CYTOIMMUNOCHEMICAL ELECTRON-MICROSCOPIC ANALYSIS OF DIFFERENCES IN IMMUNOGENICITY OF LEPTOSPIRES FOR MICE OF DIFFERENT INBRED LINES USING IODIZED ANTIBODIES

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After treatment of leptospires with iodized immune globulins from mice of lines C3H and C57BL, forming antibodies of "weak" and "strong" types respectively, the localization of the antibodies of the surface of the microorganisms was determined in the electron microscope. In both cases discrete γG-antibody-like particles were observed. γM-antibody-like particles were found only on the capsule of the leptospires when globulin from C57BL mice was used.

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After primary immunization of mice of inbred lines C57BL and C3H with leptospires of canicola fever, the agglutinin titer was 20 times higher in the first (1 : 1024 – 1 : 2048) than in the second (1 : 128 – 1 : 256) [2, 4]. It is important to note that during immunization of mice of three lines with sheep's erythrocytes, line C57BL behaved as "weak" [6], thus ruling out the possibility that the general immunological reactivity of one line is higher than that of the other. A possible explanation of this phenomenon is tolerance of mice of the "weak" line to some of the antigenic determinants of the leptospire.

The object of the present investigation was to test this hypothesis. Cytoimmunochemical analysis with the aid of iodized antibodies was chosen as principal method. In this way it was assumed that individual antigenic determinants could be identified and counted.

EXPERIMENTAL METHOD

A living culture of Leptospira canicola, grown in a 10% solution of rabbit serum in distilled water, was injected intraperitoneally in a single dose of 0.2 ml into inbred C57BL and C3H mice (males weighing 18-20 g from the "Stolbovaya" Nursery). The immune serum was obtained after sacrificing the mice on the 7th day after injection of antigen. Globulins precipitated by 40% saturation with $(NH_4)_2SO_4$ were dissolved in 1.5% $NaHCO_3$ (pH 8.3). The $(NH_4)_2SO_4$ was removed by gel-filtration through a Sephadex G-25 column equilibrated with 1.5% $NaHCO_3$ solution. The globulins were iodized by the method described previously [1]. The titers of antibodies were determined by the microagglutination reaction [5]. A suspension of leptospires fixed in 0.4% formalin and suspended in 1% CH_3COONH_4 (pH 7.0) was mixed in different proportions with iodized and noniodized globulins. The mixtures were incubated for 24 h at 37° and washed twice with an identical solution of CH_3COONH_4, and centrifuged at 16,000 rpm during cooling. A drop of the resulting suspension of leptospires was applied to a grid with Formvar backing and studied with the UÉMV-100A electron microscope with au accelerating voltage of 75 kV.

EXPERIMENTAL RESULTS

Iodization of Antibodies. As Table 1 shows, the titers of iodized globulin antibodies from the serum of the C57BL mice exceeded the titers of iodized globulins from the C3H mice by one order, just as they did before iodization.

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TABLE 1. Results Obtained by Iodization of Immune Globulins

Line of mice	No. of animals	Serum		Noniodized globulins			Iodized globulins		
		volume (in ml)	titer of antibodies	volume (in ml)	protein (in %)	titer of antibodies	volume (in ml)	protein (in %)	titer of antibodies
C57 BL	50	12	1 : 2048	2.4	1.93	1 : 512	4.75	1.00	1 : 16
C3H	20	4	1 : 256	0.7	0.87	1 : 128	2.40	0.18	1 : 2

TABLE 2. Antigen – Antibody Ratio

Index	Experiment No.			
	1	2	3	4
	line of mice			
	C3H	C57BL	C3H	C57BL
Volume of solution of iodized globulins (in ml)	0.08	0.01	0.20	0.20
Volume of standard suspension of leptospires (in ml)	0.06	0.06	0.02	0.02
Volume of buffer solution (in ml)	0.11	0.18	0.03	0.03
Protein of globulins (in mg/ml suspension of leptospires)	2.70	1.70	20.0	100.0

However, in contrast to the results obtained by iodization of rabbit and horse immune globulins [1, 3, 8], in the present case iodization led to a substantial change in the absolute titers of antibodies. It is not known whether this was due to species-specific properties of the mouse serum, the chemical structure of the antigen [12], or to a slight modification of the method. The modification was necessary because of the small initial volume of serum, and it consisted of the use of gel-filtration instead of dilution of globulin with bicarbonate solution. Unfortunately, because of loss of CO_2 during gel-filtration in this case, the pH increased, requiring the addition of solid CO_2 to the globulin solutions. Nevertheless, the results subsequently obtained showed that sufficient active antibodies remained to detect the corresponding antigens on the surface of the leptospires.

Electron Microscopy. Preparations of leptospires preliminarily incubated both with iodized (experiment) and with noniodized immune globulins (control) were examined in the electron microscope. The antigen – antibody ratio varied (Table 2).

By comparing the titers of iodized globulins given in Table 1 with the volumes of ingredients of the mixtures given in Table 2, the titer of antibodies per unit volume of antigen suspension can be determined. It is easy to see that this value was the same for experiments Nos. 1 and 2. For Expts. No. 3 and 4 this ratio was higher, being 8 times higher in Expt. No. 4 than in Expt. No. 3.

Preparations of intact leptospires treated with undiluted noniodized immune globulins, and preparations treated initially with native, undiluted immune sera and subsequently with iodized globulins ("block"; Fig. 1a-c), were used as controls.

As a result, the following electron-optically dense structures were detected on the surface of the leptospires treated with iodized antibodies, in contrast to the controls.

1. Granules roughly equal in diameter to leptospires (~ 0.1-0.15 μ). In Expt. No. 1 (line C3H) there were 22 of these granules on 50 leptospires, and 59 in Expt. No. 2 (line C57BL). Since the antigen – antibody ratio was different in Expts. Nos. 3 and 4, the number of granules was not counted in these experiments.

2. Particles corresponding in size to γG-antibodies (length ~200-300 Å; Figs. 1f and 2c). These particles were localized on the cytoplasmic membrane of the leptospires and in agglomerates which were probably the precipitate formed by γG-antibodies with lysate of the leptospire.

3. Particles corresponding in size to γM-antibodies (length ~ 700 Å; Figs. 1e, 2b, and 3). These particles were seen only in Expt. No. 4 (globulins from C57BL mice taken in maximal concentration) and were localized only on the capsule of a leptospire with clearly visible periodicity.

Fig. 1. Electron photomicrographs of leptospires. a) Intact leptospire, 60,000×; b) leptospire treated with non-iodized immune globulins (titer 1 : 512), 60,000×; c) leptospire treated successively with immune serum (titer 1 : 2048) and iodized immune globulins (titer 1 : 16), "block," 50,000×; d) leptospire treated with iodized immune globulins of low concentration (Expt. No. 2), 50,000×; e, f) leptospires treated with iodized immune globulins from C57 BL mice (Expt. No. 4), 6000×.

It has previously been shown by other methods that no γM-antibodies are formed by C3H mice in response to primary immunization with hemocyanin and with polysaccharide of type III pneumococci [12]. Our results show that in mice of this line, unlike in C57BL mice, no γM-antibodies likewise are formed in response to primary immunization with L.canicola. A noteworthy feature is the difference in number of granules which we detected on the surface of leptospires treated with iodized globulins of equal, but low titers, taken from both lines of mice. These results, in conjunction with the demonstrated ability of mice only of the "strong" line to form γM-antibodies against leptospires, support the validity of the original hypothesis. However, unequivocal proof of its validity can be obtained by experiments using different antigen – antibody proportions, even to the extent of obtaining complete saturation relative to the criterion of number of granules or of single antibodies detected. In this case, equalization of the antibody titers in mice of both lines during the secondary immune response [4, 7] could be considered as the result of reaching saturation level of antibody production, which is the same for mice of these lines.

Further investigations will be carried out to prove the validity of these statements.

In conclusion, we may note that the possibility of detecting qualitative and quantitative differences in the content and localization of single antigens on membranes demonstrated in this investigation with the use

Fig. 2. Electron photomicrographs of leptospires. a) Leptospire treated with iodized immune globulins from C3H mice (Expt. No. 3), 60,000×; b, c) leptospires treated with iodized immune globulins from C57BL mice (Expt. No. 4), 100,000×.

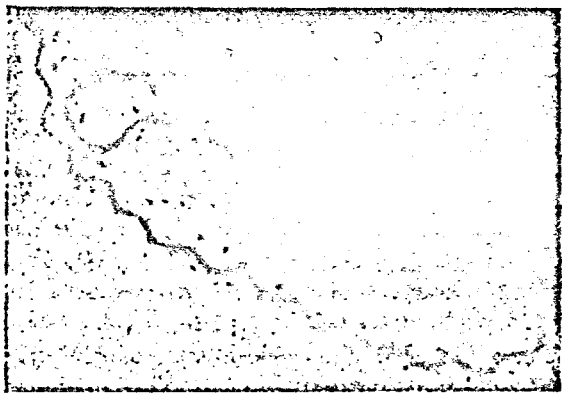

Fig. 3. Leptospires treated with iodized immune globulins from C57BL mice (Expt. No. 4), 15,000×.

of iodized antibodies gives hope that this very simple method of cytoimmunochemical analysis in conjunction with electron microscopy may be used successfully for studying the antigen topography of surface and internal structures not only of microorganisms, but also of viruses and cells. This is of great importance for many branches of biology and medicine, and primarily for elucidating the mechanism of malignant change and of immunity, both general immunity and immunity to microorganisms and viruses.

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