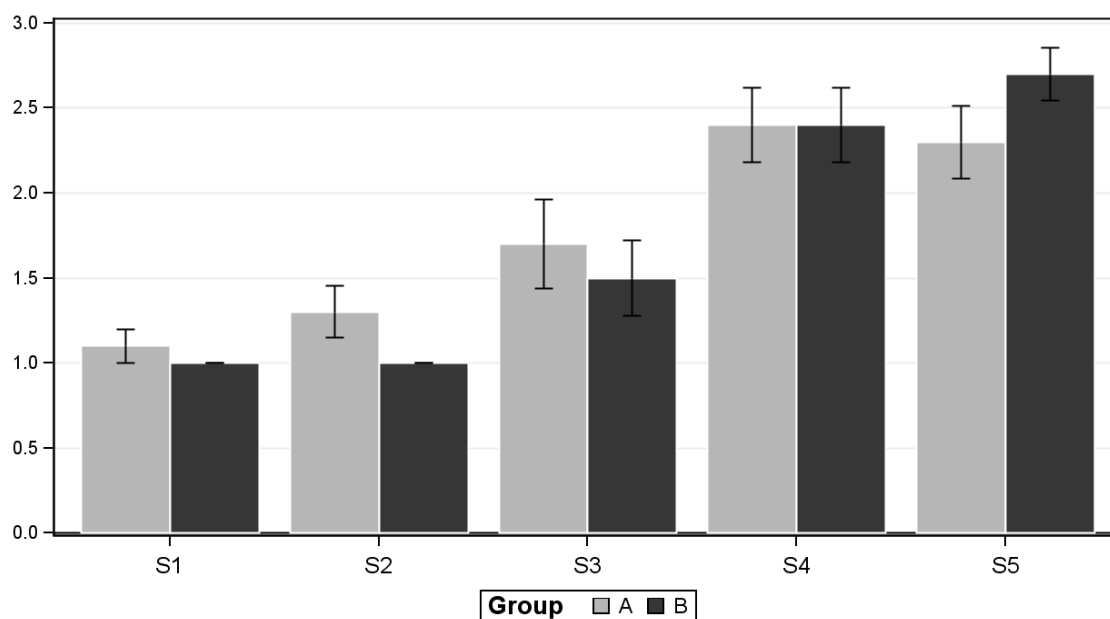
Groups	N	Age (years) avg ± SD	Height (cm) avg ± SD	Weight (Kg) avg ± SD
<b>0.75 MED group</b>				
Placebo Group (Maltodextrin)	36	25.9 ± 4.7	178.6 ± 7.5	84.7 ± 21.2
Active Group (La1)	37	27.7 ± 7.4	177.4 ± 5.9	83.0 ± 15.1
<b>2 MED group</b>				
Placebo Group (Maltodextrin)	12	21.2 ± 6.4	177.7 ± 6.7	81.5 ± 14.4
Active Group (La1)	11	21.5 ± 5.4	181.6 ± 8.7	80.0 ± 14.5

**Table 2.** Change in skin fold thickness (SFT) in 0.75 MED irradiation group.

Site	DNCB (µg/20 µL)	Placebo group			La1 group			(La1-placebo)		
		ΔSFT	Std error	p value	ΔSFT	Std error	p value	ΔSFT	Std error	p value
S1	0	-0.007	0.037	0.8505	0.040	0.036	0.2772	0.047	0.052	0.3717
S2	3.125	0.439	0.106	0.0001	0.482	0.103	<.0001	0.043	0.148	0.7723
S3	6.25	0.765	0.134	<.0001	0.776	0.131	<.0001	0.011	0.187	0.9541
S4	8.75	0.964	0.150	<.0001	0.906	0.146	<.0001	-0.058	0.210	0.7839
S5	12.5	0.989	0.155	<.0001	1.026	0.151	<.0001	0.037	0.216	0.8657

**Table 3.** Change in skin fold thickness (SFT) in 2 MED irradiation group.

Sites	DNCB (µg/20 µL)	Placebo group			La1 group			(La1-placebo)		
		ΔSFT	Std error	p value	ΔSFT	Std error	p value	ΔSFT	Std error	p value
S1	0	0.081	0.051	0.1264	0.065	0.053	0.2372	-0.016	0.073	0.8266
S2	3.125	0.113	0.040	0.0110	0.140	0.042	0.0032	0.027	0.058	0.6421
S3	6.25	0.153	0.060	0.0189	0.124	0.063	0.0616	-0.029	0.087	0.7425
S4	8.75	0.238	0.084	0.0104	0.277	0.088	0.0049	0.040	0.122	0.7476
S5	12.5	0.342	0.103	0.0034	0.326	0.108	0.0065	-0.015	0.150	0.9195



**Figure 1. Contact hypersensitivity response (CHS) in subjects who received 2 MED.**  
Group A: placebo group; Group B: probiotic treatment group.

CHS responses to DNCB than those in the placebo group ( $p = 0.04$ ).

## DISCUSSION

The CHS assay has been established for many years as a reliable method to determine the skin's immunologic status *in vivo*. In the past, the immunoprotective properties of sunscreens and other sun protection agents have been evaluated using this assay [5-7]. Although effective for the most part, people often encounter limitations such as applying an uneven or inadequate amount, skipping some areas of the skin that are difficult to reach manually, and forgetting to reapply. A systemic approach to photoprotection such as *via* dietary intake is an exciting concept as this can have longer-lasting benefits and may also improve compliance among the general population because the mode of oral intake is quite simple and easy to adapt to daily life. Previous studies have already shown that certain probiotics are not only good for the gastrointestinal tract, but also for the skin [8]. For La1 in particular, research studies have been performed showing its capacity to maintain skin homeostasis and immunologic balance *via* regulating inflammation, among other mechanisms [9, 10]. This study enrolled light-skinned male adults exclusively so as not to be

confounded by known differences in UV suppression capacity and immune reactivity secondary to inherent skin phototype and gender [11]. In our specific cohort, significant immunosuppression was achieved at twice the minimum erythema dose, but not at a sub-erythemogenic dose of 0.75 MED. This is in contrast to prior data from our group and others in which significant immunosuppression at sub-erythemogenic doses was observed in light-skinned male and female volunteers [7]. The reasons for this discrepancy remain to be tested. We speculate that this could be partially due to the normal variability encountered when studying cohorts of human subjects, or minor shifts in the spectrum of the solar simulator, specifically with the incorporation of the visible light filter (UG11) in the current study, which was not employed in our previous studies. Nevertheless, our results confirmed that La1 supplementation significantly protects the skin's immune system from UV-induced immunosuppression. This was evident in the more robust contact hypersensitivity responses to the DNCB challenge in La1-treated vs. placebo-treated subjects who were UV-exposed at a dose that is twice their MED. Maintenance of the cutaneous immune response is valuable in preventing long-term effects of UV radiation, such as tumor development, which can result when there is a lack

of appropriate immunosurveillance. Dietary La1 supplementation is an effective method of protecting the skin's immune defenses and preventing UV radiation-induced suppression of contact hypersensitivity responses.

### CONCLUSION

This study demonstrated that oral administration of probiotics could provide a method of protecting the skin from some of the detrimental effects of ultraviolet light such as cutaneous immunosuppression. Dietary supplementation should be further studied to determine its exact role as a photoprotective strategy.

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### CONFLICT OF INTEREST STATEMENT

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