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NATURE AND MECHANISM OF SYNTHESIS OF NONSPECIFIC IMMUNOGLOBULINS

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UDC 612.017.1

Mice were immunized with Vi-antigen. Suspensions of spleen cells, removed at various times after immunization, were incubated in Eagle's medium in the presence of ^{14}C-glycine. Synthesis of antibodies against Vi-antigen, autoantibodies against mouse IgG, and antigen-dependent nonspecific immunoglobulins (NIg) were determined by the use of specific immunosorbents. Immunization with Vi-antigen sharply intensified in the synthesis of antigen-dependent NIg. The formation of the proteins is thus observed not only during immunization with thymus-dependent antigens, but also in response to thymus-independent antigen. The synthesized antigen-dependent NIg were not autoantibodies against endogenous IgG.

KEY WORDS: Vi-antigen; antibodies; nonspecific immunoglobulins; autoantibodies; immunoglobulin biosynthesis.

It was shown previously [1, 6, 7] that immunization of animals leads not only to antibody synthesis, but also to the formation of antigen-dependent nonspecific immunoglobulin (NIg). It has been suggested that synthesis of the latter takes place in the same cells as antibody synthesis [4]. However, the use of thymus-dependent antigens in these experiments permitted an alternative explanation of the formation of antigen-dependent NIg by the action of nonspecific stimulating T-factors [9, 10]. Meanwhile the report on the discovery of a considerable number of cells synthesizing antibodies against endogenous IGg in the spleen of immunized mice [8] suggested that a substantial part of these antigen-dependent NIg are autoantibodies.

To test both these hypotheses, the synthesis of antibodies, autoantibodies, and NIg by spleen cells in vitro after immunization with T-independent Vi-antigen [3] was investigated.

EXPERIMENTAL METHOD

Female BALB/c and C57BL/6 mice weighing 14-16 g and Salmonella typhi Vi-antigen* were used. The Vi-antigen was injected intraperitoneally or intravenously into the animals in a dose of 1 μg per mouse. The spleen was removed on the 4th, 6th-8th, and 14th days after immunization. Spleens of normal nonimmunized animals served as the control. Cell suspensions were prepared from the spleens and batches of 40-50 million cells were incubated in Eagle's medium with the addition of ^{14}C-glycine at 37°C for 20 h [6]. At the end of incubation the cells were separated by centrifugation for 5 min at 600g and the supernatant was clarified for 30 min at 12,000g with cooling and used for determination of ^{14}C-immunoglobulins with the aid of specific immunosorbents [2]. Antibodies against Vi-antigen were determined with the aid of Vi-sorbent [5], autoantibodies

*The author is grateful to A. P. Alliluev for providing the preparation.

Laboratory of Chemistry and Biosynthesis of Antibodies, N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 10, pp. 437-439, October, 1979. Original article submitted February 8, 1979.

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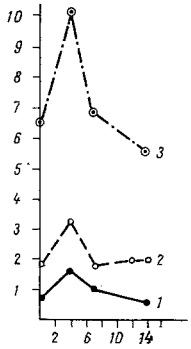

Fig. 1. Synthesis of antibodies and NIg by mouse spleen cells at different times after immunization. Abscissa, time after immunization (in days); ordinate, ^{14}C-radioactivity (in cpm per sample and 10^{-3} for curves 1 and 2, and in cpm per sample and 10^{-2} curves 3). 1) Synthesis of antibodies against Vi-antigen; 2) synthesis of antigen-dependent NIg after immunization with Vi-antigen; 3) synthesis of antigen-dependent NIg after immunization with sheep's red blood cells.

TABLE 1. Synthesis of Antibodies Against Vi-antigen, Autoantibodies, and Antigen-Dependent NIg by Spleen Cells of BALB/c Mice Immunized Intraperitoneally with Vi-antigen

Day after immunization	Increase in radioactivity, cpm per sample			
	on OA-sorbent	on Vi-sorbent	on MGG-sorbent	on anti-MGG-sorbent
—	723	1076	528	4 864
4-th	964	1322	674	8 523
8-th	1069	1882	938	10 452
14-th	784	1598	809	6 645

against mouse IgG with the aid of an immunosorbent containing mouse gamma-globulin (MGG-sorbent), and antigen-dependent NIg with the aid of a sandwich immunosorbent containing rabbit antibodies against mouse gamma-globulin (anti-MGG-sorbent). To determine nonspecific sorption, an immunosorbent containing ovalbumin (OA-sorbent) was used. To reduce nonspecific interaction between ^{14}C-proteins and immunosorbents, immunoadsorption was carried out in the presence of 0.5% Triton X-100.

The radioactivity of the samples was counted by means of an SL-40 scintillation counter (Intertechnique, France).

EXPERIMENTAL RESULTS

Determination of antibody synthesis against Vi-antigen at different stages of the immune response showed that after intraperitoneal immunization, maximal antibody formation was observed on the 8th day, compared with the 4th day after intravenous immunization. Their synthesis then gradually declined to reach the characteristic level for control unimmunized mice by the 14th day (in the case of intravenous immunization). Parallel changes were observed in the intensity of synthesis of antigen-dependent NIg (Fig. 1). Nonspecific sorption remained at about the same level throughout this period, namely about 5% of the specific sorption.

The observed increase in the intensity of synthesis of antigen-dependent NIg, as already stated, could be due to the formation of autoantibodies against mouse IgG. To determine the synthesis of these autoantibodies, after removal of antibodies against Vi-antigen from them the culture fluid was treated with MGG-sorbent, and not until then, with anti-MGG-sorbent. Data showing an increase in the radioactivity of the immunosorbents obtained in this experiment are given in Table 1.

The results indicate that the increase in radioactivity on the immunosorbents which ought to have extracted antibodies against IgG (MGG-sorbent) did not exceed that on the nonspecific immunosorbent (OA-sor-

bent) and was much less (only one-tenth) than the increase in radioactivity of the immunosorbent which extracted NIg (anti-MGG-sorbent). This means that no significant formation of autoantibodies against mouse IgG takes place as a result of immunization with Vi-antigen. Consequently, antigen-dependent NIg are not autoantibodies against endogenous immunoglobulin.

The results of these experiments showed that the formation of antigen-dependent NIg during immunization with T-independent antigen obeys the same rules as their synthesis in response to T-dependent antigen (for comparison a curve reflecting the formation of antigen-dependent NIg after immunization of the mice with sheep's red blood cells [6] is shown on the same graph). This suggests that the formation of antigen-dependent NIg is not connected with the nonspecific stimulating action of T-cells, but is due to the effect of the antigen directly on B-cells. However, this conclusion is not absolutely strict, for the possibility cannot be ruled out that, despite the T-independent character of the action of Vi-antigen, if T-cells are present in the animal, it can still induce the liberation of nonspecific stimulators of the immune response by these cells, and synthesis of antigen-dependent NIg could be attributed to their action. For a final solution to the problem of the role of T-cells and to rule out the suggestion that the synthesis of antigen-dependent NIg is due to the action of nonspecific factors secreted by them, experiments on athymic animals are therefore necessary.

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