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T-CELL IMMUNODEFICIENCY IN MICE RECEIVING LECTIN AND CYCLOPHOSPHAMIDE

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Successive injections of an antigen and cyclophosphamide (CP) can induce immunologic tolerance to that particular antigen [3]. It has been suggested [2, 6, 16] that one cause of this effect is selective elimination by the cytostatic of lymphocytes stimulated by the antigen. It can accordingly be postulated that the use of the T-cell mitogen, lectin, instead of the specific antigen in the analogous situation would induce general T-cell immunodeficiency, but without at the same time disturbing B-cell functions. The aim of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL/6)F_1 hybrid mice weighing 18-20 g. Lectin from *Lens culinaris* (LcA), obtained and generously provided by Dr. I. Hilgert of the Czechoslovak Institute of Molecular Genetics, and concanavalin A (ConA), from Difco (USA), were used in the experiments. The LcA (1 mg) and conA (100 μg) were injected intravenously in a volume of 0.5 and 0.2 ml respectively in sterile physiological saline.

Cyclophosphamide (Cyclophosphan, from Saransk Medical Preparations Factory) was dissolved in sterile distilled water and injected intraperitoneally in a dose of 200 mg/kg, 2 days after the lectin.

TABLE 1. Suppression of Immune Response to SRBC Induced by Consecutive Injections of T-Mitogens and CP (immunization 7 days after CP)

Series of experiments	Pretreatment of animals	Number of mice	Number of AFC to SRBC in spleen 4 days after immunization
I	LcA+CP	17	4 894
	LcA	17	75 242
	CP	16	56 612
	—	10	221 109
II	ConA+CP	11	12 236
	ConA	12	142 144
	CP	11	73 620
	—	11	248 593

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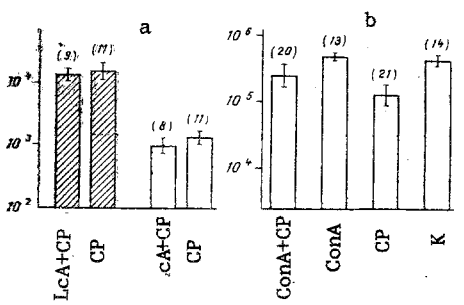

Fig. 1

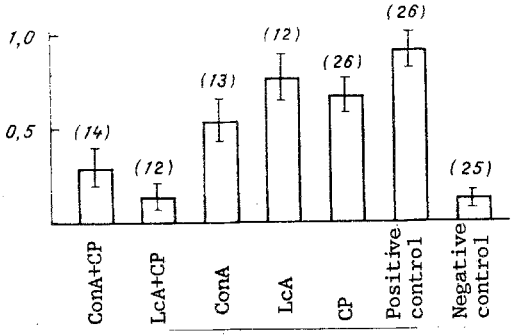

Fig. 2

Fig. 1. Antigen-specific and polyclonal response of mice receiving lectin and CP to LPS. a) Ordinate, number of AFC to LPS from *E. coli* (shaded columns) and AFC to SRBC (unshaded columns). K) Control; b) ordinate, number of AFC to LPS from *S. marcescens*. Here and in Fig. 2, number of experiments shown in parentheses. Here and in Figs. 2 and 3, mean values with confidence limits are shown.

Fig. 2. Formation of DTH reaction to SRBC in mice receiving lectin and CP. Ordinate, level of DTH reaction (in mm).

Sheep's red blood cells (SRBC) in a dose of 5×10^8 and lipopolysaccharide (LPS) from *E. coli* or *S. marcescens* (Difco), in a dose of 10 μg, were injected intravenously, as a rule 7 days after CP. After 4 days the number of antibody-forming cells (AFC) in the spleen of the experimental animals was determined. AFC to SRBC were determined by the local hemolysis in gel test, AFC to LPS by the passive local hemolysis in gel test, using SRBC sensitized by LPS from *E. coli* or *S. marcescens*.

In some experiments the nonspecific polyclonal response to LPS (50 μg/mouse) was evaluated by determining AFC to SRBC 3 days after immunization.

The delayed-type hypersensitivity reaction to SRBC was carried out by the usual method [14]: experimental animals were sensitized intravenously with 0.5×10^6 SRBC, and 4 days later 10^8 SRBC were injected subcutaneously into a hind foot (as reacting injection) and physiological saline into the opposite foot. Animals receiving the reacting injection of SRBC only were used as the control. After 24 h the level of the DTH reaction was read as the difference in thickness of the experimental and contralateral limbs. The thickness of the limbs was measured with a micrometer.

Antigen-specific T suppressors of DTH were induced by the method in [5]: 12 days after CP the experimental mice were given an intraperitoneal injection of 6.2×10^9 SRBC. After 5 days splenocytes of these mice were injected intravenously into intact syngeneic recipients. These recipients and intact control mice were sensitized 3 h later with SRBC (0.5×10^6), and this was followed by setting up the DTH reaction as described above.

EXPERIMENTAL RESULTS

In the first two series of experiments production of antibodies to thymus-dependent antigen of SRBC was investigated. Mice were immunized 7 days after injection of LcA and CP (experiments of series I) or of ConA and CP (series II). As Table 1 shows, AFC formation was considerably depressed in the experimental animals — by 20-40 times compared with the control. In animals receiving lectin only or CP only, AFC formation was significantly less strongly depressed: by 2-4 times compared with the control. Additional tests showed that these differences persisted for 4 weeks, although they gradually diminished toward the end of this period.

In the next series of experiments animals receiving LcA and CP were immunized with a thymus-independent antigen, namely LPS from *E. coli*, which is at the same time a polyclonal B-cell activator. Both effects of LPS were taken into consideration: antigen-specific (AFC to LPS) and polyclonal (AFC to SRBC). It will be clear from Fig. 1a that there were no significant differences in either parameter between animals receiving LcA and CP and mice receiving CP alone. Investigation of the response of mice receiving preliminary injections of ConA and CP likewise revealed no significant change in the response to thymus-independent antigen — LPS from *S. marcescens* (Fig. 1b) — compared with the control.

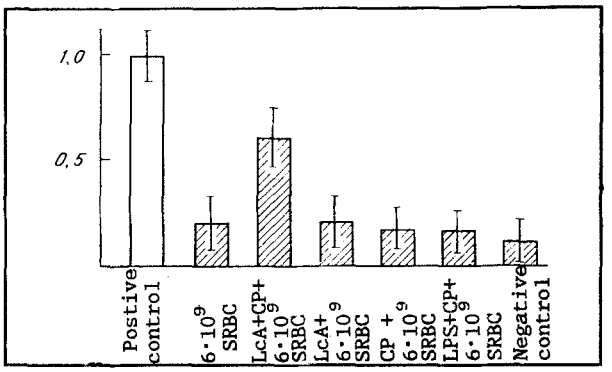

Fig. 3. Effect of successive injections of T-mitogen and CP to formation of antigen-specific suppressors of DTH. Ordinate, level of DTH reaction to SRBC (in mm).

In the next two series of experiments DTH formation to SRBC was studied in mice receiving lectin (LcA or ConA) and CP. It will be clear from Fig. 2 that mice receiving lectin or CP alone formed DTH rather less strongly than the control animals. Mice receiving combined injections of lectin and CP either did not form DTH at all or formed it much less strongly than animals receiving lectin alone or CP alone.

In the last series of experiments the formation of suppressor cells of DTH was investigated in mice receiving lectin and CP. As Fig. 3 shows, transfer of spleen cells from donors immunized with 6×10^9 SRBC prevents DTH formation to that antigen. This effect is known [5, 11, 15, 19] to be due to antigen-specific suppressor T cells (T_S). It is also clear from Fig. 3 that pretreatment of the donors with lectin alone or CP alone did not prevent T_S formation. However, if the donors received lectin and CP, T_S formation was appreciably disturbed. Combined injections of LPS and CP had no effect on T_S formation.

The results indicate unequivocally that various T-cell functions (helper, suppressor, DTH formation) are disturbed in mice receiving lectin (LcA or ConA) and CP. Injection of these preparations separately either had no such effect at all (Fig. 3) or had a much weaker action (Table 1; Fig. 2). A combination of lectin with CP acts only on T cells and not on B cells (Fig. 1).

We know that lectins can induce an immunodepressive effect by forming antigen-specific suppressor T cells [8-10, 13]. Since ability to cooperate in the formation of antigen-specific suppressor cells is a property of CP also [17, 18], this could be the explanation of the mutual potentiation of their immunodepressive effects. Such an explanation, however, disagrees with the results given in Fig. 3, where depression of suppressor cell formation as a result of combined injections of lectin and CP can be clearly seen.

The results of the present investigation should be compared with those obtained in response to combined administration of various antigens [3] and the B-cell mitogen LPS together with CP [1, 4]. This comparison reveals a general rule: those cells which were initially stimulated are mainly affected. For instance, injection of CP preceded by antigen-specific stimulation induces specific immunologic tolerance [2].

Injection of CP after the B-cell mitogen (LPS) induces anergy of B cells of varied immunologic specificity, while preserving T-cell activity [1, 4]. Finally, injection of CP after T-cell mitogens (LcA, ConA), as is evident from the results described in this paper, induces anergy of T cells of varied immunologic specificity and function (DTH helper and effector T cells, DTH suppressor T cells) while preserving B-cell activity.

It can be tentatively suggested that all the effects described above are based on increased toxicity of CP for proliferating lymphoid cells — a fact established previously for certain lymphomas [7, 12]. Meanwhile, elucidation of the nature of the T-cell immunodeficiency described in this paper must evidently await further research.

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IMMUNOCHEMICAL PROPERTIES OF IMMUNOGLOBULIN G, CONJUGATED WITH DEXTRAN

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Theoretical developments in physicochemical biology have demonstrated the possibility of deliberate modification of the pharmacologic properties of various drugs. As a result of progress in engineering enzymology (a rapidly developing field of physicochemical biology), success has been achieved in the creation of unique therapeutic substances by conjugating medically important enzymes with various polymers [6, 8, 10].

Such a deliberate change in the physiological and pharmacological activity of immunoglobulins [5, 14], proteins with an important role in the maintenance of homeostasis, could be of great practical value. The good prospects for the creation of long-acting immune preparations (of the immobilized enzyme type) are largely determined by preservation of the therapeutic and prophylactic activity of antibodies after incorporation into the polymer matrix.

The aim of this investigation was to study specific antigen-binding activity of antibodies and their effector functions after conjugation of immunoglobulin molecules with dextran.

EXPERIMENTAL METHOD

Immunoglobulin G (IgG) from the blood serum of hyperimmunized rabbits, specific for sheep's red blood cells (SRBC), and IgG from horse serum, isolated by ion-exchange chromatography in DEAE-cellulose [3], served as the model proteins in our experiments. The IgG were conjugated as described previously [9] (with certain modifications), with dextran (mol. wt. 35-50 kD), with protein and dextran in the reacting mixture in a ratio (w/w) of 1:6.

The physicochemical properties of the conjugates were studied by gel-chromatography on Toyopearl gel, ultracentrifugation in a sucrose density gradient [12], and polyacrylamide gel

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