SYNCHRONIZATION OF DNA SYNTHESIS IN SPLEEN CELLS FOLLOWING A SECOND ANTIGENIC STIMULATION

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Immunocompetent cells in mice begin to synthesize DNA in the first hours after secondary antigenic stimulation with diphtheria toxoid, and DNA synthesis takes place in three successive waves. Among the intensively multiplying cells, a group of pyroninophilic small lymphocytes can be distinguished, characterized by a high rate of protein synthesis and a relatively low rate of RNA synthesis. Following intensive proliferation of the pyroninophilic small lymphocytes, reticular cells begin to participate in the process.

In recent years, synthesis of antibodies and DNA in lymphoid cells during immunogenesis has been investigated cytologically and biochemically [3,9,10,12,14,19]. Plasma cells, hemocytoblasts, and in some cases medium-sized and small lymphocytes are active producers of antibodies [17,19]. However, the importance of the lymphocyte in antibody formation is small, and its role is considered to be that of precursor of the plasma cells [4,17]. The function of storage of immunologic memory has been ascribed to the lymphocyte [7,15]. Reticular cells are also regarded as ancestors of antibody-forming cells [21]. Hence, despite the many investigations of this problem, the points of conflict regarding its principal aspects remain unresolved and the kinetics of DNA synthesis in cells participating in the antigenic response has not been studied in sufficient detail.

The object of this investigation was to study the dynamics of DNA synthesis during the secondary immune response and to assess the participation of cells of different types in DNA synthesis at different times after injection of antigen.

EXPERIMENTAL METHOD

Experiments were carried out on 119 male mice of line Balb, weighing 18-22 g. The mice were immunized by a single injection of diphtheria toxoid in a dose of 20 Lf per animal. Between 2 and 3 months later, the animals received a second injection of diphtheria toxoid in the same dose. At different times after stimulation the animals were sacrificed and the spleens transferred to a medium (Eagle's medium + Hank's solution, 1:1) containing one of the radioactive precursors of nucleic acids and protein (uridine-H^3, 10 μCi/ml; thymidine-H^3, 4 μCi/ml; leucine-H^3, 20 μCi/ml). The cells with precursors were incubated at 37° for 30 min in the case of thymidine, 45 min for uridine, and 60 min for leucine, with periodic shaking. After incubation and washing with cold Hanks's solution, films were made of the cells which were fixed in a 3:1 mixture of alcohol and acetic acid for 10 min. The films were treated with 5% TCA for 20 min at 4° and then covered with type M (NII KhIMFOTO) emulsion. After development, the films were stained with methyl green–pyronine by Brachet's method and with azure II–eosin by Romanovsky's method. As a control of incorporation of nucleic acid precursors and staining, some of the preparations were treated with ribonuclease and deoxyribonuclease. The number of cells labeled with thymidine-H^3 was determined by analysis of 2000-5000 cells. The results were subjected to statistical analysis by the method of confidence intervals (from 6 to 12 animals were taken for each period of the immune response). To rule out the influence of the circadian rhythm of mitosis, the animals were sacrificed at the same time of day.

EXPERIMENTAL RESULTS

Investigation of films of spleen cells from sensitized animals not receiving a secondary stimulation (control) revealed a fairly standard picture. The proportion of cells synthesizing DNA was 1.2 ± 0.22%, and DNA was synthesized by blast cells and medium-sized and small lymphocytes. The picture changed

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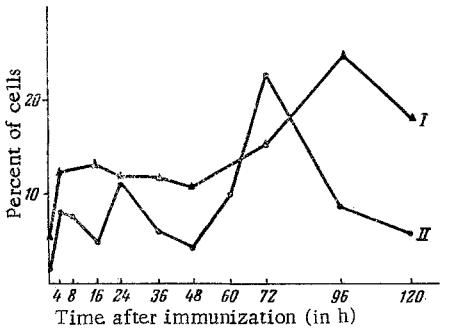

Fig. 1. Percentage of pyroninophilic small and medium lymphocytes (I) and cells labeled with thymidine-H^3 (II) in the mouse spleen at various times after a second injection of antigen.

abruptly 4 h after secondary stimulation. A large proportion of the cells (8.2 ± 0.74%) began to synthesize DNA. After 8 h the proportion of labeled cells fell slightly (differences not significant), but by 16 h after stimulation the response had fallen to 4.7 ± 1.2%. Fresh increases in the proportions of labeled cells took place at the end of the first (11.8 ± 0.36%) and third (22.6 ± 1.4%) days after injection of antigen, the maximum occurring on the third day (Fig. 1.). At intermediate points (36 and 48 h) a decrease in the number of cells in the S period was observed, with some increase to 9.7% 60 h after secondary stimulation. In the other papers we have consulted, no such pattern was described, probably because of the techniques used. In most investigations thymidine-H^3 was injected into the animals either repeatedly over a long period of time or as a single injection, with a long interval between the times of injection and taking the material.

A momentary picture of DNA synthesis in the population of lymphoid cells was thus obtained at various times after the second injection of antigen, disclosing synchronization of DNA synthesis in cells participating in the response to the antigen. Cell proliferation in the spleen of intact animals is known to be desynchronized [6], but some investigators describe fluctuating or cyclic processes taking place in lymphoid organs under the influence of an antigenic stimulus [13,16]. It may be postulated that synchronization of periods of DNA synthesis during the secondary response is due to the fact that metabolism in the cells in contact with the antigen is activated at precisely the same time. According to the findings of Cohen and Talmage [12], proliferation of cells participating in the response begins 5-6 h after the second injection of antigen. On the basis of the present observations and bearing in mind that the length of the S-period in most mammalian cells is 7-8 h [11], it may be postulated that contact with antigen leads within the first few hours to activation of the processes necessary for entry of the cells into the S-period. The results of this investigation are confirmed by those of others. The work of Dutton and co-workers [14], for instance, showed that for activation of DNA synthesis the time of contact of the cells with the antigen is not important. Grutman [3], who investigated nucleic acid synthesis during immunogenesis, showed that the amount of DNA is increased 2 h after injection of the antigen.

An analysis was next carried out of cells labeled with thymidine-H^3 belonging to various types. The proportion of plasma cells increased in the course of the immune response from 0.05% in the control to 1.5% on the 4th day after stimulation. The number of blast cells increased significantly on the 2nd day after immunization to reach a maximum at the 72nd hour of the response. At this time about 64% of all the blast cells were labeled with thymidine-H^3.

In the nonstimulated animals, the number of labeled reticular cells was small, amounting to 0.1-0.5% of the total number of reticular cells in the film and to 21.4% of the total number of labeled cells. During development of the immune response, the number of labeled reticular cells increased to 14.8 ± 1.3% (of the total number of reticular cells), and their proportion of the total number of labeled cells increased to 39.6 ± 1.12% on the 2nd day after injection of antigen.

In the group of intensely proliferating cells, cells with structural features of small and medium lymphocytes but differing from them in the distinct pyroninophilia of their cytoplasm could be distinguished. Such cells had a high rate of DNA synthesis and a characteristic morphology. As a rule these cells were round or oval in shape and of the same size as the small or medium lymphocytes. Large chromatin granules were visible in their nuclei, but less frequently the nucleus of these cells appeared vitreous and homogeneous. Often binuclear forms or cells with a deep indentation of their nucleus were seen. The volume of the cytoplasm was larger than in typical small and medium lymphocytes, and it is clearly stained by pyronine (this effect was abolished by ribonuclease).

The number of these cells was small in the nonstimulated animals (3.8 ± 0.22%). The number of pyroninophilic small lymphocytes 4 h after stimulation increased to 12.6 ± 0.38%, and it remained at this level, with slight fluctuations, until 60 h, thereafter increasing to reach a maximum 96 h after stimulation (Fig. 1).

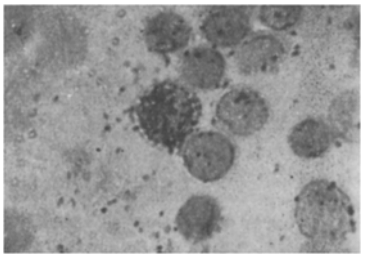

Fig. 2. Pyroninophilic lymphocyte intensely labeled with leucine-H^3. Methyl green-pyronine, 1200 ×.

At all stages of the immune response, among the pyroninophilic lymphocytes was a small number of cells with pycnotic and fragmented nuclei. This group of cells proliferated rapidly. With an increase in the total number of labeled cells 4 h after stimulation, the number of cells of this type among them was particularly high, namely 64.3 ± 2.6%. At later periods the relative proportion of these cells in the population of labeled cells diminished, but nevertheless remained fairly high (32.4 ± 1.3% 72 h after stimulation). The rate of protein synthesis (reflected by incorporation of leucine-H^3) in the population of pyroninophilic lymphocytes was more than twice as high as the velocity of protein synthesis in typical small lymphocytes (Fig. 2, preliminary data). The velocity of RNA synthesis in these cells was much lower than in typical small lymphocytes, but in some cells of this type the rate of RNA synthesis was sharply increased, and was comparable with that in the blast cells.

The appearance of pyroninophilic small lymphocytes during immunogenesis has been observed by some investigators [2,5,17] who associate the increase in the ribonucleoprotein content in these cells with antibody synthesis. Fridenshtein and Lebedev [8], observed the appearance of a large number of pyroninophilic small lymphocytes in the late stages of the immune response.

On the other hand, at certain stages of immunogenesis, typical (not pyroninophilic) small lymphocytes with a high velocity of RNA synthesis appear [1,9,18], possibly a reflection of the conversion of such lymphocytes into the pyroninophilic form.

The facts described above provide an adequate justification for a future more detailed investigation of the metabolism, structure, and functions of the pyroninophilic lymphocytes described.

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