MICROBIOLOGY AND IMMUNOLOGY

Immunological Characteristics of Skin Preparations

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Effects of three preparations from porcine skin on the number of antibody-producing mouse splenocytes at the peak of primary immune response and on the restoration of the sensitivity of background rosette-forming splenocytes to inhibitory effect of azathioprine were studied in thymectomized mice. All three preparations were active in the first test, while in the second test only the preparation with molecular weight below 1.4 kD was active. The effects of preparations on B and T immunity have been demonstrated.

Key Words: *immunomodulators; cytokins; skin; B immunity; T immunity*

Development of new immunomodulators is a perspective problem of modern immunology. Endogenous preparations, elements of natural metabolic processes, are of particular interest. The skin, a unique immune organ capable of developing local and systemic immune responses [4,6], is the source of immunomodulators. The overwhelming majority of cytokins were detected in the skin [4].

We previously showed that porcine skin contains factors modifying the proliferation and differentiation of human keratinocytes in primary culture [5]. A method for isolation of these factors has been developed [1] and the physicochemical properties of these preparations were studied [2]. Here we investigated the effects of these preparations on some immunological parameters.

MATERIALS AND METHODS

Porcine skin was fragmented with scissors and homogenized in 0.14 M NaCl (pH 7.0) at 4°C in a 1094 Homogenizer (Tecator). The homogenate was centrifuged at 2500*g* for 30 min at 4°C (J-6M/E centrifuge, rotor 4.2, Beckman). The precipitate was discarded and supernatant was subjected to thermal denaturation at 75°C for 20 min in a water bath at constant shaking, after which the mixture was cooled to 4°C. Denaturated material was precipitated by centrifugation at 10,000*g* for 20 min at 4°C (centrifuge J2-21M, rotor JA-14, Beckman) and the supernatant was slowly added to acetone. The precipitate was washed with acetone by centrifugation at 2500*g* for 15 min at 4°C and dried at 18-20°C. The acetone powder was dissolved in 0.01 M Tris-HCl buffer with 0.15 M NaCl (pH 8.0) at 18-20°C. Unsoluble substances were removed by 30-min centrifugation at 4000*g* at 4°C and the supernatant was layered onto a 2.5×100 cm Sephadex G-50 column. Fractions with molecular weights above 15 kD (preparation 1, P1), 1.4-15 kD (preparation 2 – P2), and below 1.4 kD (P3) were collected, desalinated, and lyophilized.

Protein content in the preparations was measured by Lowry's method [10] and biuret method [9], carbohydrates were measured by anthrone method [14], RNA as described elsewhere [13], and total lipids as described previously [15].

Antibody-producing cells (APC) in the spleen of CBA mice were counted at the peak of immune response [3]. Eleven experimental series were performed, each series comprised with at least 20 animals. Sheep

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erythrocytes (400×10^6/ml) were injected intraperitoneally in 0.5 ml normal saline. Simultaneously the test preparations were injected in doses of 1, 10, and 100 μg/mouse. On day 5 the animals were sacrificed, spleens were isolated, and zones of hemolysis in Petri dishes were counted. The percentage of APC in comparison with the control (100%) was estimated.

Restoration of sensitivity of background rosette-forming splenocytes (bRFC) from thymectomized mice to inhibitory effect of azathioprine was evaluated as described previously [8]. Six experimental series were carried out on male C57Bl/6 mice. For creating a model of partial T immunodeficiency, the mice were thymectomized at the age of 6-8 weeks and were taken into experiment 2-8 weeks postoperation. Splenocyte suspension was prepared. Azathioprine and test agents were added to experimental samples in final concentrations of 1, 5, 10, 20, 50, and 100 μg/ml. The number of splenic bRFC per 10^4 nucleated cells was counted. The results were expressed in percentage of bRFC by the formula:

$$A=M_E/M_K\times100\%,$$

where M_K and M_E are the mean numbers of bRFC in control and experimental samples.

Fractions containing less than 50% bRFC were considered active.

The significance of differences was evaluated using unpaired parametrical Student's *t* test.

RESULTS

The elution profiles of supernatant on a Sephadex G-50 column is shown in Fig. 1. The data on protein, carbohydrates, RNA, and total lipid content in the preparations are presented in Table 1.

Protein and total lipid content decreased, while the content of carbohydrates and RNA increased from P1 to P3. Protein content in P1 and P2 measured by Lowry's and biuret methods was virtually the same. Protein content in P3 measured by biuret method was 1.8 times higher than that measured by Lowry's method. Presumably the biuret method detects proteins undetectable by Lowry's method.

The effects of test preparations on the number of APC in mouse spleen were studied at the peak of primary immune response (Fig. 2). P1 in a dose of 10 μg/mouse significantly increased the number of APC in comparison with the control (by 1.21 times, $p<0.05$), P2 in a dose of 1 μg/mouse increased the number of APC by 1.40 times and in a dose of 100 μg/mouse by 1.35 times ($p<0.01$ and $p<0.05$, respectively). P3 significantly increased the number of APC in all doses studied: 1 μg/mouse by 1.58 times, 10 μg/mouse by 1.44 times, and 100 μg/mouse by 1.92 times ($p<0.01$).

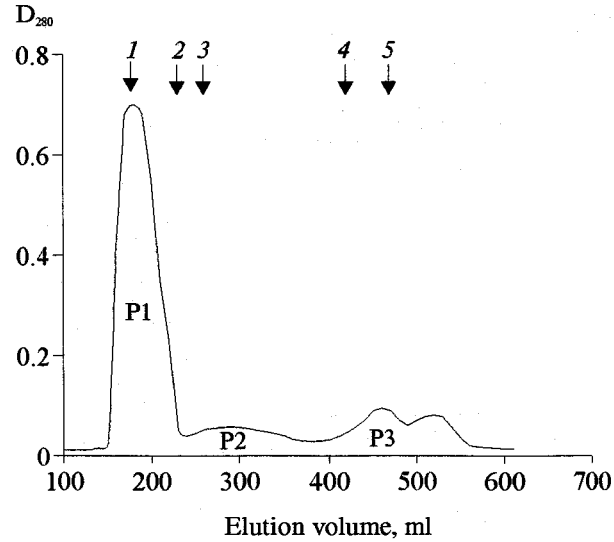

Fig. 1. Supernatant elution profile on Sephadex G-50 column. Arrows show markers: *1*) dextrane blue (2000 kD); *2*) chymotrypsinogen (25 kD); *3*) cytochrome C (12.3 kD); *4*) vitamin B_{12} (1357 D); *5*) DNP-alanine (255 D). Here and in Figs. 2 and 3: P1, P2, P3 — preparations 1, 2, and 3.

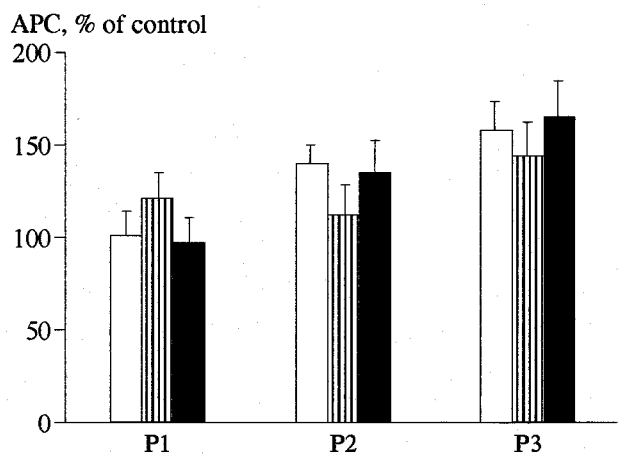

Fig. 2. Effects of porcine skin preparations on the number of antibody-producing cells (APC) in the spleen of CBA mice at the peak of primary immune response. Concentrations of preparations: 1 μg/mouse (light bars), 10 μg/mouse (hatched bars), and 100 μg/mouse (dark bars).

Since APC are derived from B lymphocytes, we can speak about stimulating effect of the preparations on B immunity. Further studies will help to elucidate the mechanism of this effect.

Then the effects of the preparations on restoration of the sensitivity of splenic bRFC to inhibitory effect of azathioprine were studied (Fig. 3).

After addition of 10 μg/ml P3 the number of bRFC was 56%. The arithmetic means of several experiments are shown in Fig. 3; the percentage of bRFC in each experiment was no more than 50%. P3 was active in concentrations 10 and 20 μg/ml, while P1 and P2 were inactive in all the studied concentrations.

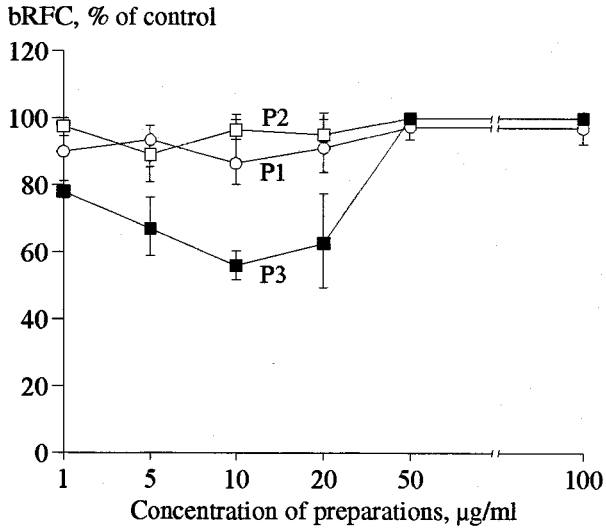

Fig. 3. Effect of porcine skin preparations on recovery of sensitivity of background rosette-forming cells (bRFC) in the spleen of thymectomied mice to inhibitory effect of azathioprine.

TABLE 1. Content (in %) of Proteins, Carbohydrates, RNA, and Total Lipids in Skin Preparations (*M±m*)

Component	P1	P2	P3
Protein			
Lowry's method	91±2.9 (8)	51±3.2 (6)	8.2±1.1 (10)
Biuret method	87±7.3 (5)	56±6.0 (3)	15±2.0 (5)
Carbohydrates	0.74±0.30 (5)	8.0±1.4 (4)	9.0±2.7 (4)
RNA	2.0±0.4 (6)	5.4±1.0 (5)	14±2.4 (7)
Total lipids	2.2±0.1 (6)	1,4±0.3 (3)	1.4±0.2 (6)

Note. Number of measurements is shown in parentheses.

Azathioprine sensitivity recovery test are often used for assessing the activities of thymic factors [8]. Thymectomy drastically decreased the sensitivity of splenic bRFC forming rosettes with sheep erythrocytes to azathioprine, which is caused by decreased number of mature T lymphocytes. Thymic factors restore this capacity by stimulating the maturation of T cells. In this study P3 restored sensitivity to azathioprine, which indicates its effect on T cell differentiation.

The skin and thymus possess common properties [6]. Skin keratinocytes and thymic epitheliocytes express common surface markers; both possess keratin. Keratinocytes release factors affecting T lymphocyte differentiation [6,11]. It was suggested that keratinocytes produce thymic hormones, but this hypothesis has been proven only for embryonal skin producing thymopoietin [7]. More profound studies with polyclonal and monoclonal antibodies detected no thymic hormones in skin keratinocytes from adult donors [12]. However, keratinocytes produce other factors affecting T cell differentiation [6,11].

Our findings indicate the presence of factors modifying both B and T immunity in the skin.

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