# FORMATION OF HYPERSENSITIVITY OF DELAYED TYPE TO SHEEP'S RED BLOOD CELLS AFTER SEPARATE AND COMBINED INJECTION OF ANTIGEN AND CYCLOPHOSPHAMIDE

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Ability to form hypersensitivity of delayed type (HDT) to the corresponding antigen was investigated in mice receiving an injection of a massive dose of sheep's red blood cells and cyclophosphamide at various times before the experiments. Depression of HDT formation as a result of injection of either the cytostatic alone or the massive dose of antigen alone was studied at the same times. Combined treatment was shown to lead to the formation of tolerance as reflected in various tests for HDT (skin test and inhibition of macrophage migration). This form of tolerance is based on a true deficiency of the corresponding clone of T cells. Injection of cyclophosphamide alone leads to some degree of depression of HDT. Injection of the massive dose of antigen alone leads to a different form of areactivity, due to suppressor cells, the nature of which is not yet clear.

KEY WORDS: immunologic tolerance; cyclophosphamide; hypersensitivity of delayed type.

Previous investigations showed that during tolerance to sheep's red blood cells (SRBC) obtained with the aid of cyclophosphamide (CP) the absence of antibody formation is due to a specific deficiency of T cells competent with respect to this antigen [2, 6].

The object of this investigation was to determine whether tolerance arises in the subpopulation of effector cells of hypersensitivity of delayed type (HDT) in animals treated in this way. If tolerance does develop, the next step was to study whether this was connected with a deficiency of effectors of HDT or with active inhibition of their function.

## EXPERIMENTAL METHOD

(CBA × C57BL/6)$F_1$ mice weighing 20-22 g were used. To obtain tolerance the mice were given an intraperitoneal injection of $6 \cdot 10^9$ SRBC, followed 42-46 h later by an intraperitoneal injection of CP in a dose of 200 mg/kg. The mice were sensitized at different times either by intravenous injection of $10^8$ SRBC in physiological saline [7] or by intradermal injection of $10^8$ SRBC in 40 µl Freund's complete adjuvant (FCA) [9]. In the first case HDT was determined by skin tests [7]. For this purpose, on the 4th day after sensitization $10^8$ SRBC in 40 µl physiological saline was injected into a footpad of one hind limb of the mice. The difference between the thickness of the footpads of the two limbs characterized the level of HDT. Animals injected with $6 \cdot 10^9$ SRBC only (AG-control) or with CP only (CP-control), sensitized animals (positive control), and animals receiving only the reacting injection of SRBC (negative control) served as the controls to these experiments. Each group consisted of 17-20 mice.

During sensitization by SRBC in FCA, the inhibition of macrophage migration test was set up by Suslov's modification [1] on the 10th day. Culture medium for immunocompetent cells *in vitro* [3], kindly supplied by Professor A. E. Gurvich, was used as the medium for the test. The concentration of water-soluble erythrocytic antigen, prepared by the method of Simon et al. [12], was 50 µg/ml. Peritoneal exudate cells were incubated at 37°C for 20 h in an atmosphere containing 5% $CO_2$. The inhibition of migration index (IMI) was calculated by the equation:

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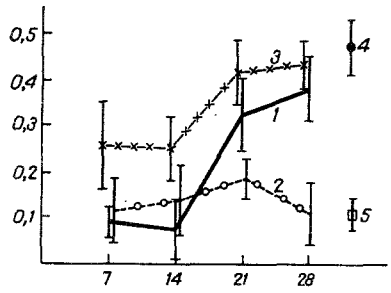


Fig. 1. Restoration of ability to give HDT after tolerogenic treatment. 1) AG + CP; 2) AG; 3) CP; 4) positive control; 5) negative control. Abscissa, time after tolerogenic treatment (in days); ordinate, thickness of edematous area of skin 24 h after reacting injection of antigen (in mm).

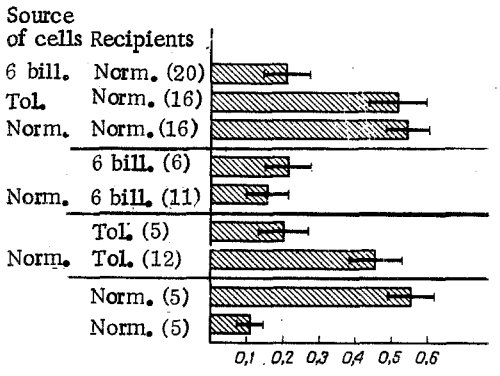


Fig. 2. Level of HDT against SRBC in experiments with crossed transplantation of syngeneic lymphocytes. Tol.) Mice receiving tolerogenic treatment ($6 \cdot 10^9$ SRBC + CP) 6 bill.) mice receiving $6 \cdot 10^9$ SRBC Norm.) intact mice. Donors treated 7 days before transplantation of cells, recipients 14 days beforehand. All recipients and their controls (except last group) received sensitizing injection 1 h after transplantation of cells. Number of recipients indicated in parentheses. Abscissa, thickness of edematous area of skin 24 h after reacting injection of antigen (in mm).

$$IMI = \left(1 - \frac{\text{Weight of migration zone in experimental group}}{\text{Weight of migration zone in control group}}\right) \times 100.$$

In the experiments with adoptive transplantation, spleen cells from different donors (11-20 mice in the group) were washed once with medium No. 199, resuspended in the same medium, and injected intravenously in a dose of $10^8$ into the corresponding recipients. The recipients were sensitized 1 h after injection of the cells by intravenous injection of SRBC.

## EXPERIMENTAL RESULTS

As Fig. 1 shows, after combined tolerogenic treatment the experimental animals were unable to form HDT against the corresponding antigen for the first 2 weeks.

The level of the skin tests in this group differed significantly from that in the CP-control and positive-control groups. At later stages the difference largely disappeared.

Similar results were obtained in the IMMT. As Table 1 shows, in the animals of the main experimental group cells producing the factor inhibiting macrophage migration were not formed. On the 14th day, migration of peritoneal exudate cells in the main experimental group was practically indistinguishable from migration of these cells in intact animals. By the 28th day the population of precursors of cells producing macrophage migration inhibition factor was partly restored.

The lower level of sensitization in the CP-control group than in the positive control group (Fig. 1) is evidence that CP acts not only on the effectors of HDT [8], but also on their precursors. It follows from these results and also from those of special additional experiments that preliminary (1-14 days before sensitization) injection of CP in doses of 20-200 mg/kg did not potentiate HDT if the optimal dose of antigen ($10^5$ SRBC) was used for sensitization. Meanwhile, there are data in the literature [5, 9] to show that CP can potentiate HDT in the case of sensitization with supraoptimal doses of antigen through the elimination of HDT suppressors.

It will be noted that in the group of animals receiving a supraoptimal dose of antigen, the function of HDT effectors also was depressed (Fig. 1). These results are in agreement

TABLE 1. IMI of Peritoneal Exudate Cells at Various Times After Tolerogenic Treatment or Injection of CP (IMI for intact animals is 0)

| Treatment | IMI | |
|---|---|---|
| | 14th day | 28th day |
| $6 \cdot 10^9$ SRBC + CP | —12±6,1 (11) | 36,3±2,2 (10) |
| CP-control | 55,0±2,4 (6) | 59,2±1,7 (6) |

Legend. Number of animals given in parentheses.

with data in the literature [4, 11]. Unlike in animals receiving combined treatment with antigen and CP, in animals receiving only a supraoptimal dose of SRBC, ability to form HDT was not restored on the 21st day.

The ability of animals into which cells of intact donors or of donors receiving a massive dose of antigen, or combined injections of antigen and CP 1 h before sensitization to respond by a reaction of HDT is illustrated in Fig. 2. As Fig. 2 shows, transplantation of cells in the first and third cases did not affect the level of HDT of the sensitized recipients. Conversely, transplantation of cells from mice receiving $6 \cdot 10^9$ SRBC caused suppression of HDT. Cells of intact donors completely restored ability to respond with an HDT reaction in animals receiving antigen and CP, but not in animals previously receiving only a massive dose of SRBC. These results are evidence that the cells of animals which received a large dose of SRBC, unlike spleen cells of animals receiving combined treatment with antigen and CP, can actively suppress the response of sensitized recipients. Lagrange et al. [5] connected suppression of HDT by a high dose of antigen with the action of antibodies or antigen—antibody complexes [5]. The results obtained by other workers [10, 13] led to the conclusion that in some cases HDT can be suppressed by cells of thymic origin. It is impossible to determine on the basis of the present experiments whether the immunosuppressive effect of an excessive dose of antigen is connected with the action of antibody-forming cells, T-helpers, or T-suppressors on HDT.

Disturbance of HDT formation after combined treatment with antigen and CP is evidently due to a deficiency of effectors of HDT because of elimination of their precursors. Summing up the results of the present investigation and those obtained previously [2], it can be concluded that as a result of massive antigenic stimulation precursors of widely different subpopulations of effector cells start to proliferate. Subsequent administration of CP eliminates all cells involved in proliferation and leads to the development of tolerance, which is based on a true deficiency of cells competent with respect to the given antigen. The times of injection of CP are of essential importance in this case. For instance, injection of CP 24 h after antigenic stimulation does not affect ability to form HDT [11], although it considerably reduces ability to form antibodies [2]. Increasing the interval between injection of the antigen and CP to 42-46 h causes the formation of tolerance not only in the population of B cells and T-helpers but also, as the results of the present investigations show, in the population of T cells responsible for HDT. This subpopulation of T cells is evidently stimulated to proliferate by the antigen later than precursors of the T-helpers, and for that reason it can be eliminated by CP only if it is injected later.

## LITERATURE CITED


1. A. P. Suslov, A. K. Yazova, A. I. Gusev, et al., Byull. Éksp. Biol. Med., 84, No. 10, 1225 (1976).
2. L. N. Fontalin, T. K. Novikova, I. A. Kondrat'eva, et al., Byull. Éksp. Biol. Med., 84, No. 4, 445 (1976).
3. R. E. Click, L. Benek, and B. J. Alter, Cell. Immunol., 3, 264 (1972).
4. P. H. Lagrange and G. B. Mackaness, J. Exp. Med., 141, 82 (1975).
5. P. H. Lagrange, G. B. Mackaness, and T. E. Miller, J. Exp. Med., 139, 528 (1974).
6. J. F. A. P. Miller and G. F. Mitchell, J. Exp. Med, 131, 675 (1970).
7. T. E. Miller, G. B. Mackaness, and P. H. Lagrange, J. Natl. Cancer Inst., 51, 1669 (1973).

8. A. Mitsuoka, M. Baba, and S. Morikawa, Nature, 262, 77 (1976).
9. I. Ohmichi, K. Nomoto, H. Yamada, et al., Immunology, 31, 101 (1976).
10. P. Phanuphak, W. Moorhead, and H. Claman, J. Immunol., 112, 849 (1974).
11. S. A. Ramshaw, R. A. Bretsher, and C. R. Parish, Eur. J. Immunol., 6, 674 (1976).
12. S. Simon, S. C. Mazie, and A. E. Bussard, J. Immunol., 1, 387 (1971).
13. M. Zembala and J. Asherson, Nature, 244, 227 (1974).

# MYOGLOBIN CONCENTRATION AND PARTIAL OXYGEN PRESSURE IN MUSCLE TISSUE DURING ALLERGIC REACTIONS

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The effect of sensitization to horse serum, brucellas, and BCG, and also the effect of allergic reactions induced by these antigens on the partial pressure of oxygen and myoglobin concentration in muscle tissue were studied. The myoglobin concentration was found to be reduced during the formation of increased sensitivity but only in the case of sensitization by living microbial strains. Allergic reactions of immediate and delayed types caused a decrease in the myoglobin concentration both in the myocardium and skeletal muscles. The partial pressure of oxygen in the skeletal muscles was lowered.

KEY WORDS: sensitization; allergic reactions; myoglobin; partial pressure of oxygen.

The basic biochemistry of altered reactivity and metabolic disturbances developing in allergic reactions has been inadequately studied, although the elucidation of these problems is of great interest for the understanding of the pathogenesis of allergic diseases and for the development of pathogenetically based methods of their treatment. An important role in the formation and manifestations of allergic reactions is played by muscle tissue which, according to some workers, is the target tissue for the antigens [3, 12].

The object of the present investigation was to study some indices of muscle tissue metabolism during the formation of increased sensitivity to various antigens.

## EXPERIMENTAL METHOD

The myoglobin concentration was studied in cardiac and skeletal muscles and the partial pressure of oxygen was determined in the skeletal muscles during primary contact of animals with antigens (horse serum, tuberculin, therapeutic brucellosis vaccine — TBV), and at the height of the development of sensitization to horse serum, BCG, and *Brucella abortus* during realization of the allergic reaction to these antigens (Table 1). Altogether 10 series of experiments were carried out on 442 guinea pigs.

The combined determination of the myoglobin concentration and partial pressure of oxygen in skeletal muscles is of undoubted interest for myoglobin is a component of the oxygen transport system in muscle tissue [14], and it also takes part in the equalization of the partial pressure of oxygen, increasing its rate of diffusion within the muscle [9, 11, 13], and in electron transport and oxidative phosphorylation [8].

Determination of the partial pressure of oxygen reveals the character of the supply of oxygen to the body tissues and serves as an index of their oxygenation [2, 5].

The myoglobin concentration was determined spectrophotometrically [10] and the partial pressure of oxygen polarographically [1]. The experimental results were subjected to statistical analysis [7].

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