MODEL OF A STABLE FORM OF DELAYED-TYPE HYPERSENSITIVITY TO CHEMICAL ALLERGENS

A. D. Chernousov and N. V. Medunitsyn

UDC 616-056.43-02;66]-092.9

KEY WORDS: delayed-type hypersensitivity; chemical allergen.

During cutaneous sensitization of mice with the contact allergen trinitrochlorobenzene or sensitization with cutaneous Ia^+-cells, dendritic spleen cells (SC), and peritoneal exudate cells (PEC) obtained after stimulation with Freund's complete adjuvant (FCA) and modified by 2,4-6-trinitrophenylsulfonic acid (TNPSA), a stable form of delayed-type hypersensitivity (DTH), lasting more than 3 weeks, developed [11]. A different picture is observed after intravenous injection of TNPSA-modified syngeneic SC (TNPSC) or PEC obtained after stimulation with mineral oil. In that case a transient form of sensitization to the contact allergen developed, lasting not more than 10-14 days [1, 11]. These differences in DTH responses can be explained by the presence of Ia-proteins of antigen-presenting cells, activating different T cell subpopulations. In the stable form of DTH $I\text{-}A^+$-antigen presenting cells activate T-helper and T-effector cells of DTH (T_{dth}); in the transient form the number of antigen-presenting cells includes both $I\text{-}E/C^+$- and $I\text{-}J^+$-cells, which leads to activation of T suppressor cells (T_s), limiting the intensity and duration of DTH reactions [3, 5, 12]. The creation of conditions under which no T_s are formed, in response to injection of an antigen (AG) in FCA [9] or to injection of AG after preliminary injection of cyclophosphamide (CP), ought therefore to induce the development of stable forms of DTH.

The aim of this investigation was to develop a model of a stable form of DTH to chemical allergens in mice.

EXPERIMENTAL METHOD

Female BALB/c mice weighing 18-20 g, obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, were used. To sensitize the animals, 60 µl of a 10 mM solution of TNPSA in Hanks' solution (pH 7.5), emulsified in an equal volume of FCA (RIA 815, from Calbiochem, USA) was injected into the base of the tail. The intensity of sensitization was determined at various times after injection of TNPSA in FCA by means of skin tests. For this purpose 40 µl of a 10 mM solution of TNPSA in Hanks' solution, pH 7.2-7.4 (130 mg) was injected intradermally into the footpad of a hind limb [4]. The reaction was read after 6, 18, and 24 h, and subsequently daily for a maximum of 11 days. The size of the zone of edema was measured by means of an MK 0-25 engineers' micrometer [4]. The difference in thickness of the paws reflected the intensity of the reaction. The control for these experiments consisted of intact animals and mice receiving an injection of an emulsion of Hanks' solution in FCA, and skin tested at the same time. Mice immunized by intradermal injection of $4 \cdot 10^7$ TNPSC, or of the same dose of TNPSC together with CP served as the sensitization control. CP was injected intraperitoneally in a dose of 50 mg/kg 2 days before injection of AG. The formation of DTH effectors in the lymph nodes (LN) of sensitized mice was tested by the method of local transfer to intact syngeneic recipients [1]. Under these circumstances different doses of unfractionated cells from inguinal LN or of T cells, enriched on plastic dishes covered with rabbit antibodies against mouse immunoglobulins [2], were injected intradermally into the footpad of a hind limb 2 h after injection of a 10 mM solution of TNPSA. In this case the reaction was read 22 h after cell transfer.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that skin reactions, much greater in intensity than those in mice sensitized with TNPSC or a combination of TNPSC with CP, developed on the 5th day in mice

---

Laboratory of Molecular Immunology, Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 6, pp. 722-724, June, 1987. Original article submitted April 10, 1986.

0007-4888/87/0006-0825$12 50

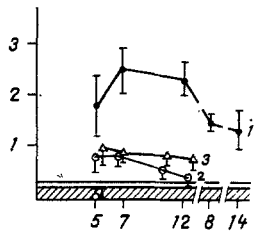

Fig. 1

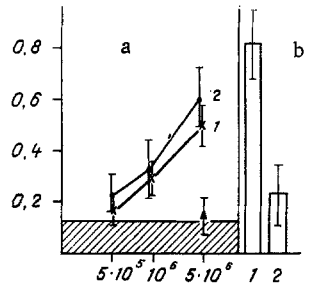

Fig. 2

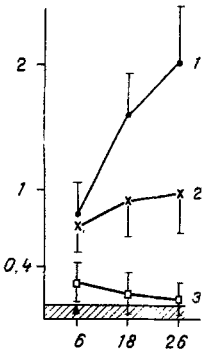

Fig. 3

Fig. 1. Intensity of skin reactions on testing at various times after sensitization with TNPSA in FCA (1), with TNPSC (2) and with TNPSC combined with CP (3). Abscissa, time after sensitization (5, 7, and 12 days, 8 and 14 weeks); ordinate, size of edema (in mm). Horizontal line indicates size of edema in animals receiving injection of Hanks' solution in FCA. Size of edema in intact animals corresponds to shaded zone.

Fig. 2. Local transfer of hypersensitivity by unfractionated LN cells (a) from donors sensitized with TNPSC (1) and with TNPSA in FCA (2). Abscissa: a) dose of cells transferred; b: 1) transfer of enriched T-cell population ($5 \cdot 10^6$), 2) transfer of same dose of T cells of normal mice; ordinate, size of edema (in mm). Size of edema in mice injected with TNPSA only corresponds to shaded zones; triangle — size of edema in mice into which $5 \cdot 10^6$ LN cells from normal animals were transferred after injection of TNPSA.

Fig. 3. Course of development of skin reactions in mice sensitized with TNPSA in FCA (1) and size of edema in mice receiving injection of emulsion of Hanks' solution in FCA (2) and into which 10^8 SC from mice immunized intravenously with 10^5 TNPSC were transferred before testing (3). Abscissa, time after testing (in h); ordinate, size of edema (in mm).

sensitized with TNPSA in FCA. Whereas after subcutaneous sensitization with modified cells reactions were observed only for 2 weeks, after sensitization with TNPSC combined with CP they were observed for over 3 weeks, and after sensitization with TNPSA in FCA they were discovered throughout the period of observation (14 weeks) and were distinguished by their extremely high intensity. The duration of the reactions after testing also was unusual. According to data in the literature [8-10] the duration of the reactions in mice sensitized by different methods was 24-48 h. The inflammatory reactions in mice sensitized with TNPSA in FCA were detected for 72 h after skin tests done on the 5th day, for 120 h after tests on the 7th day, for 244 h after those on the 12th day, and for 120 and 48 h after tests in the 8th and 14th weeks, respectively.

During sensitization with TNPSA in FCA, DTH effectors were formed in the regional LN. This is shown by the results of local transfer of unfractionated inguinal LN cells (Fig. 2a) and T cells of sensitized animals into intact syngeneic recipients.

Sensitization with the water-soluble form of a chemical allergen in FCA was thus followed by the development of allergic reactions an essential component of which was DTH, due to the formation of T_{dth} in LN (Fig. 2b). However, in the course of the skin reactions in sensitized mice, certain differences were observed.

As Fig. 3 shows, skin reactions of considerable intensity developed in mice sensitized with TNPSA in FCA after only 6 h (earlier, after 2 h, an intensive reaction was observed also in control mice, into which only an emulsion of Hanks' solution in FCA had been injected). The size of the zone of edema increased at 18 h and reached a maximum after 24 h. These data suggest that with this method of sensitization, allergic reactions caused by antibodies also develop. This component always accompanies the development of DTH in mice to a greater or lesser degree. Meanwhile the early component of the reaction could be due to the formation of a larger number of T_{dth}, giving rise to a definite inflammatory reaction after only 6 h. This possibility cannot be ruled out because the level of the skin reactions in recipients into which cells were transferred from mice sensitized with TNPSA in FCA, was higher, although

not significantly, than the level of the skin reactions in recipients of LN cells from mice sensitized with TNPSC (Fig. 2a). Finally, the early component in the skin reactions may be connected with the fact that after immunization with AG in FCA the different precursors of T_{dth} are activated, including some responsible for the early component of DTH, tested in some models 2 h after performance of skin tests [12]. This T_{dth} subpopulation is sensitive to the action of CP, and its activity is not inhibited by T_s, which are formed on the 12th day after intravenous injection of TNPSC, themselves unable to inhibit skin reactions detectable 6 h after testing, although they inhibit by 40 and 70% skin reactions detectable after 18 and 24 h, respectively. Whatever the case, all the hypotheses mentioned above require special experimental verification.

The results as a whole are evidence that in response to injection of chemical allergens in FCA, intensive and prolonged sensitization develops in mice. This model can evidently be used to test the activity of preparations intended for use in the treatment of allergy due to chemicals.

LITERATURE CITED

1. A. D. Chernousov, N. V. Molodtsov, and N. V. Medunitsyn, Immunologiya, No. 5, 20 (1982).
2. A. D. Chernousov, N. V. Molodtsov, and N. A. Medunitsyn, Byull. Éksp. Biol. Med., No. 12, 62 (1983).
3. A. D. Chernousov, A. V. Chervonskii, P. R. Poznakhirev, et al., Byull. Éksp. Biol. Med., No. 4, 450 (1986).
4. P. W. Askenase, Progress in Immunology, IV, Vol. 1, London (1980), p. 829.
5. J. S. Britz, P. W. Askenase, and W. Ptak, J. Exp. Med., 155, 1344 (1982).
6. H. N. Claman, S. D. Miller, and M. S. Sy, Immunol. Rev., 120, 105 (1980).
7. M. I. Green, M. Sugimoto, and B. Benacerraf, J. Immunol., 120, 1604 (1978).
8. S. Jarraman and C. J. Bellone, J. Exp. Med., 155, 1810 (1982).
9. P. H. Lagrange, G. B. Mackaness, and T. Miller, J. Exp. Med., 139, 1529 (1974).
10. G. B. Mackaness, P. H. Lagrange, and T. Ishibashi, J. Exp. Med., 139, 1540 (1974).
11. W. Ptak, D. Rozycka, and P. W. Askenase, J. Exp. Med., 151, 362 (1980).
12. H. Van Loveran and P. W. Askenase, J. Immunol., 133, 2397 (1984).