Immunomodulating Effect of Neurotropin on Delayed Type Hypersensitivity Response in Mice

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Abstract—Effect of Neurotropin on the delayed type hypersensitivity (DTH) response to sheep red blood cells was examined in mice. Neurotropin suppressed the high DTH responses in ddY and BALB/c mice, while it enhanced the low DTH response in C57BL/6 mice when administered after sensitization. In addition, Neurotropin exerted a suppressive effect on the enhanced DTH response by cyclophosphamide pretreatment in C57BL/6 mice, but had a restorative effect on the suppressed DTH response in ddY mice loaded with restraint stress.

In a previous report (1), we have demonstrated that Neurotropin enhanced the DTH response to sheep red blood cells (SRBC) in low responder C57BL/6 mice through the induction of $\text{Lyt } 1^{+}2^{-}$ DTH helper T cells. To clarify the immunopharmacological action of Neurotropin more precisely, its effect on the DTH response was examined further.

Outbred ddY, inbred BALB/c and C57BL/6 male mice were purchased from Shizuoka Laboratory Animal Center, Shizuoka, Japan. Mice of 7–10 weeks of age were used for all experiments. Neurotropin, a non-proteinaceous and standardized extract containing biologically active principle(s) isolated from the inflamed dermis of rabbits inoculated with vaccinia virus, has been safely and widely used as an analgesic and antiallergic drug in Japan (2). The concentrated form of commercially available Neurotropin (10 mg dry weight/ml; Nippon Zoki Pharmaceutical Co., Osaka, Japan) was diluted with saline and administered at 10, 20 and 50 mg/kg/day. Cyclophosphamide (CP) was purchased from Nakarai Chemical Co., Ltd., Kyoto, Japan. SRBC were obtained from Nippon Bio-supp. Center, Tokyo, Japan. In stress loading experiments, ddY mice were confined in restraint cages (25 mm diameter, 90 mm length, made of steel wire net) from 5:00 PM to 9:00 AM for 2 consecutive days. DTH

responses to SRBC were measured as follows: Mice were sensitized with 1×10^7 SRBC in the left footpad and were challenged with 1×10^8 SRBC 4 days after sensitization in the right footpad. The degree of swelling compared to the uninjected footpad was measured with a dial thickness gauge at 24 hr after the challenge.

Whether Neurotropin would modulate different levels of DTH response in ddY, BALB/c and C57BL/6 mice when administered after sensitization was examined. As shown in Fig. 1, 10–50 mg/kg/day Neurotropin tended to suppress the high DTH response in ddY when administered p.o. for 4 consecutive days. Neurotropin had the same effect with a peak suppression at 20 mg/kg/day in the ddY mice even if administered i.p. for 4 consecutive days after sensitization (data not shown). Similarly, 10–50 mg/kg/day Neurotropin dose-dependently suppressed the high DTH response in BALB/c mice in the i.p. route. Conversely, 10–50 mg/kg/day Neurotropin clearly enhanced the low DTH response in C57BL/6 mice, with a peak enhancement at 20 mg/kg/day. These results indicate that Neurotropin administered after sensitization exerts suppressive effects on the high DTH responses, but has an enhancing effect on the low response in mice. However, the possibility exists that Neurotropin may have modulating

actions on DTH response only in intact mice of particular strains. In order to explore this possibility further, whether Neurotropin would modulate the experimentally altered DTH response by CP pretreatment or restraint stress loading was investigated. As shown in Table 1, Neurotropin tended to suppress the

CP-induced enhancement of DTH response in C57BL/6 mice in a dose-dependent manner, whereas it clearly enhanced the low DTH response in intact C57BL/6 mice, with a peak enhancement at 20 mg/kg/day. On the other hand, 10–20 mg/kg/day Neurotropin exerted a restorative effect on the stress-induced suppression of DTH response, while it had a suppressive effect on the high DTH response in intact ddY mice as demonstrated in Fig. 1. These results indicate that Neurotropin can modulate not only the different levels of DTH responses of genetically pre-determined nature but also the experimentally modified DTH response in mice.

Although the data were not presented, the administration of Neurotropin at any dose on the last two days of the 4 consecutive days administration had no modulating effects on the DTH responses. Furthermore, Koda et al. (2) and our group (3) have already shown that Neurotropin exerted no effect on the effector phase of DTH responses such as picryl chloride-induced contact dermatitis and cell transfer-induced local graft-versus-host reaction in mice. Therefore, Neurotropin seems to have a modulating effect on the induction phase, but not on the inflammatory process in the effector phase of the DTH responses.

Neurotropin suppressed the high DTH responses in intact ddY and BALB/c mice as well as the enhanced DTH response by CP

Fig. 1. Effect of Neurotropin on DTH response to SRBC in three strains of mice. ddY, BALB/c and C57BL/6 mice were sensitized with 1×10^7 SRBC on day 0 and challenged with 1×10^8 SRBC on day 4. Neurotropin or saline was administered p.o. into ddY mice or i.p. into BALB/c and C57BL/6 from day 0 to day 3. Each column represents the mean $\pm$ S.E. of 6–10 mice. *: $P < 0.05$, ***: $P < 0.001$ as compared to the control by Student's *t*-test.

Table 1. Modulating effect of Neurotropin on experimentally altered DTH response to SRBC in C57BL/6 and ddY mice

Group (mg/kg/day)	C57BL/6 mice		ddY mice	
	Intact	CP-treated	Intact	Stressed
Control (saline)	36.1 $\pm$ 2.6	64.0 $\pm$ 4.3†††	99.0 $\pm$ 6.8	74.4 $\pm$ 8.1†
Neurotropin 10	52.9 $\pm$ 5.0**	56.0 $\pm$ 5.7	85.5 $\pm$ 6.9	96.4 $\pm$ 5.2*
20	59.3 $\pm$ 5.1***	52.2 $\pm$ 4.3	73.0 $\pm$ 5.4**	106.8 $\pm$ 7.1**
50	56.4 $\pm$ 5.7**	51.4 $\pm$ 4.2*	77.5 $\pm$ 6.4*	81.7 $\pm$ 6.1

C57BL/6 mice (20–25 mice per group) were injected i.p. with 200 mg/kg CP on day –4. The CP-treated mice and intact mice were administered i.p. with Neurotropin or saline from day –4 to day –1. In another experiment, ddY mice (10 mice per group) were restrained for 16 hr per day for 2 days (day –1 and day 0). The restrained mice and intact mice were administered p.o. with Neurotropin or saline from day 0 to day 3. All mice were sensitized with 1×10^7 SRBC on day 0 and challenged with 1×10^8 SRBC on day 4. Each value (footpad swelling, $\times 0.01$ mm) represents the mean $\pm$ S.E. †: $P < 0.05$, †††: $P < 0.001$, as compared to the intact control, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, as compared to the corresponding control by Student's *t*-test.

administration in C57BL/6 mice. It has been demonstrated that CP pretreatment damaged the precursor for suppressor T cells (Ts) involved in the DTH response, resulting in enhancement of the DTH response in mice (4, 5). Neurotrophin has been reported to restore the Ts activity in aged NZB/NZW F₁ female mice (6). Therefore, it is suggested that Neurotrophin suppresses these DTH responses via the augmentation of Ts activity. On the other hand, Neurotrophin enhanced the low DTH responses in intact C57BL/6 mice or in ddY mice loaded with the stress when administered after sensitization. Okimura et al. (7) and Komori et al. (8) have reported that restraint stress caused suppression of immune responses in mice through the depression of helper T cell (Th) activity. We have already demonstrated that pretreatment with Neurotrophin induced Th involved in DTH response in low responder C57BL/6 mice (1). Inagaki et al. (9) have also reported that Neurotrophin administration restored the decreased number of OKT 4⁺ T lymphocytes in a hemophilia patient with acquired immune deficiency syndrome-related complex. Therefore, Neurotrophin administered after sensitization may enhance the low DTH response via the augmentation of Th activity. From all of these findings, it is suggested that Neurotrophin may directly or indirectly modulate the levels of DTH response through the regulation of number and/or activity of immunoregulatory T cells such as Th and Ts. Further studies will be necessary to examine the mechanism by which Neurotrophin modulates the DTH response at cellular levels.

References

- 1 Yoshii, H., Suehiro, S., Watanabe, K. and Yanagihara, Y.: Immunopharmacological actions of an extract isolated from inflamed skin of rabbits inoculated with vaccinia virus (Neurotrophin): Enhancing effect on delayed type hypersensitivity response through the induction of Lyt-1⁺2⁻ T cells. *Int. J. Immunopharmacol.* **9**, 443–451 (1987)
- 2 Koda, A., Nagai, H., Kurimoto, Y., Yamada, T., Mori, H., Nishiyori, T. and Inagaki, N.: Effect of Neurotrophin (NSP) on allergic reactions. *Folia Pharmacol. Japon.* **78**, 319–334 (1981) (Abs. in English)
- 3 Yanagihara, Y., Shida, T. and Yoshii, H.: Immunopharmacological actions of Neurotrophin (2): Effect of Neurotrophin on graft-versus-host reaction. *Folia Pharmacol. Japon.* **78**, 451–458 (1981) (Abs. in English)
- 4 Gill, H.K. and Liew, F.Y.: Regulation of delayed-type hypersensitivity III. Effect of cyclophosphamide on the suppressor cells for delayed-type hypersensitivity to sheep erythrocytes in mice. *Eur. J. Immunol.* **8**, 172–176 (1979)
- 5 Mitsuoka, A., Morikawa, S., Baba, M. and Harada, T.: Cyclophosphamide eliminates suppressor T cells in age-associated central regulation of delayed type hypersensitivity in mice. *J. Exp. Med.* **149**, 1018–1028 (1979)
- 6 Naiki, M., Imai, Y., Nakanishi, A., Hishinuma, A. and Osawa, T.: Effect on autoimmunity developed in (NZB/NZW) F₁ mice of substance(s) extracted from inflammatory dermis of rabbits induced by inoculation of vaccinia virus. *Clin. Immunol.* **16**, 506–516 (1984)
- 7 Okimura, T., Satomi-Sakaki, Y. and Ohkuma, S.: Stress and immune response II. Identification of stress-sensitive cells in murine spleen cells. *Japan. J. Pharmacol.* **40**, 513–525 (1986)
- 8 Komori, T., Nakano, T. and Ohsugi, Y.: Alleviation of depressed immunity caused by restraint-stress by the immunomodulator, lobenzarit disodium (disodium 4-chloro-2,2'-iminodibenzoate). *Int. J. Immunopharmacol.* **9**, 433–442 (1987)
- 9 Inagaki, M., Taki, M., Miura, T., Saitoh, N. and Yamada, K.: Effect of Neurotrophin on helper lymphocyte proliferation in hemophiliacs with immune deficiency. *Proc. 5th Int. Symp. on Hemophilia Treatment*, p. 303–309. Kyoritsu Printings, Tokyo (1986)