ANALYSIS OF THE DYNAMICS OF ANTIBODY FORMATION EXEMPLIFIED BY ANTIBODY PRODUCTION IN RABBITS IMMUNIZED WITH Vi-ANTIGEN OF *Salmonella typhi*

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When determining the characteristics of an immune response it is important to establish the quantity of antibodies synthesized at the various stages of the reaction. The absolute titer of antibodies present at a particular moment in an animal's serum cannot provide an adequate index, because it depends not only on the intensity of antibody formation at that time, but also on the quantity of antibodies formed previously and the rate of their destruction in the body.

In the present investigation a method of estimating the quantity of antibodies synthesized is proposed, enabling the dynamics of the immunological response to be determined accurately.

EXPERIMENTAL METHOD

Rabbits weighing 2.0-2.8 kg were immunized intravenously with 400 μg of a chemically purified preparation of Vi-antigen obtained from microorganisms of the strain Salmonella typhi Ty_2 [1, 21]. The Vi-antibodies were determined by the passive hemagglutination reaction, and their physicochemical nature was established by treating the test with a 0.2 M solution of cysteine [3]. In the passive immunization experiments the rabbits were injected intravenously with 5-15 ml of homologous Vi-serum with a titer of 1:5000-1:20,000. The Vi-antibodies in these sera completely lost their serological activity after treatment with cysteine.

EXPERIMENTAL RESULTS

The dynamics of the antibody titer in the rabbits' serum after a single injection of Vi-antigen is shown in Fig. 1. The quantity of antibodies formed in successive time intervals (Δt) was assessed on the basis of the index of antibody production (ΔA).

The derivation of the formula for calculating this index is given below*. Let A_t be the quantity of antibodies in the serum at the end of the period of time Δt, and let $A_{t-\Delta t}$ be the quantity of antibodies at the beginning of Δt (the initial antibodies). If the antibodies entering the serum remained in it indefinitely, the increase in the antibodies after a time Δt would be $A_t - A_{t-\Delta t}$. However, in fact, a natural destruction of the antibodies takes place in the organism, and two corrections must therefore be made in the calculation.

Correction for natural destruction of initial antibodies. It has been shown experimentally that the natural destruction of the antibodies is expressed by an exponential equation of the type:

$$N_t = N_0 e^{\frac{-0.698\Delta t}{T}},$$

where N_0 and N_t are the quantities of the substance at the beginning and end of Δt, e is the base of the natural logarithms, and T is the half-life period of the particular substance. Correspondingly, of the initial antibodies $A_{t-\Delta t}$, after a period of time Δt, an amount

$$A_{t-\Delta t} \cdot e^{\frac{-0.693\Delta t}{T}}.$$

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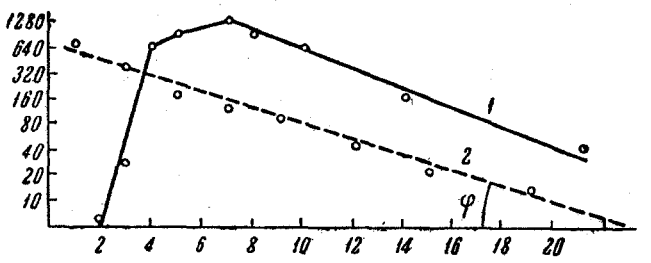

Fig. 1. Dynamics of circulating Vi-antibodies in rabbits after active and passive immunization. 1) Titer of Vi-antibodies after intravenous injection of 400 μg Vi-antigen; 2) titer of antibodies after intravenous injection of Vi-serum. Along the axis of ordinates – quantity of antibodies in serum (reciprocal of the titer); along the axis of abscissas – time after immunization (in days); The period of natural destruction of antibodies φ is equal to the cotangent of the angle φ.

Correction for natural destruction of antibodies formed during the period Δt. The value of this correction depends on how the intensity of antibody formation varied during the period Δt. Let us take extreme cases. If the whole antibody production took place at the beginning of Δt, the correction for the destruction of these antibodies would be $e^{\frac{0.693\Delta t}{T}}$. If, on the other hand, all the antibodies were formed at the end of Δt, the correction would be $e^{\frac{0.693\cdot 0}{T}} = 1$. Accepting that the value of log A changed in a straight line throughout the period Δt,* the value of this correction must be $e^{\frac{0.693\Delta t}{2T}}$.

With the corrections for the natural destruction of the initial and the newly formed antibodies, the formula for the calculation appears as follows:

$$\Delta A = e^{\frac{0.693\cdot\Delta t}{2T}}\cdot(A_t - A_{t-\Delta t}e^{\frac{0.693\Delta t}{T}}),$$

or in a simplified form:

$$\Delta A = kA_t - k^{-1}A_{t-\Delta t},$$

where

$$k = e^{\frac{0.693\Delta t}{2T}}.$$

The last formula may be transformed as follows. Since $e^{-0.693} = 2$, $k = 2^{\Delta t/2T}$. Taking logarithms of both parts of the equation, we obtain:

$$\log k = \frac{\Delta t}{2T}\log 2 \approx 0.3\frac{\Delta t}{2T} = 0.15\frac{\Delta t}{T}.$$

Hence, for calculating ΔA the period of the natural half-life of the antibodies (T) must be determined. For the γ_2 antibodies of rabbits, T = 4.6-6 days [4, 8, 17]. However, in the present experiments the Vi-antibodies lost their serological activity after treatment with cysteine, i.e., they were macroglobulins. In passive immunization

*This assumption is essential because it determines the choice of the rational value of Δt when planning the experiment. The maximal error of calculation $(\frac{\Delta A_{calculated}}{\Delta A_{total}})$ when $\Delta t = 0.5$ t is $\times 1.2$, when $\Delta t =$ t it is $\times 1.4$, and when $\Delta t = 2T$ it is $\times 2$.

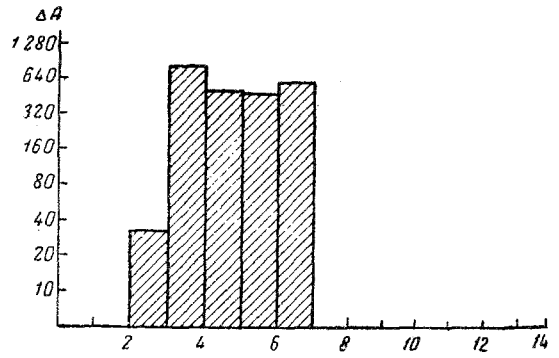

Fig. 2. Dynamics of antibody production in rabbits after intravenous immunization with 400 μg Vi-antigens. Along the axis of ordinates – ΔA (in hemagglutinating units); along the axis of abscissas – time after immunization (in days).

experiments on 5 rabbits it was found (Fig. 1) that for antibodies of this type the value of T varied from 2.7 to 3.4 days (mean 3.1±0.2 days). This is in agreement with figures given by other authors [19]. When T = 3.1 days and Δt = 1 day, we obtain:

$$\log k = 0.15\frac{\Delta t}{T} = 0{,}0485, \text{ and } k = 1.1.$$

Consequently, $\Delta A = 1.1A_t - 0.9A_{t-\Delta t}$.

The results of the calculation using this formula provide an accurate picture of the dynamics of antibody formation. It is clear from Fig. 2 that the productive phase of antibody formation included two successive stages: a stage of logarithmic increase in the quantity of synthesized antibodies and a stage of stationary production. The logarithmic increase in antibody synthesis was observed during the 3rd and 4th days of the immune reaction, and the period of doubling of the intensity of synthesis varied from 4 to 7 h for the different animals. Having reached its maximal level, antibody production continued for the next 3 days at roughly constant intensity (the stationary stage). These two stages in antibody production are analogous to those described by Ingraham on the basis of his analysis of his own findings and data in the literature [11].

These special features which were observed in the process of antibody formation are interesting from the point of view of the cellular mechanisms of antibody production. In the course of the logarithmic stage the quantity of antibodies synthesized increased by 4-6 $\log_2$ (i.e., by 16-64 times) per day. Meanwhile, the minimal interval between successive cell divisions in the plasma-cell series is 12 h [7, 13, 15, 20]. Hence, the rate of proliferation of the antibody-forming cells (twice in the course of 24 h) cannot account for the observed rates of increase in antibody synthesis. Another factor in the development of the reaction must evidently be qualitative changes in the cells, their functional transformation, revealed by the appearance of or a sharp increase in antibody production in each cell. These qualitative changes may be represented in two ways: a) all the cells enter the productive phase at once; subsequently, besides proliferation of the cells, there is a progressive increase in synthesis of antibodies by each cell; b) the cells differ in the duration of their inductive phase, [12]. In these circumstances the number of cells entering the productive phase increases progressively throughout the logarithmic stage, and reaches its maximum at its end.

Attention is drawn to the quick cessation of antibody production at the end of the first week, which was disclosed by this analysis. A similar pattern was observed by other authors in experiments in vivo [11, 18] and in vitro [6, 10, 16] with different antigens. Evidently, this dynamic pattern of antibody production is typical.

The scheme suggested by Nossal explains the cessation of antibody synthesis by death of the producing cells [14]. It is known, however, that the main producers of antibodies (especially during the primary response) are cells which are not terminal forms – transitional cells, blast cells, immature plasma cells [2, 9, 15, 22]. It is, therefore difficult to explain the rapid cessation of antibody synthesis by massive death of the cells, more especially because such a phenomenon has not been described morphologically.

The arguments given above suggest that the lymphoid cell, which begins to synthesize antibodies intensively in response to the action of the antigen, may then revert to an inactive state. This hypothesis of the reversibility of the process complicates the interpretation of antibody formation from the standpoint of the clonal selection hypothesis [5]. It requires direct experimental verification.

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