Abnormality of the Delayed Type Hypersensitivity (DTH) Response to *Bordetella Pertussis* in Spontaneously Hypertensive Rats (SHR)

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Abstract—The delayed type hypersensitivity (DTH) response of spontaneously hypertensive rats (SHR) was compared with that of Wistar Kyoto rats (WKY), a normotensive strain. SHR showed a lower DTH response to *Bordetella pertussis* than WKY, especially 48 to 72 hr after antigenic challenge. These results were observed before appearance of abnormality of antibody formation or blood pressure. The reduced DTH responses of SHR were partially restored by either transfer of WKY thymocytes or treatment with levamisole. Conversely, the transfer of SHR thymocytes into WKY rats tended to diminish the DTH response. These findings suggest that SHR have a dysfunction of T lymphocytes involved in DTH response (e.g., increase of suppressor cells and/or decrease of helper cells).

It has recently been described that SHR show depressed T cell functions measured as the percentage of rosette-forming cells, the number of plaque-forming cells (PFC) against sheep red blood cells (SRBC) and the blastogenesis response to mitogens. Ba et al. (1) and Takeichi et al. (2) have suggested that the development of hypertension in spontaneously hypertensive rats (SHR) is related to depression of T cell function; namely, the depressed T cell functions and high blood pressure of SHR were normalized by a histocompatible thymus graft (1). In addition, Bendich et al. (3) showed that treatment with levamisole or anti-thymocyte serum (ATS) reduces the blood pressure of SHR. On the other hand, it was shown that T cell-dependent chronic arthritis could not be induced in SHR by muramyl dipeptide (4). Takeichi et al. (2) also studied delayed type hypersensitivity (DTH) induced by SRBC and observed a reduced response in SHR, although the DTH response varies depending on the antigen (5).

*Bordetella pertussis* (*B. pertussis*) is generally utilized for evaluating the DTH response in rats (6–9). It is also reported that levamisole and D-penicillamine modulate these DTH responses (6–8). In this study, we compared the DTH response to *B. pertussis* of SHR with that of normotensive rats, Wistar Kyoto rats (WKY), and showed that the DTH response of SHR was markedly diminished and that it was restored by either the transfer of WKY thymocytes or the treatment with levamisole.

Materials and Methods

**Animals:** Male SHR and WKY aged 4 or 8 weeks old were used. Animals were obtained from Charles River Japan, Inc., kept in an air conditioned room and given standard chow and water ad libitum.

**Assay of DTH:** *B. pertussis* Tohama Phase I (5×10¹⁰ cells/ml) was emulsified with the same volume of Freund’s complete adjuvant (FCA, Difco). Eight weeks old rats were sensitized with 0.2 ml of the emulsion, containing 5×10⁹ cells, by the injection into
the dorsal surface of the left hind footpad and the right fore footpad. Twelve days later, animals were challenged by subcutaneous injection of *B. pertussis* (5×10⁹ cells/ml of foot volume) into the right hind footpad. The foot volume was measured 24, 48 and 72 hr after challenge by the water displacement method (9) and expressed as percentage increase. Rats were sensitized when they were 8 weeks old, except the experiments using 4 weeks old animals. In the latter, rats were sensitized with 0.1 ml of the emulsion, containing 2.5×10⁹ cells.

**Assay of PFC response:** Four or 8 weeks old rats were sensitized with 1×10⁸ or 2×10⁸ SRBC (Nihon Bio-Sap Center), respectively, by the injection into the tail vein. The rats were sacrificed 4 days after immunization, and a spleen cell suspension was prepared for hemolytic plaque assay (10).

**Thymocyte transfer:** Thymocytes were obtained aseptically from rats and suspended in balanced salt solution, and viable cells were counted by trypan blue exclusion. The cells (2.5×10⁸) were injected intravenously into rats under ether anesthesia through the tail vein. The ages of the donor and recipient rats were matched.

**Measurement of blood pressure:** The blood pressure was measured directly using a polygraph (RM-85, Nihon Kohden Kogyo Co.) 2 hr after insertion of a canula into the femoral artery. Canulation was performed under ether anesthesia.

**Drug treatment:** Levamisole and D-penicillamine were dissolved in saline and administered orally once daily. Two drug regimes were used: a long-dosing regime, in which drug administrations were started four weeks before sensitization and drugs were stopped on the day of challenge, and a short-dosing regime, in which drugs were given from the day of sensitization to the day of challenge.

**Statistics:** Statistical significance was evaluated by Student's *t*-test.

**Results**

**DTH in SHR:** Comparative results on DTH to *B. pertussis* in SHR and WKY are shown in Fig. 1. Maximum edema formation was observed 24 to 48 hr after challenge. These DTH reactions were specific since rats immunized with *B. pertussis* showed a

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**Fig. 1.** Comparison of delayed type hypersensitivity induced by pertussis vaccine in SHR and WKY. Rats were sensitized with a FCA emulsion of *B. pertussis* (2.5×10⁹ cells for young and 5×10⁹ cells for adult rats) at 8 weeks (A) or 4 weeks (B) of age. On day 12, animals were challenged by injection of *B. pertussis* (5×10⁹ cells/ml of foot volume) into a hind footpad. The foot volume was measured 24, 48 and 72 hr after challenge and the percentage increase in volume was determined. ●, SHR; ○, WKY; ---, no sensitization; —, sensitization. Points represent means±S.E.M, for the numbers of animals shown in parentheses. *, ** Significantly different from the WKY control group at *P*<0.01 and *P*<0.001.
strong reaction compared to nonimmunized controls. In an experiment in which rats were sensitized at 8 weeks of age, the percentage increases in foot volume 24, 48 and 72 hr after challenge were 75.8, 72.4 and 58.8% in WKY, and 60.1, 42.4 and 33.3% in SHR, respectively (Fig. 1A). Thus, the DTH response was much less in SHR, especially 48 to 72 hr after challenge. Similar results were obtained in 4 weeks old rats (Fig. 1B).

The PFC responses and blood pressures of these animals are shown in Table 1. SHR sensitized at 4 weeks of age showed a slightly decreased PFC response to SRBC and slightly increased systolic blood pressure. These changes were much greater in SHR sensitized at 8 weeks of age.

Effect of thymocyte transfer on DTH in SHR: We examined the effect of cell transfer to determine whether the depressed DTH reactions in SHR were due to dysfunction of T cells. The results are shown in Table 2. When WKY thymocytes were transferred to 3 weeks old SHR, they had no effect on the DTH reaction (Table 2, Exp. 1). When they were transferred on the day of sensitization, they showed a slightly decreased PFC response to SRBC and slightly increased systolic blood pressure. These changes were much greater in SHR sensitized at 8 weeks of age.

| Table 1. Comparison of immune responses and blood pressures of SHR and WKY |
|---------------------------------------------------------------|
| **Age at sensitization (weeks)** | **Strain** | **PFC/10^6 1) spleen cells** | **Blood pressure (mmHg) 2)** | **Heart rate 2)** (beats/min) |
|-------------------------------|------------|-------------------------------|-----------------|------------------|
| 4                             | WKY        | 381±78 3)                   | 154.0±2.4       | 387±6            |
|                               | SHR        | 234±36                       | 172.5±4.2*      | 421±18           |
| 8                             | WKY        | 748±108                      | 165.0±3.4       | 378±21           |
|                               | SHR        | 200±21**                     | 220.0±4.5**     | 407±11           |

1) Rats were sensitized with SRBC (1 x 10^6 cells for young rats and 2 x 10^6 cells for adult rats). 2) The blood pressure and heart rate were measured through a cannula inserted into the femoral artery. SBP: systolic blood pressure, DBP: diastolic blood pressure. 3) Values are mean±S.E.M. for 5 or 6 rats. **Significantly different from WKY rats at P<0.01 and P<0.001, respectively.

| Table 2. Effect of allogenic thymocyte transfer on delayed type hypersensitivity (DTH) in SHR and WKY |
|-----------------------------------------------------------------------------------------------------|
| **Strain** | **Transferred 1) thymocytes** | **DTH (% increase in foot volume 2)** | **Time (hr) after challenge** |
|-------------------------|-------------------------------|--------------------------------------|-----------------|
| Exp. 1                  |                               |                                      | 24              | 72              |
| SHR                     | None                          |                                      | 55.2±3.7 3)     | 35.5±2.8        |
|                         | WKY                           |                                      | 48.1±6.2 (67.1) 4) | 34.4±2.1 (96.9) |
| WKY                     | None                          |                                      | 62.6±5.4        | 65.8±6.2        |
| Exp. 2                  |                               |                                      | 42.3±2.5        | 28.5±2.4        |
| SHR                     | None                          |                                      | 48.3±2.6 (114.2) 4) | 32.1±1.3 (112.6) |
|                         | WKY                           |                                      | 50.4±2.9        | 43.8±3.3        |
|                         | SHR                           |                                      | 50.2±3.9 (99.6) 4) | 41.2±4.0 (94.1) |
| Exp. 3                  |                               |                                      | 27.3±2.2        | 18.7±1.2        |
| SHR                     | None                          |                                      | 31.4±2.6 (115.0) 4) | 27.9±1.6 (149.2) |
|                         | WKY                           |                                      | 33.3±2.5        | 50.4±4.9        |
|                         | SHR                           |                                      | 28.5±1.2 (85.6) 4) | 42.4±8.2 (84.1) |

1) Thymocytes (2.5 x 10^6) were injected intravenously into 3 weeks old rats (Exp. 1) on the day of sensitization (Exp. 2) or three times at 6-day intervals from the day of sensitization to the day of challenge (Exp. 3). 2) The experimental procedure was as described for Fig. 1. 3) Values are mean±S.E.M. for 5 to 7 rats. 4) Percent of non-treated group is shown in parentheses. *Significantly different from the untreated group at P<0.001.
they slightly increased the DTH reaction in SHR (Table 2, Exp. 2). This potentiating effect was greater after repeated thymocyte transfer. When WKY cells were injected three times at 6-day intervals from the day of sensitization to the day of challenge, they potentiated DTH about 50% (Table 2, Exp. 3). In Exp. 2 and 3, the DTH response of untreated animals was lower than those seen in other experiments. This may have been due to the effect of use of ether anesthesia during cell transfer.

Effects of immunomodulators on DTH in SHR: The effects of the immunomodulators levamisole and D-penicillamine on the diminished DTH response in SHR were studied using two dose regimes as shown in Fig. 2. With a long dosing regime (Fig. 2B), levamisole (5 mg/kg) tended to stimulate the DTH response of SHR by 24–42% at 24–72 hr after challenge. D-Penicillamine (25 mg/kg) had no effect on the DTH response of SHR. With a short dosing regime (Fig. 2A), neither levamisole nor D-penicillamine had any obvious influence on the DTH response.

Discussion
The DTH responses to B. pertussis of SHR and a normotensive strain (WKY) were compared. SHR showed a lower DTH response than WKY, especially 48 to 72 hr after antigenic challenge. These results were obtained when the rats were sensitized at both 4 and 8 weeks of age. In the former, the abnormality of the DTH response was greater than abnormalities of the PFC response and hypertension. These findings suggest that the DTH response may be influenced at an earlier stage of maturation than the PFC response and the blood pressure. Takeichi et al. (2) studied SRBC-induced DTH by measuring penetration of 125I-labeled human serum albumin 24 hr after antigenic challenge and observed a diminished response in SHR. However, no obvious differences in B. pertussis induced DTH between SHR and WKY were observed 24 hr after challenge. This may be due to the difference of antigen and/or assay system used.

DTH is regulated by effector (11, 12), helper (13) and suppressor T cells (14, 15). Ba et al. (1) reported that the depressed T cell functions of SHR are restored by an allogenic thymus graft with reduction in the blood pressure. Thus, the reduced DTH responses in SHR are postulated to be associated with the disfunction of T lymphocytes. Therefore, we studied the effect of thymocyte transfer on DTH response in SHR. Results showed that the reduced DTH responses were restored by transfer of thymocytes from
WKY rats, which were injected intravenously three times at 6 day intervals from the day of sensitization. Conversely, injection of SHR thymocytes tended to reduce the DTH response in WKY. These results suggest that SHR has a disfunction of T lymphocytes involved in the DTH response (e.g., increase of suppressor cells and/or decrease of helper cells), and WKY thymocytes restoring DTH response of SHR are short-lived cells and have more influence on the effector phase of DTH response than the induction phase. It is thought that the effect of WKY thymocytes is counteracted by the intrinsic immune system of SHR. Therefore, the reduced DTH response might be mildly restored by transfer of thymocytes from WKY rats.

Recently, Nash and Gell (15) reported that splenic T cells that suppress the induction of DTH response to herpes simplex virus are characterized as cells having both Lyt 1+2* and Lyt 1-2+. The early stage is regulated by Lyt 1+2* and the later stage by Lyt 1-2*. Thus, the diminished response in SHR observed in the later phase suggests that the DTH response to B. pertussis may also be regulated by different cell types in the two phases.

It is reported that hypertension in SHR is normalized by a thymus graft (1) or levamisole treatment (3). We observed that hypertension was suppressed only by the transfer of WKY thymocytes in immature SHR (Table 2, Exp. 1); other transfer of thymocytes (Table 2, Exp. 2, 3) had no effect on hypertension in the present experiments (data not shown). No exact correlation was found between DTH and hypertension. Further studies are needed to assess the role of T cell function in regulating DTH and the correlation between DTH and hypertension in SHR.

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References
1 Ba, D., Takeichi, N., Kodama, T. and Kobayashi, H.; Restoration of T cell depression and suppression of blood pressure in spontaneously hypertensive rats (SHR) by thymus grafts or thymus extracts. J. Immunol. 128, 1211–1216 (1982)
2 Takeichi, N., Suzuki, K., Okayasu, T. and Kobayashi, H.; Immunological depression in spontaneously hypertensive rats. Clin. Exp. Immunol. 40, 120–126 (1980)
3 Bendich, A., Beilisie, E.H. and Strausser, H.R.; Immune system modulation and its effect on the blood pressure of the spontaneously hypertensive male and female rat, Biochem. Biophys. Res. Commun. 99, 600–607 (1981)
4 Kohashi, O., Kohashi, Y., Kotani, S. and Osawa, A.; A new model of experimental arthritis induced by an aqueous form of synthetic adjuvant in immunodeficient rats (SHR and nude rats). Ryumachi 21, Supp. 149–156 (1981)
5 Fulton, R.A., Souteyrand, P. and Thivolet, J.; Influence of retinoid Ro 10–9358 on cell-mediated immunity in vivo. Dermatologica 165, 568–572 (1982)
6 Dieppe, P.A., Willoughby, D.A., Huskisson, E.C. and Arrigoni-Martelli, E.; Pertussis vaccine pleurisy: A model of delayed hypersensitivity. Agents Actions 6, 618–621 (1976)
7 Arrigoni-Martelli, E. and Bramm, E.; Development of models for penicillamine-like drugs. Rheumatol. Rehabil. 15, 207–210 (1976)
8 Cunningham, F.M., Ford-Hutchinson, A.W., Oliver, A.M., Smith, M.J.H. and Walker, J.R.; The effects of D-penicillamine and levamisole on leucocyte chemotaxis in the rat. Br. J. Pharmacol. 63, 119–123 (1978)
9 Komoriya, K., Tsuchimoto, M., Naruchi, T., Okimura, T. and Yamamoto, I.; Immunopharmacological profile of TEI-3096: a new immunomodulator. J. Immunopharmacol. 4, 285–301 (1983)
10 Jerne, N.K. and Nordin, A.A.; Plaque formation in agar by single antibody-producing cells. Science 140, 405 (1963)
11 Zinkernagel, R.M.; H-2 restriction of virus-specific T-cell-mediated effector functions in vivo. II. Adoptive transfer of delayed-type hypersensitivity to murine lymphocytic choriomeningitis virus is restricted by the K and D region of H-2. J. Exp. Med. 144, 776–787 (1976)
12 Leung, K.N. and Ada, G.L.; Cells mediating delayed-type hypersensitivity in the lungs of mice infected with an influenza A virus. Scand. J. Immunol. 12, 393–400 (1980)
13 Leung, K.N., Ada, G.L. and McKenzie, I.F.C.; Specificity, Ly phenotype, and H-2 compatibility requirements of effector cells in delayed-type hypersensitivity responses to murine influenza virus infection. J. Exp. Med. 151, 815–
14 Sunday, M.E., Benacerraf, B. and Dorf, M.E.: Hapten specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. VIII. Suppressor cell pathways in cutaneous sensitivity responses. J. Exp. Med. 153, 811–822 (1981)

15 Nash, A.A. and Gell, P.G.H.: Membrane phenotype of murine effector and suppressor T cells involves in delayed hypersensitivity and protective immunity to herpes simplex virus. Cell Immunol. 75, 348–355 (1983)