SUPPRESSION OF THE IMMUNE RESPONSE TO OVALBUMIN *IN VIVO* BY ANTI-IDIOTYPIC ANTIBODIES

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Depending on the conditions of injection of anti-idiotypic (AID) antibodies development of the immune response may be either intensified or suppressed [7-9, 11, 12, 15]. Development of methods of inhibition of the immune response with the aid of AID antibodies is a promising step toward the creation of new clinical methods of correction of immunogenesis in allergic and autoimmune processes.

In the investigation described below conditions of suppression of the immune response to a food allergen (ovalbumin) were studied with the aid of AID antibodies.

EXPERIMENTAL METHOD

Hen ovalbumin (OA, from Olaine Factory, Lavtia), additionally purified on DEAE-cellulose [1], was used.

Experiments were carried out on BALB/c and CBA mice weighing 18-20 g. The mice were immunized with OA in a dose of 100 μg per mouse, with $Al(OH)_3$ as adjuvant. The number of IgG-antibody-forming cells (AFC) was determined on the 9th-10th day in the suspension of splenocytes from immune mice by a modified [2] method of indirect passive hemolysis in gel [10].

The source of antibodies to OA was the pooled ascites fluid from 25 hyperimmune mice [1], which was subjected to preparative isoelectric focusing [3]. Antibodies were isolated from the resulting protein fractions and tested for inhibitor activity by the method of direct radioimmunologic analysis (RIA): the test system consisted of the reaction of binding the globulin fraction (AID-sera) to the total preparation of antibodies to OA from balb/c mice and a ^{125}I-labeled total preparation of antibodies to OA of these same animals [1, 3]. The maximal inhibitory action on this reaction was observed with antibodies from fractions with pI of 6.2-6.7 [3]. Rabbits were immunized repeatedly with antibodies of these fractions in order to obtain serum (AID_{pI} sera). The resulting serum was thoroughly adsorbed on normal serum globulins of BALB/c mice immobilized on CNBr-Sepharose (Pharmacia, Sweden). Proof of the specificity of the AID_{pI} serum was obtained by inhibition of binding of a test system of the indirect RIA, which consisted of an immune complex of a total preparation of mouse antibodies to OA and the serum to be tested, on polyvinyl chloride wells with which the ^{125}I-labeled sheep's antibodies against rabbit globulin fraction interacted. Simultaneously with the test serum, during formation of the immune complexes antibodies to OA, normal serum globulins of BALB/c mice, or plasmacytoma immunoglobulins (including the IgA fraction from MOPC315 ascites fluid, possessing specificity toward trinitrophenyl) were added. These experiments showed that about 200 ng of antibodies was needed to obtain 50% inhibition of binding in the indirect RIA test system, whereas addition of 10,000 ng of normal or plasmacytoma immunoglobulins did not inhibit binding significantly (Fig. 1).

Addition of the AID_{pI} serum in a final dilution of 1:200 in situ during Jerne's test [2, 10] with immune splenocytes of BALB/c mice inhibited the appearance of AFC on average by 30-40%. This reaction was antigen-specific, for similar treatment, in the system for determining AFC to sheep's red blood cells (SRBC) inhibited not more than 5% of AFC. On the basis of these data and others in the literature [5, 14] it was concluded that the effect of inhibiton was due to neutralization of antibodies with an idiotype (idiotypes) detectable by

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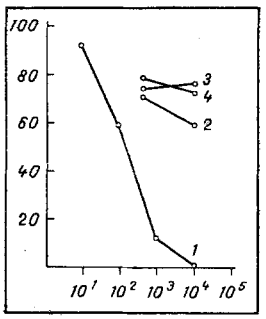

Fig. 1. Inhibition of binding of AID_{pI} serum with total preparation of antibodies to OA of BALB/c mice in indirect RIA reactions (1), with normal immunoglobulins of BALB/c mice (2), with IgA fractions of ascites fluid from plasmacytoma MOPC315 [3], and with IgG_1 fraction of ascites of plasmacytoma MOPC21 (4). Abscissa, concentration of inhibitors (in ng per well); ordinate, percentage binding of ^{125}I-labeled sheep's antibodies against globulin fraction of rabbit serum. Binding of label in absence of inhibitors (about 5,000 cpm) was taken as 100%, binding of label to wells covered with normal globulin fraction of BALB/c mice (about 550 cpm) taken as 0%.

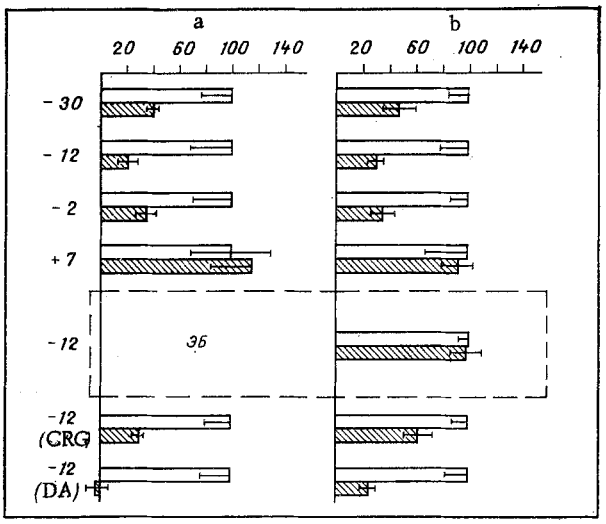

Fig. 2. General (a) and positive idiotypic (b) IgG-antibody formation to OA or SRBC in BALB/c mice after injection of 1000 μg of serum globulin fractions of an intact rabbit (unshaded columns) or of AID_{pI} serum (obliquely shaded columns). Vertical axis, time of injection of globulin fractions (in days) relative to day of immunization with OA (+7 corresponds to injection of preparations into primed animals with injection of reacting dose of OA on day +20). CRG) injection of Carrageenan in a dose of 500 μg per mouse 5 h before globulin fractions, DA) injection of globulin fractions in deaggregated form; horizontal axis — percentage of AFC (response to injection of globulin fraction of an intact rabbit taken as 100%).

AID_{pI} serum, and the number of AFC inhibited by the addition of serum was interpreted by us as a positive idiotypic response.

All the experimental data were subjected to statistical analysis and the results are given in the form $M \pm m$.

EXPERIMENTAL RESULTS

The globulin fraction of AID_{pI} serum (AID_{pI} antibodies), if injected intraperitoneally in a dose of 1000 μg per mouse at different times before immunization, induced suppression not only of the positive idiotypic response, but also of the general response to OA (Fig. 2).

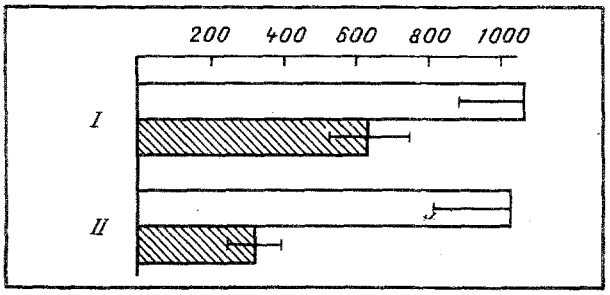

Fig. 3. Effect of serum globulin fractions of intact rabbit (unshaded columns) or of AID_{pI} serum (obliquely shaded columns, injected in dose of 1000 μg 12 days before immunization with OA, on IgE antibody titer in BALB/c mice. I) Native globulins, II) de-aggregated globulins. Horizontal axis — reciprocals of titers of IgE antibodies.

Normal rabbit globulin was injected into control animals at the same times. As will be clear from Fig. 2, the maximal effect was observed when AID_{pI} antibodies were injected 12 days before immunization; however, after injection of the preparation either 2 or 30 days beforehand, marked suppression was observed. This effect on the response to OA also was antigen-specific. Proof of this was obtained when the action of AID_{pI} antibodies (Fig. 2) was studied on the response to SRBC in BALB/c mice. Injection of AID_{pI} antibodies into primed BALB/c mice did not induce suppression of the IgG response (Fig. 2). The effect of suppression of the response to OA was absent in CBA mice after injection of AID_{pI} (the response in the control and experiment was 10,106 ± 3,236 and 9,927 AFC per spleen, respectivley).

Since the AID_{pI} antibodies which were used were a xenogeneic protein for mice, we considered that reducing the rate of utilization of this preparation as the antigen would enhance the immunosuppressive effect. Two methods were used to test this hypothesis. The first was injection of Carrageenan (from Serva, West Germany), which, according to data in the literature [4], significantly reduces the phagocytic activity of macrophages. However this treatment did not give the expected increase, but on the contrary, it reduced suppression of the general response (Fig. 2), which can be attributed to a side effect of Carrageenan on other immunocompetent cells. The other method was to use AID_{pI} antibodies in the de-aggregated form [6] (two-thirds of the topmost zone of the supernatant after ultracentrifugation for 1.5 h at 150,000g was taken). The preparation thus obtained gave the strongest immunosuppressive effect in these experiments: total suppression of the positive idiotypic response and suppression of the general response by almost three-quarters (Fig. 2).

Considering that OA is an allergen and that its injection gives a marked IgE response, we studied the action of AID_{pI} antibodies on synthesis of these immunoglobulins by the method in [13]. The results showed that if the AID_{pI} antibodies were not de-aggregated beforehand, IgE synthesis was depressed by less than half, and this difference was on the borderline of statistical significance. Injection of AID_{pI} antibodies in the de-aggregated form lowered the titer of IgE antibodies by more than two-thirds (Fig. 3).

In our view, the AID_{pI} serum which was obtained is directed against the immunodominant idiotype represented most strongly in fractions of antibodies to OA with pI = 6.2-6.7. With the aid of this preparation it was therefore possible to obtain suppression not only of the positive idiotypic response, but also of the general response. It will be noted that the de-aggregated preparation possessed stronger inhibitory properties against IgE synthesis, and also against the positive idiotypic IgG response, and this could be of practical importance in the future. On the basis of data on the times of development of suppression, we consider that its development depends, not on induction of anti-idiotypic antibodies of suppressor cells *in vivo*, but on tolerance of the corresponding clone of lymphoid cells by these antibodies.

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MODIFICATION OF MONOCLONAL ANTIBODIES BY POLYMERS POSSESSING CHELATING PROPERTIES

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The use of monoclonal antibodies in diagnosis has increased steadily in recent years. Their use is based on the production of corresponding antibodies against characteristic components of a diseased organ or tissue, labeling the antibodies thus obtained with a radioactive or other label, which can be visualized by noninvasive methods, intravenous injection of labeled antibodies into the patient, and subsequent recording of accumulation of the label in the target zone. This approach has already been tested in the diagnosis of myocardial infarction [4], when antibodies against cardiac myosin are used to visualize the zone of necrosis, in the diagnosis of thrombosis [2], when antifibrin antibodies are used, and in the diagnosis of certain tumors [10], when antibodies against components of the tumor cells are used as carriers of the label. To label antibodies, γ-radioactive isotopes (^{111}In, ^{99m}Tc, ^{123}I, ^{131}I) or metals with a strong NMR signal (Mn, Cd) may be used. In the first case, to obtain an image of the affected zone, a Gamma-camera is used; in the second case an NMR tomograph is used. Several methods of binding the label to antibodies have now been developed, the most effective being preliminary modification of the antibodies by groups chelating heavy metals, such as ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA) [7].

Unfortunately, all existing labeling methods have obvious disadvantages. When the radioactive marker and radioisotope diagnosis with an antibody is used, in principle a sufficient amount of radioactivity can be bound; but to obtain a good picture a long exposure is needed, in order to remove unbound label from the bloodstream, since it is impossible to distinguish the activity specifically bound with the affected zone against such a background. In the case of NMR diagnosis, to obtain a clear picture a high local concentration of the metallic label must be obtained because of the relatively low sensitivity of the method. Since in general it is impossible to bind more than three or four atoms of a metal with one molecule of an antibody (because of loss of activity and specificity by the antibody as a result of "remodification"), to accumulate a sufficient quantity of marker in the required site a large quantity of antibodies has to be injected, and this is highly undesirable. The ideal marker of a pathological zone would be antibodies capable of carrying a large number of atoms of a marker metal per molecule, without losing their activity and specificity in so doing, and one which

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