DIFFERENCES BETWEEN CYTOCHEMICAL INDICES OF HUMAN BLOOD LYMPHOCYTES AFTER ADMINISTRATION OF TETANUS TOXOID AND BCG VACCINE

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Activity of succinate dehydrogenase, α-glycerophosphate dehydrogenase, and acid phosphatase was investigated cytochemically in the circulating blood lymphocytes before and after (4, 7 and 14 days) administration of tetanus toxoid and BCG vaccine. In the period of antibody synthesis the activity of the dehydrogenases increased while that of acid phosphatase showed no significant change. BCG vaccination led to a significant increase in acid phosphatase activity and a decrease in succinate dehydrogenase activity, with a compensatory increase in α-glycerophosphate dehydrogenase activity. The high acid phosphatase activity and the decrease in succinate dehydrogenase activity are regarded as criteria of hypersensitivity of delayed type during its formation and at the height of its expression. During antibody synthesis there is a correlation between activity of the dehydrogenases which is absent after BCG vaccination.

Previous investigations [1, 2] showed that injection of antigenic preparations is accompanied by significant changes in the metabolism of the peripheral blood lymphocytes. This is expressed as a change in activity of succinate, α-glycerophosphate, and lactate dehydrogenases. Acid phosphatase activity of the lymphocytes also undergoes significant changes. The level of the changes in acid phosphatase activity is most closely connected with the character of the course of the period of immunogenesis. The appearance of complications of various types after administration of diphtheria and tetanus toxoids (second to ninth day) is accompanied by a marked increase in hydrolase activity [4]. Meanwhile, in the absence of clinical abnormalities, acid phosphatase activity fluctuates only slightly during the period of immunogenesis [4]. An increase in dehydrogenase activity and slight changes in acid phosphatase in the peripheral lymphocytes are observed during the formation of hypersensitivity of delayed type [2]. Conversely, administration of Freund's adjuvant with BCG vaccine (to simulate adjuvant disease) is accompanied by a simultaneous decrease in dehydrogenase activity and an increase in acid phosphatase activity, which coincide with the period of possible transfer of the autoimmune processes [3].

The object of the present investigation was to study the cytochemical indices of human blood lymphocytes after administration of a monoantigen (tetanus toxoid) and of BCG vaccine.

EXPERIMENTAL METHOD

Tests were carried out on 42 children aged 7 and 14 years undergoing routine revaccination; 22 children received tetanus toxoid by subcutaneous injection and 20 received BCG vaccine intradermally. During the first 24 h neither local nor general reactions to injection of the antigens were observed.

Cytochemical investigations were carried out on films of blood taken from the finger before and on the 4th, 7th and 14th days after vaccination. Activity of acid phosphatase (3. 1. 3. 2) in the blood lymphocytes

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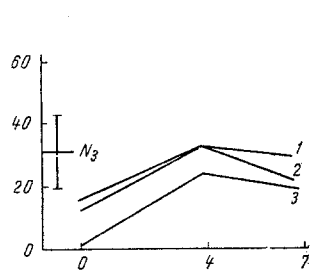

Fig. 1

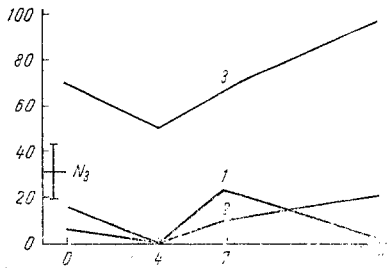

Fig. 2

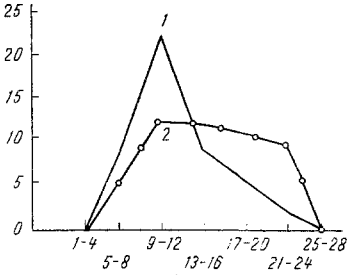

Fig. 3

Fig. 1. Activity of succinate dehydrogenase (1), α-glycerophosphate dehydrogenase (2), and acid phosphatase (3) in blood lymphocytes before and after injection of tetanus toxoid. Here and in Fig. 2: ordinate, number (in percent) of cells with high and moderate activity; abscissa, day after vaccination; N_3) normal level of acid phosphatase activity ($\pm\sigma$); 0) before vaccination.

Fig. 2. Activity of succinate dehydrogenase (1), α-glycerophosphate dehydrogenase (2), and acid phosphatase (3) in blood lymphocytes before and after injection of BCG vaccine.

Fig. 3. Polygon of frequencies of lymphocytes with different levels of succinate dehydrogenase activity before (1) and on the fourth day after (2) injection of tetanus toxoid. Ordinate, number of cells; abscissa, number of granules per cell.

was studied by the method of Goldberg and Barka [11], and activity of succinate (1. 3. 99. 1) and α-glycerophosphate (1. 1. 99. 5) dehydrogenases by Nartsissov's method [5, 6]. The results were expressed as percentages of lymphocytes with high and moderate enzyme activity and the number of granules in 50 lymphocytes [6]. In the statistical analysis of the results the mean value (M) and the error of the mean (m) were calculated and the significance of the differences assessed.

EXPERIMENTAL RESULTS

The tests showed that the activity of these enzymes in the peripheral blood lymphocytes before vaccination varies, especially the acid phosphatase activity. After injection of the toxoid the dehydrogenase activity increased (fourth day) and then gradually fell until the seventh day. Acid phosphatase activity during the same period followed the course of the changes in dehydrogenase activity (Fig. 1). The changes in activity of the enzymes were statistically significant ($P < 0.05$).

After BCG vaccination the dynamics of activity of these enzymes was rather different in character (Fig. 2). The increase in acid phosphatase activity ($P < 0.01$), normalization of α-glycerophosphate dehydrogenase activity, and decrease in succinate dehydrogenate activity ($P < 0.05$) toward the 14th day should be noticed in particular. Microscopic examination of the films at these times showed not only a decrease in the number of cells with moderate and high succinate dehydrogenase activity, but also a substantial decrease in the deposition of formazan in the lymphocytes with low enzyme activity and the complete disappearance of enzyme activity in individual cells.

Investigation of the acid phosphatase activity showed that whereas before vaccination the reaction product was deposited as distinct granules, after injection of BCG vaccine it was diffusely distributed in some of the lymphocytes, and on the 14th day enzyme activity was found in all the cells. For this reason it should be noted that lymphocytes in which acid phosphatase activity could be detected but succinate dehydrogenase activity was absent were observed in the circulating blood.

After injection of tetanus toxoid, besides an increase in the number of cells with moderate and high succinate dehydrogenase activity, the deposition of formazan was also increased in the cells with low activity. Analysis of the population structure by the use of a quantitative method of assessment of enzyme activity (counting the number of formazan granules in individual lymphocytes) showed that after vaccination there was a regular increase in the number of granules in each lymphocyte (Fig. 3).

Statistical analysis of the results showed that not only the dynamics of dehydrogenase activity after administration of antigens but also the level of correlation between these changes is important. In particular, the level of succinate dehydrogenase activity in the lymphocytes before revaccination with toxoid was independent of the corresponding values of α-glycerophosphate dehydrogenase activity. On the 4th and 7th

days after injection of toxoid a tendency was found for these indices within the group to be directly dependent on one another. At the same time after BCG vaccination, despite parallel changes in the activity of the dehydrogenases, no such tendency toward correlation of the indices could be found. These results confirm once again the character of the differences between the dynamics of dehydrogenase activity after administration of tetanus toxoid and BCG vaccine. After these results had been obtained it was necessary to compare the results of BCG vaccination with the classical manifestations of immunogenesis, during which there is increased antibody formation. Restoration of the normal cytochemical indices on revaccination with toxoid appears by the seventh day and remains unchanged for the following week [1, 4]. The increase in activity of the dehydrogenases (mitochondrial markers) developing after administration of tetanus toxoid is in full agreement with the increase in the number of mitochondria in the peripheral lymphocytes which is known to take place during active antibody synthesis [9]. The change in succinate dehydrogenase and acid phosphatase activity found in children after BCG revaccination is very reminiscent of the dynamics of the relationship between the enzymes (at the corresponding times) in the cells of animals receiving injections of Freund's complete adjuvant with BCG vaccine [3]. In children, however, these changes are less marked. In addition, injection of BCG vaccine alone induced only a partial decrease in succinate dehydrogenase activity and, moreover, it was accompanied by definite normalization of α-glycerophosphate dehydrogenase activity. From the point of view of energetics, this level of α-glycerophosphate hydrogenase activity must compensate for the isolated decrease in succinate dehydrogenase activity.

The cytochemical data described in this paper are further confirmation of the morphological differences found in lymphoid tissue during the period of formation of hypersensitivity of delayed type and of antibody synthesis [8]. If it is remembered that the blood lymphocytes reflect to a definite degree the character of the processes taking place in lymphoid tissue, this tendency toward synchronization of the activity of the dehydrogenases which can provide optimal energetic conditions for antibody synthesis after administration of tetanus toxoid can be understood.

Meanwhile, after BCG revaccination, which in certain stages is accompanied by a decrease in dehydrogenase activity, relatively unfavorable conditions for anabolism are created in the lymphocytes. Under these conditions additional factors leading to a decrease in activity of oxido-reduction enzymes must evidently contribute to a still greater decrease in the production of high-energy compounds and an increase in membrane permeability. Changes of this type are evidently among the factors provoking the onset of pathological processes induced by lysosome destruction [7, 10].

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