DYNAMICS OF FORMATION OF DIFFERENT IMMUNOCHEMICAL TYPES OF ANTIBODIES AGAINST CERTAIN L FORMS AND MYCOPLASMAS

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Antibodies of both IgM and IgG types were found in the sera of rabbits immunized with L forms of group A hemolytic streptococcus and Mycoplasma fermentans; in the course of the immune response the hemagglutinin titer rose and the hyperimmune sera contained mainly antibodies of the IgG type.

KEY WORDS: L forms of bacteria; mycoplasmas; antibodies; immunoglobulins.

It is now established that antibodies belonging to different types of immunoglobulins differ significantly in their functional activity in different serologic immunologic reactions.

Antibodies of the IgM class are much more active in agglutination and lysis reactions and in the clearance of corpuscular antigens by cells of the RES than IgG antibodies. The latter exhibit greater activity in the case of neutralization of toxins and in the passive anaphylaxis reaction. Finally, antibodies of the IgA type, because of their high avidity, are effective in neutralization reactions and, being synthesized by lymphocytes of the mucous membranes of the intestinal and respiratory tracts, they participate in the mechanisms of local immunity [2, 5, 13].

It has also been shown that the functional activity of antibodies of the same class varies in the course of the immune response depending on the character, times, and frequency of injection of the antigen [1, 15].

With these considerations in mind it becomes clear that it is no longer possible to judge the character of developing immunity without a correct idea of the immunochemical type and activity of the antibodies.

However, data in the literature on antibody formation against infectious agents with a defective cell wall or completely without it are very scanty. A few papers are devoted to the study of relations between antibodies of different immunochemical types in the sera of patients infected with Mycoplasma pneumoniae [11, 12, 16].

As regards the characteristics of antibodies formed under the influence of L forms of bacteria, no information of this kind could be found in the accessible literature.

The object of this investigation was to study the order of formation of antibodies belonging to different classes of immunoglobulins and changes in the types of antibodies in the course of formation of specific immunity induced by L forms of group A hemolytic streptococcus and Mycoplasma fermentans.

EXPERIMENTAL METHOD

Chinchilla rabbits weighing 2-2.5 kg were immunized. Antigens to produce the specific immune sera were prepared by the method described in [7] from L forms of hemolytic streptococcus L-406 isolated by G. Ya. Kagan et al. (Moscow) from a patient with rheumatic fever, and a strain of Mycoplasma fermentans (from Edward's Museum, England).

The cycle of immunization of the animals, by the method suggested by the present writers [7], lasted 3 months. Periodic tests were carried out on 18 rabbits (10 immunized with the culture of L forms, 8 with the culture of mycoplasmas). Sera taken 6-7 days after the first injection of antigen were conventionally described as "early primary" and those taken 4 weeks later as "late primary." "Secondary" sera were obtained 1 week

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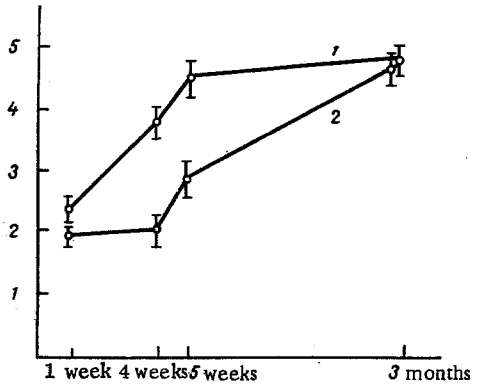

Fig. 1

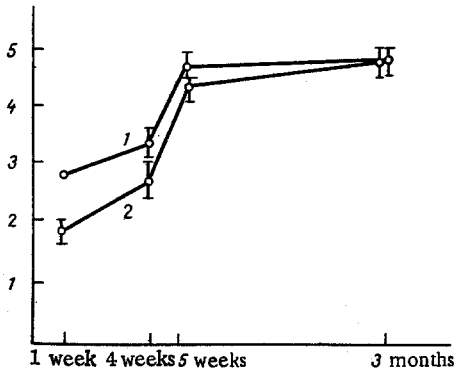

Fig. 2

Fig. 1. Hemagglutinating activity of sera against L-406 antigen. Here and in Fig. 2: abscissa, time of investigation (in weeks); ordinate, log of mean geometric titers. 1) IgM + IgG antibodies; 2) IgG antibodies.

Fig. 2. Hemagglutinating activity of sera against Mycoplasma fermentans.

TABLE 1. Distribution of Hemagglutinating Antibodies in Chromatographic Fractions of Rabbit Sera at Different Stages of Immune Response

Antigen	Serum	Titer of antibodies, log			
		original serum	fraction 1	fraction 2	fraction 3
L-406	Primary:				
	"early"	2,4	0	1,5	2,3
	"late"	3,7	1,5	1,8	2,1
	Secondary	4,5	3,4	3,1	2,9
	Hyperimmune	4,7	4,1	3,8	3,3
Mycoplasma fermentans	Primary:				
	"early"	2,8	0	2,1	2,5
	"late"	3,3	0,5	2,8	2,2
	Secondary	4,6	2,8	3,2	2,8
	Hyperimmune	4,8	5,0	4,7	2,8

after the 2nd immunization, which was given 1 month after the 1st, and "hyperimmune" sera were obtained after revaccination.

Antibodies in the sera were determined by the passive hemagglutination test (PHT) in the writers' modification [6, 8].

To establish the type of antibodies the sera were treated with 0.1 M cysteine by Chernokhvostova's method [4] and fractionated on DEAE-cellulose columns by the stepwise elution method of Adinolfi et al. [9], using the following phosphate buffers: 1) pH 6.6, M-0.02; 2) pH 8.0, M-0.08; 3) pH 8.0, M-0.25.

To characterize the protein composition of the three fractions, an immunoelectrophoretic investigation was carried out with sheep antirabbit serum. Fraction 1 was found to contain mainly slow IgG-globulins, fraction 2 fast IgG-globulins and also, perhaps IgA-globulins, whereas fraction 3 contained IgM globulins and traces of IgG-globulins.

EXPERIMENTAL RESULTS

Data showing the hemagglutinating activity of the sera in the various stages of the immune response are given in Figs. 1 and 2.

Titers of antibodies against L forms of streptococcus (Fig. 1), expressed in log of the mean geometric titer, were relatively low in the "early primary" sera (2.4 ± 0.14). After treatment with cysteine they fell a little, evidence of the presence of both IgM- and IgG-antibodies, although the latter were present in small amounts (1.9 ± 0.06).

The sera of rabbits immunized with mycoplasmas (Fig. 2) had higher over-all titers in the early stages (2.8), but also contained a small quantity of IgG-antibodies (1.9 ± 0.1). The total antibody content in the "late" L-sera 4 weeks after the first immunization was increased (3.7 ± 0.3, whereas the level of IgG-antibodies remained comparatively low (2.1 ± 0.14). The over-all titer of hemagglutinins in the antimycoplasma sera also was raised at these times (3.3 ± 0.1), and the titer of IgG-antibodies also was increased (2.7 ± 0.4).

Repeated immunization led to a sharp increase in the overall titers of the sera (4.5 ± 0.25 and 4.6 ± 0.2); IgM-antibodies still predominated at this period in the sera of the rabbits receiving L forms of streptococcus, whereas in the "secondary" sera against M. fermentans the titer of IgG-antibodies was sharply increased (4.4 ± 0.2).

The hyperimmune sera of both groups of animals obtained after intensive revaccination (intramuscular, intradermal, and intravenous injection of antigens) contained antibodies in high titers (4.7 ± 0.3 to 4.8 ± 0.2). Treatment with cysteine did not lower the titers, indicating a sharply increased level of synthesis of antibodies of the IgG class. However, some idea of the actual content of IgM-antibodies in the "early" and "hyperimmune" sera could be obtained only after their fractionation, the results of which are given in Table 1.

IgM-Antibodies predominated in the "early" sera of rabbits immunized with L forms, and their titers were almost equal to the total titer of antibodies detected in the PHT. When IgG-antibodies were determined it became evident that their titer was almost equal to that obtained after the reaction with cysteine (Fig. 1); moreover, it was due to the presence of IgG-antibodies of the fast type. In the "late" sera the over-all titer of hemagglutinins consisted of the sum of the increased IgG-antibodies, including slow (fraction 1) and fast (fraction 2), whereas the IgM-antibody level was rather lower.

An increase in the titer of antibodies belonging to both classes of immunoglobulins was observed in the secondary and hyperimmune sera, mainly on account of the IgG-antibodies of both types.

When the results of fractionation of the anti-mycoplasma sera are examined it must be noted that in the "early" and, in particular, in the "late" primary sera the formation mainly of IgG-antibodies was observed, initially fast and later slow. Antibodies of the IgG type predominated in the secondary and hyperimmune sera, just as in the first group.

Antibodies belong to both IgM and IgG types were thus found in the sera of rabbits immunized with microorganisms lacking in a cell wall; in the course of immunization, the titer of hemagglutinins rose, with predominance of antibodies of the IgG type in the hyperimmune sera.

This character of the immune response is due in all probability to the special features of the structure of the L forms and myoplasmas deficient in a cell wall and, consequently, in the antigens of lipopolysaccharide type belonging to the cell wall [3].

It is now well known that the nature of the antigen has a considerable effect on the kinetics of appearance of antibodies belonging to the different classes of immunoglobulins. It has been shown, for example, that some polysaccharides and complex cellular antigens induce the prolonged synthesis of 19S antibodies, whereas protein antibodies and, in particular, preparations obtained from the flagella of Salmonella adelaidae and other organisms induce principally the production of IgG-antibodies [1, 5, 10, 14].

It can be concluded from these observations that sera containing antibodies of both the macro- and microimmunoglobulin type, possess hemagglutinating properties.

The further study of the activity of different types of antibodies in immune reactions such as inhibition of growth and metabolism, the most accurate indicators of immunity in infections caused by microorganisms deficient in the cell wall, will be of great interest.

LITERATURE CITED

1. É. Z. Rukhadze, Zh. Mikrobiol., No. 7, 90 (1971).
2. É. Z. Rukhadze and E. V. Chernokhvostova, Zh. Mikrobiol., No. 8, 53 (1971).
3. V. D. Timakov and G. Ya. Kagan, L-Forms of Bacteria and the Mycoplasmataceae Family in Pathology [in Russian], Moscow (1973).
4. E. V. Chernokhvostova, Lab. Delo, No. 6, 323 (1965).
5. E. V. Chernokhvostova, Usp. Sovrem. Biol., 73, No. 3, 444 (1972).
6. N. V. Chumachenko and S. A. Goncharova, Lab. Delo, No. 11, 683 (1973).
7. N. V. Chumachenko and T. D. Smirnova, Lab. Delo, No. 1, 39 (1974).

8. N. V. Chumachenko, A. S. Labinskaya, E. P. Ponomareva, et al., Sov. Med., No. 7, 31 (1974).
9. M. Adinolfi, P. Mollison, and M. Polley, J. Exp. Med., 123, 951 (1966).
10. D. Bauer and A. Stavitsky, Proc. Nat. Acad. Sci. USA, 4, 1667 (1961).
11. G. Biberfeld, J. Immunol., 100, 338 (1968).
12. G. Fernald, W. Clyde, and F. Denny, J. Immunol., 98, 1028 (1967).
13. J. Lo Spalluto, W. Miller, B. Dorward, et al., Clin. Invest., 4, 415 (1962).
14. J. Robbins, K. Kenny, and E. Suter, J. Exp. Med., 122, 385 (1965).
15. A. Shirai, O. Sobeslavsky, and R. Chanoen, Am. J. Epidemiol., 84, 301 (1966).
16. G. Torrigiani, I. Roitt, and D. Taylor-Robinson, J. Exp. Med., 122, 81 (1965).

COMPATIBILITY OF F-LIKE PLASMID FB1drd WITH STANDARD F-GROUP PLASMIDS IN STRAINS OF *Escherichia coli* K12

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Compatibility of derepressed F-like plasmid FB1drd, integrated into the chromosome of *Escherichia coli* K12 cells with standard plasmids of compatibility groups FI-FVI was studied. The results show that such plasmids can coexist in a stable state in the same cell with plasmid FB1drd. This suggests that it belongs to a new compatibility group (FVII).

KEY WORDS: bacterial plasmids; compatibility; plasmid transconjugants.

One of the chief criteria for determination of the degree of phylogenetic kinship between different bacterial plasmids is the compatibility test, based on the inability of two closely related plasmids to coexist in a stable state in the same cell [3, 4]. According to this criterion, all currently known F-like plasmids can be divided into six compatibility groups (FI-FVI) [3-5].

The object of this investigation was to study compatibility of the F-like plasmid FB1drd, previously identified by the writers in cells of *Escherichia coli* serogroup O6 [2].

EXPERIMENTAL METHOD

Cells of *E. coli* strain AP106, containing standard plasmids belonging to groups FI-FVI were used as donors of the genetic material. The original strains carrying plasmids of groups FI-FV were obtained from Dr. Dennison (England); the strain with plasmid Hly-P212 (FVI) was obtained from Dr. Monti-Bragadin (Italy). Strain *E. coli* AP117 trp, thi, lac, nal (derived from strain 200PSF$^-$), bred by the present writers, and its derivatives AP118 and AP119 were used as recipients. Strain AP118 contains integrated plasmid FB1drd and is a lac$^+$-recombinant obtained by conjugating *E. coli* AP3Hfr [2] with AP117 cells. Strain AP119 was bred by the writers as a lactose-negative mutant from an AP118 population treated with nitrosoguanidine.

The bacteria were conjugated by the following standard method. 18-Hour broth cultures were diluted 1:10 in fresh nutrient broth (NB) and grown for 3 h at 37°C, after which they were mixed in the proportion of 1:4 by volume in 50-ml flasks. The conjugation mixtures were incubated for 2 h at 37°C and then seeded on media for selecting clones of recipient cells receiving the corresponding plasmid marker from the donor (plasmid transconjugants).

To study compatibility of the plasmids, the isolated transconjugants (at least three from each conjugation) were grown for 18 h in NB not containing any of the selective agents (at 37°C), and then seeded on dishes with nutrient agar (NA). The resulting clones (at least 50 from each sample) were replicated on dishes with selective agar in order to determine preservation of the corresponding plasmid markers.

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