Individual Variations of the Immune Response in BALB/c Mice

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The nature and the causes of variations of the immune response to thyroid hormones are analyzed in BALB/c mice.

Key Words: *immune response; thyroid hormones*

In an animal population, the immune response to the same antigen varies in intensity and specificity. It has been shown that purebred animal lines differ in sensitivity to various antigens. Little attention has been paid to the variations of the individual immune response of purebred animals, and yet such information is very important for the production of monoclonal antibodies. BALB/c mice respond well to most antigens, and therefore they are often used for monoclonal antibody production. In this work we studied the immune response in BALB/c mice to thyroid hormones.

MATERIALS AND METHODS

Thyroxine, triiodothyronine, and hemocyanin from the *Fissurella* mollusk (KLH), bovine serum albumin (BSA), Freund's adjuvant, peroxidase-labeled anti-rabbit Ig antibodies, Tween-20, glutaraldehyde, sodium borohydride, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (Sigma), o-phenylenediamine (H-Roche), and 96-well polystyrene plates (Nunc) were used. Thyroxine and triiodothyronine were conjugated with protein carriers (hemocyanin from *Fissurella*, T-KLH), as described elsewhere [1,2] and used for immunization. The conjugation reaction was carried out with the use of glutaraldehyde.

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Thyroxine and triiodothyronine conjugates with BSA (T-BSA) were employed in immunoenzyme assay for the determination of antibodies. These conjugates were prepared with the use of hydrochloride 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

The mice were immunized intraperitoneally and subcutaneously with emulsion (2:1, 0.2 ml) consisting of complete Freund's adjuvant and solution containing T-KLH (0.1 mg) in phosphate-buffered saline (PBS, pH 7.4). Six weeks later the mice were again immunized with the antigen in incomplete Freund's adjuvant and then once a month for 5 months. For the determination of antibodies to the hapten, blood was collected from the retroorbital sinus 7 days after the last immunization.

For the determination of antibodies to triiodothyronine and thyroxine in solid-phase immunoenzyme assay the antigen T-BSA in 0.05 M sodium carbonate-bicarbonate buffer (pH 9.6) in a concentration of 2 μg/ml was incubated in 96-well polystyrene plates (120 μl per well) for 3 h at room temperature. The solid phase was then washed with water and dried.

Immune serum (100 μl per well, 10-fold dilution in PBS containing 0.05% Tween-20 and 0.2% BSA) was incubated for 1 h at 20°C with plastic-adsorbed antigen. The plates were washed and incubated for 45 min with horseradish peroxidase-labeled antibodies to mouse Ig (100 μl per well). After the incubation the plate was washed with water, and the enzyme activity was measured. For this purpose substrate solution (100 μl) con-

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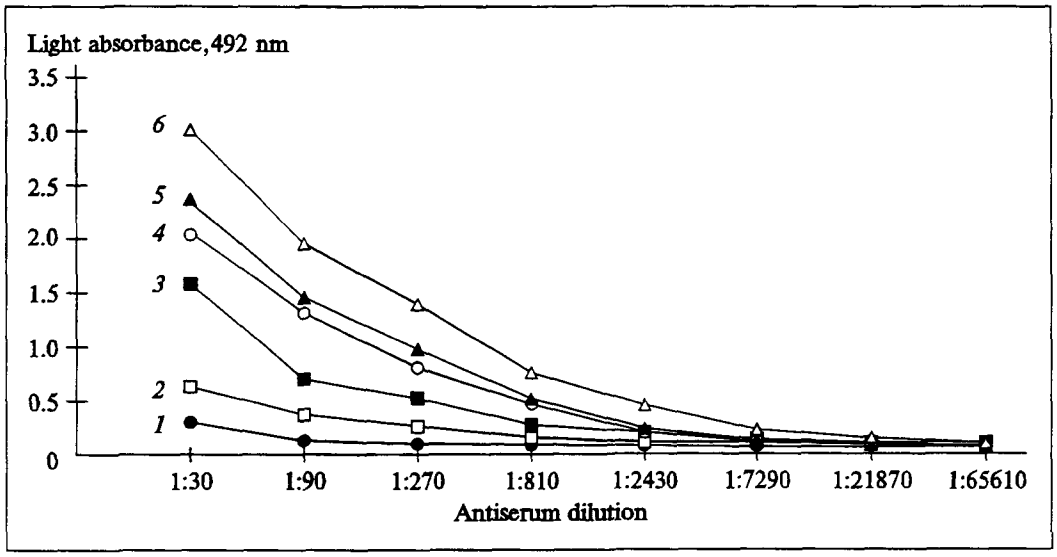

Fig. 1. Determination of the titer of antitriiodothyronine antibodies in mice immunized with triiodothyronine–KLH. *1*) control; *2–6*) different mice.

taining 4 mg orthophenylenediamine and 4 μl 30% hydrogen peroxide in 10 ml 0.1 M sodium-citrate buffer (pH 5.0) was added to each well and incubated for 5-10 min. The reaction was stopped by the addition of 10% sulfuric acid (100 μl), and light absorbance was recorded at 492 nm. The antibody titer and its dilution corresponding to 50% binding were calculated from these data.

The antibody specificity was assessed by inhibition of the antibody binding to immobilized antigen by thyroxine. The solutions were prepared in 0.01 M NaOH. Hapten dilutions of 0.1-1000 ng/ml in the buffer for analysis (50 μl) were prepared in the plate and incubated for 1 h at room temperature with 50 μl antigen in the dilution corresponding to 50% binding. Further analysis was carried out as described above.

RESULTS

Previously, we demonstrated that mice immunized with the thyroxine-KLH conjugate differ considerably in the anti-thyroxine antibody titers [1]. Similar results were obtained after immunization of mice with the triiodothyronine-KLH conjugate: the antibody titer varied from 1:500 to 1:20,000 (Fig. 1).

The antibody titer is a general parameter depending both on the amount of antibodies and on their affinity. The decrease in the amount of antibodies to thyroid hormones in some animals can be partially explained by the fact that the animals had been exposed to different competing immunogens, for example, to a latent infection. These variations may be caused by differences in thyroid status, since some antibodies to endogenous antigens are blocked by endogenous matter circulating in the blood. It should be noted that the mice differed not only in the titer of antithyroxine and antithyronine antisera, but also in the affinity and specificity of the forming antibodies. This was demonstrated previously for antithyroxine antisera [1], but a similar situation was observed in the population of mice immunized with the triiodothyronine-protein conjugate, as can be seen from the curves illustrating inhibition of the antibody binding to immobilized triiodothyronine-BSA conjugate by free hormones (Fig. 2). Obviously, antisera *A*, *B*, and *C* have a strong affinity for triiodothyronine but differ considerably in specificity, antiserum *A* noticeably cross-reacts with thyroxine, antiserum *B* does not distinguish between thyroxine and triiodothyronine, and antiserum *C* has the highest specificity for triiodothyronine. Some antisera recognize well only the triiodothyronine-protein conjugate but do not react with free triiodothyronine and thyroxine (antiserum *D*). Presumably, antibodies to the complex antigenic determinant containing triiodothyronine and a fragment of the protein molecule predominate in such antisera.

Thus, although a homogeneous animal population was immunized, all parameters of the immune response to thyroid hormones varied considerably. In order to account for this it is necessary to remember that the immune response develops in several steps:

- the conjugate processing;
- specific recognition of the antigen by immunoglobulins on the B-cell surface;
- activation of B cells initiated by rapid oligoclonal proliferation, which is accompanied by hypersomatic mutation of variable regions of the genes coding for immunoglobulins, which results in the formation of up to 10,000 B cells during a 3-day period [3].

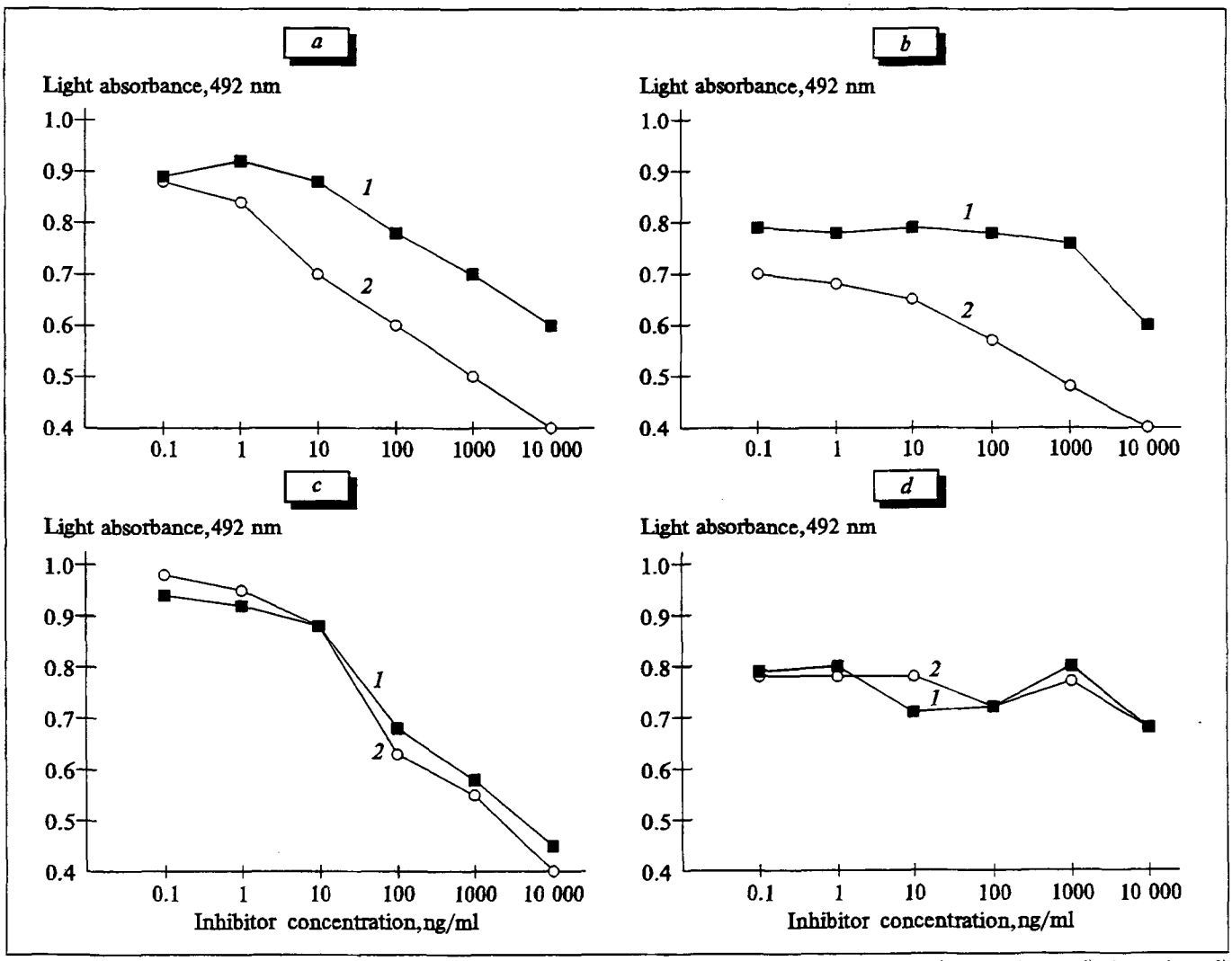

Fig. 2. Production of specific anti-triiodothyronine antibodies by different mice. *a*, *b*, *c*, and *d*: antisera; *1*) thyroxine; *2*) triiodothyronine.

The first two steps are practically the same in pure-strain animals, but the third step is different. The results of mutations do not coincide in different animals, since this is a random process. Therefore, irrespective of interplay of the same embryonal genes in pure-strain animals, the antibodies encoded by genes that have undergone mutations can diverge markedly.

Interestingly, mice immunized with conjugate of another hapten, digoxin, produce a more homogeneous immune response. Presumably, for this degree of divergence the immune response to various haptens also depends on the number of embryonal genes involved in the activation process during the primary immune response.

From the results obtained in this study it can be concluded that the animals with the most pronounced immune response should be selected for the production of monoclonal antibodies; this selection will increase the probability of obtaining monoclonal antibodies with the desired affinity and specificity.

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