IMMUNOGLOBULIN-POSITIVE HUMAN SPLEEN AND THYMUS CELLS DURING EMBRYOGENESIS

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The relative percentages and types of luminescence of immunoglobulin-positive cells were investigated by the indirect immunofluorescence method in the spleen and thymus of 32 human fetuses between the 11th and 32nd weeks of development. The number of Ig-positive cells in the spleen increased during embryogenesis from 13 to 33.7%, but in the thymus it remained at 0-2% regardless of the stage of development of the fetus. For the first time a differential count of Ig-positive cells based on types of luminescence was carried out in the course of embryogenesis. Thymus cells had solitary points of luminescence on their surface. Lymphocytes with single, and later with multiple points, progressing to solid luminescence over the whole surface and, finally, cells with "caps" of luminescence appeared successively in the spleen during development of the fetus. The differences in the density of immunoglobulin receptors on the surface reflect the degree of differentiation of the lymphocyte.

KEY WORDS: spleen; thymus; embryogenesis; B-lymphocytes; immunofluorescence.

Investigators are currently paying great attention to the formation of immunocompetence of lymphocytes in developing organs of the human immune system. The presence of plasma cells in the spleen of a fetus from a sick mother was first reported in 1965 [4]. Since that time further data have been collected which have shed light on some aspects of the formation and differentiation of the population of immunoglobulin-positive (Ig-positive) B-lymphocytes during the period of prenatal development.

It has recently been shown that B-lymphocytes first appear in the spleen and thymus at 11.5-12 weeks of embryonic development [3, 6]. At the same time, their rather later appearance in the spleen has also been reported [5]. As regards quantitative studies of B-lymphocytes in organs of lymphopoiesis, data in the literature are fragmentary and contradictory. For instance, by the use of serum against $F(ab)_2$-fragments of immunoglobulins, 12% of B-lymphocytes was found in the spleen of a human fetus at the 12th week of development, whereas with an increase in age from 16 to 24 weeks, the mean number was 63%. Other workers [5] consider that there is no correlation between the number of B-lymphocytes in the spleen and age of the fetus. Up to 5% of Ig-positive cells have been found in the human fetal thymus [6-8].

There is thus as yet no satisfactory idea on how the population of B-lymphocytes is formed during human prenatal ontogeny, nor have the properties of lymphocytes been characterized in the period of formation of their immunocompetence.

The object of this investigation was to study the times of appearance, relative percentages, and types of immunofluorescence of B-lymphocytes in the human spleen and thymus during embryogenesis.

EXPERIMENTAL METHOD

Experiments were carried out on 32 human fetuses (11-32 weeks of development), obtained during termination of pregnancy on medical grounds. The age of the fetus was determined from the presumed time of ovulation of the woman and the body length of the fetus. Cell suspensions were obtained from the spleen and thymus with the aid of a liquid disintegrator [1]. The fraction of mononuclear cells was isolated from cell suspensions on a Ficoll–verografin gradient. Subsequent processing of the cell suspensions and conditions for the immunofluorescence reaction were as described previously [2]. To detect B-lymphocytes with surface IgG-receptors, a hyperimmune rabbit serum against human class G immunoglobulin, prepared in the writer's

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TABLE 1. Relative Percentages of Ig-Positive Cells of Human Spleen and Thymus during Embryogenesis (M ± m)

Age of fetus, weeks	Spleen		Thymus	
	No. of fetuses	No. of Ig positive cells, %	No. of fetuses	No. of Ig-positive cells, %
11	4	0	4	0
12—14	3	13,0±2,0	3	1,3±0,6
16—17	4	26,3±2,4	4	0,4±0,3
19—20	4	21,0±5,0	4	0,3±0,3
22—24	7	28,7±1,7	7	2,0±0,7
25—27	5	33,7±4,0	4	0,8±0,4
29—32	6	31,0±2,9	6	0,6±0,4

laboratory with the aid of V. S. Rukosuev, and also a commercial donkey serum against rabbit immunoglobulins, labeled with fluorescein isothiocyanate, were used. The optimal dilution of serum (1:10) was determined by the cytotoxic test and immunodiffusion test in agar. Two-stage staining was used as the control: with native inactivated rabbit serum and with luminescent donkey serum against rabbit immunoglobulins. One-stage staining of the cell suspension with luminescent donkey serum also was used. The relative percentage of B-lymphocytes was determined by counting 200 lymphocytes in the spleen and 1000 in the thymus. In the course of analysis of the results three types of cellular immunofluorescence were distinguished. Lymphocytes with single points of fluorescence on their surface were classes in type I, those with fluorescence ranging from multiple punctate to continuous affecting half or more of the cell surface were placed in type II, and those with local fluorescence at one pole of the cell, in the form of a "cap," were classed in type III.

EXPERIMENTAL RESULTS

At the 12th week of fetal development the proportion of B-lymphocytes with surface IgG-receptors in the spleen at the 12th week of fetal development was 13% (Table 1). After the 16th-17th weeks of development the number of Ig-positive cells increased appreciably, to reach 33.7% at the 25th week.

Differential counting of Ig-positive cells with respect to types of immunofluorescence gave the following results. At the 12th week of fetal development the splenic lymphocytes had type I fluorescence exclusively. Between the 14th and 22nd weeks of development, cells with type II fluorescence also were found. At the age of 22-24 weeks, lymphocytes forming local fluorescence of the "cap" type at one pole (type III) began to appear in the spleen. Later, all three types of fluorescence were found on the spleen cells.

As Table 1 shows, Ig-positive cells with surface IgG-receptors first appeared in the thymus at the 12th week of fetal development. At all times of testing their number was between 0 and 2%, and differential counting revealed lymphocytes with only type I immunofluorescence.

The control tests showed that native nonimmune rabbit serum and the corresponding labeled serum gave no immunofluorescence of thymus or spleen cells. One-stage staining of the cell suspension with luminescent serum likewise gave no fluorescence.

The experiments thus showed that B-lymphocytes with surface IgG-receptors first appear in the spleen and thymus at the 12th week of embryonic development. Their kinetics, studied in the present investigation, shows that the number of these IgG-positive cells increases with age of the fetus.

For the first time in this investigation lymphocytes were counted differentially depending on the type of their fluorescence. For instance, at all times of fetal development thymus cells showed only single points of fluorescence on their surface. In the spleen, however, with an increase in age of the fetus, lymphocytes belonging to all three types of fluorescence, including the formation of "caps" on their surface, appeared consecutively with an increase in age of the fetus. These results indicate that lymphocytes in the thymus and spleen differ in the density of immunoglobulin receptors on their surface in the course of embryonic development. A similar rule was found by the writer previously in rats [2].

In agreement with the view [9] that the degree of maturity of the lymphocyte correlates with the density of immunoglobulin receptors on its surface, and supported by the discovery that splenic lymphocytes form "caps" of fluorescence in the presence of antigen at the 22nd-24th week, the writer suggests that this period of embryonic development is responsible for the formation of immunocompetence of IgG-positive lymphocytes in the human fetus. It may also be noted that the number of IgG-positive cells in the thymus also increases a

little at this period. The view is held [3] that the appearance of immunoglobulins on T-lymphocytes may reflect a certain stage of their maturation and activation.

Formation of the B-lymphocyte population in man thus begins in early prenatal ontogeny; Ig-positive cells with surface IgG-receptors are present both in the thymus and in the spleen, but the density of immunoglobulin receptors on their surface and the ability of the lymphocytes to form "caps" of fluorescence in the presence of antigens are evidence of the degree of differentiation of the lymphocyte.

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COOPERATION OF ANTIGENS, IMMUNOGLOBULINS, COMPLEMENT, AND ANTIMICROBIAL ENZYMES IN REGULATION OF MOBILITY OF BLOOD GRANULOCYTES

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Cooperation between specific and nonspecific factors of humoral immunity in the regulation of granulocyte mobility was studied. Bacterial antigens of dental plaque, immunoglobulins, lysozyme, peroxidase, ribonuclease, and trypsin, each separately, were shown to produce moderate stimulation of chemotaxis and chemokinesis of granulocytes. The strongest chemotaxic effect was given by ribonuclease and the strongest chemokinetic effect by lysozyme. The strongest chemotaxic stimulus was generated on activation by complement in the classical way. Lysozyme sharply enhanced whereas ribonuclease and trypsin depressed the formation of the chemotaxis factor of complement in the classical way. Treatment of granulocytes with antimicrobial enzymes lowered their sensitivity to this factor.

KEY WORDS: chemotaxis and chemokinesis of granulocytes; bacterial antigens; immunoglobulins; complement; antimicrobial enzymes.

In the intact organism complement activation takes place parallel with an increase in the activity of antimicrobial enzymes in a focus of inflammation [1, 2, 6]. Because of the absence of data in the literature on relations between these two manifestations of humoral immunity in the generation of the chemotaxic stimulus, their separate and combined effect on granulocyte chemotaxis and chemokinesis was studied.

EXPERIMENTAL METHOD

Mobility of granulocytes isolated from 30 blood samples from clinically healthy donors was studied. The granulocytes were isolated from whole blood, diluted 1:2, by centrifugation in a two-step density gradient of

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