ROLE OF A LOW-MOLECULAR-WEIGHT BONE MARROW FACTOR IN INDUCTION OF THE PRIMARY AND SECONDARY IgE-RESPONSE TO OVALBUMIN

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The study of regulation of biosynthesis of IgE antibodies in connection with their role in the development of allergic reactions has attracted the attention of many research workers [1, 3, 13]. The immune response can also be regulated by soluble factors [6, 8, 12, 15]. The immunoregulatory activity of several low-molecular-weight factors has been demonstrated [5, 8, 11], including those produced by bone marrow (BM) cells [6, 8, 12]. We demonstrated previously that BM cells can exert a regulatory action on the IgE-response, manifested as a fall of the serum IgE level and suppression of IgE production by antibody-producing cells in response to injection of BM cells at the time of induction of the primary and secondary IgE response [10]. It has also been shown that BM cells produce a soluble mediator, whose action on the IgE response has not been studied [9]. Accordingly, in the investigation described below we studied the effect of the BM cell factor described previously on the IgE response at the moment of its induction.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL/6)F_1 hybrid mice weighing 18-20 g, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. The IgE response was induced by intraperitoneal injection of ovalbumin (OA) in a dose of 5 μg per mouse. Aluminum hydroxide in a dose of 2 mg per mouse was used as the adjuvant.

The suppressor factor (SF), obtained by incubation of BM cells ($5 \cdot 10^6$ cells/ml) for 72 h in an atmosphere with 5% CO_2 at 37°C in medium RPMI-1640 with 10% fetal calf serum and in serum-free Iskov's medium was injected intravenously in a volume of 0.5 ml and a dose of 10 μg/g at different times after immunization of the mice. Both media were used with standard additions of 2 mM L-glutamine, $5 \cdot 10^{-5}$ M mercaptoethanol, and 50 μg/ml gentamicin. The source of SF consisted of supernatants of the above cultures, passed through millipore filters with a pore diameter of 22 μ whose activity was tested on a "Mark 3" counter, by measuring suppression of proliferative activity of mastocytoma P-815 cells or spleen cells, stimulated by peanut phytohemagglutinin ("Difco,") in a dose of 10 μg/ml [2, 9]. SF obtained on Iskov's medium was fractionated on Sephadex G-25 ("Pharmacia") on a column 1.4 cm in diameter and 86 cm high. Fractions were collected on a "Pharmacia" fraction collector (Sweden). The optical density of the portions was measured on an SF-26 spectrophotometer at a wavelength of 280 nm [4]. The content of substances with a peptide bond in SF was determined by the biuret method according to Lowry [14].

The serum IgE antibody level of the mice was determined by the passive cutaneous anaphylaxis (PCA) method on rats 1-2 weeks after immunization [16].

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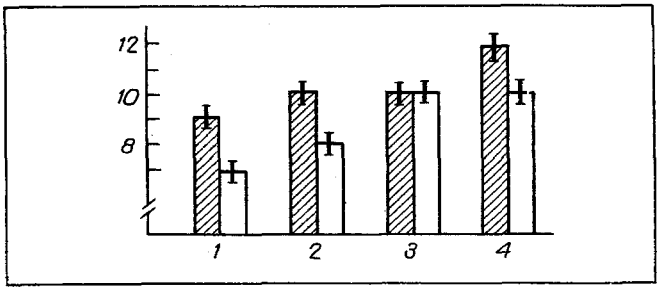

Fig. 1. Depression of IgE response at different times after primary and secondary immunization with OA. Here and in Fig. 2, ordinate – $\log_2$ of titer^{-1} of PCA reaction. SF injected intravenously in a dose of 10 μg/g and in a volume of 0.5 ml per mouse. Level of IgE-antibodies determined on 7th (1), 14th (2), and 21st (3) days after immunization and on 7th day after reimmunization, undertaken on 20th day (4). Shaded columns – control, unshaded – experiment.

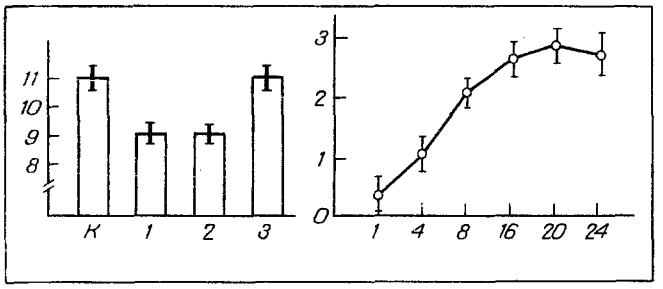

Fig. 2 Fig. 3

Fig. 2. Effect of unfractionated SF and SF fractionated by molecular weight on IgE-response in mice. 1) 10 μg/g of SF fraction with mol. wt. of 8000 daltons, 2) 10 μg/g of unfractionated SF, 3) 10 μg/g of SF fraction with mol. wt. of 700 daltons, K) control (0.5 ml of Iskov's medium).

Fig. 3. Depression of IgE-response to OA in mice as a function of injected dose of SF. Abscissa, dose of SF, μg/g; ordinate, index of suppression of IgE-response (difference between serum IgE levels of control and experimental animals, expressed in $\log_2$ titer^{-1} of PCA reaction).

EXPERIMENTAL RESULTS

The action of SF of the BM cell cultures was assessed at different stages of development of the primary and secondary IgE-response to OA. A fall of the serum IgE antibody level of the mice was discovered when SF was injected simultaneously with OA, as early as 1-2 weeks after immunization (Fig. 1). Reduction of IgE-antibody production was observed for 14 days. The action of SF, injected 2-3 weeks after primary immunization, did not affect the intensity of development of the IgE response. When SF was injected simultaneously with reimmunization, which was carried out 3 weeks after primary immunization, the IgE-antibody level was found to fall (Fig. 1). These results are in good agreement with those of previous studies [2, 7, 9], in which the ability of SF of BM cell cultures to abolish the development of antibody production and the proliferative response in vitro on the addition of SF in the initial stages of culture, was demonstrated. In the present investigations SF was characterized as thermostable, stable in an acid medium, species-nonspecific, and not a prostaglandin [9].

To obtain precise characteristics of SF, it was fractionated by gel filtration on Sepharose G-25. Two peaks (Fig. 2) of an increase in optical density were found, corresponding to a molecular weight of 8000 and 700 daltons respectively (the column was calibrated beforehand relative to contrykal, bradykinin, insulin, and peptides with known molecular weight). When the CF fractions were injected simultaneously with immunization, the effect of suppression of the IgE-response was found to be mediated by the low-molecular-weight fraction with mol. wt. of 8000 daltons (Fig. 2).

The action of SF and its fractions on the initiating stages of formation of the IgE response suggests that the targets for its action are precursor cells of antibody producers, which are present in sufficient numbers in the early stages of the response. Mention was made previously [6, 7] of the existence of a mechanism acting through suppression of proliferation of B-cell precursors. An effect of BM cells themselves was observed at the corresponding times in our previous investigations [10]; this fact indicates that the action of bone marrow on the IgE response, which we discovered previously, is effected with the aid of SF. Other possible targets for SF are the memory cells, which would account for suppression of the secondary IgE response when SF was injected simultaneously with reimmunization at the third week. The absence of activity of SF when injected at the third week after primary immunization can be attributed to the absence of target cells at this stage of the response because of the transition from precursor cells into mature antibody producers.

Incidentally, the action of SF depends on its dose. When different quantities of SF were injected (from 1 to 20 μg/g body weight) into mice simultaneously with immunization with OA, a suppressor effect of varied intensity was recorded: the greater the dose of SF injected, the stronger inhibition of the IgE response (Fig. 3).

It can thus be tentatively suggested that the suppressor action of mouse BM cells on the IgE response to OA is mediated through a humoral factor with molecular weight of 8000 daltons. The action of SF is realized at the moment of initiation of the primary or secondary IgE response and it continues for 2 weeks.

These results are of great interest in connection with the possibility of regulating biosynthesis of IgE antibodies.

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