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Spectrum of Anti-Measles Immunoglobulin G Subclasses in Convalescents after Measles

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A simple semiquantitative method for measuring anti-measles IgG subclasses is developed on the basis of commercial diagnostic test system for measurements of anti-measles IgG and a kit of peroxidase-labeled monoclonal antibodies to human IgG subclasses. During the acute phase of the disease specific antibodies are presented mainly by IgG2 antibodies, while in subjects with a history of measles more than 10 years before 2 subgroups were detected, which responded by production of IgG2 or IgG1 subclasses.

Key Words: *IgG subclasses; measles*

Despite mass vaccination against measles in industrial and developing countries, this infection is responsible for almost one million deaths annually [2]. This sad statistics is caused by extremely high virulence of measles virus, on the one hand, and by unique capacity of this virus to induce pronounced immunosuppression in the patients, on the other [1,6,8,9].

The mechanisms of formation of anti-measles immunity was extensively studied, but many important problems remain not quite clear, for example, the problem of distribution of specific IgG antibodies into subclasses. There are few reports about the spectrum of specific anti-measles IgG subclasses [3,7]. This can be explained by the lack of simple and reliable methods for detection of specific antibody subclasses. Uneven distribution of specific anti-measles antibodies into subclasses was demonstrated by means of a sophisticated qualitative method based on antibody binding to cultured *Vero* cells infected with the virus [4,5]. Difficulty of the method and impossibility of quantitative evaluation of subclasses of specific anti-measles IgG in the serum prompted us to develop a simple semiquantitative method, which can be used for screening studies.

MATERIALS AND METHODS

A commercial diagnostic kit for detection of anti-measles IgG antibodies (Vektor-Best) and peroxidase-labeled monoclonal antibodies to individual subclasses of human IgG (IgG1, IgG2, IgG3, IgG4) (Polignost) were used in the study. The test sera in different dilutions were put into wells of a 96-well panel with measles antigens fixed on the plastic. After 30-min incubation at 37°C and washing, labeled monoclonal antibodies to different IgG subclasses were added (1 μg/well) instead of the standard conjugate (peroxidase-labeled polyclonal antibodies to human IgG). The panels were incubated for 30 min, washed, and the substrate with tetramethylbenzidine stain (from the main kit) was added. Staining intensity was evaluated at λ=450 nm against 630 nm on a Humareader photometer (Human).

The group of donors consisted of 10 subjects (5 men and 5 women) aged 33-65 years. Specific anti-

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measles IgG were detected in all examined volunteers with a history of measles more than 10 years before the study. In addition, 7 patients (4 men and 3 women) aged 31-45 years were examined during the acute phase of measles. None of these patients was immunized against measles. All patients had specific IgM antibodies and increase of specific IgG titers in paired sera. The sera for studies were collected on day 12 after efflorescence and stored at -20°C.

RESULTS

The method for detecting specific immunoglobulin subclasses was developed on a collection of immune donor sera containing antibodies to measles virus in different titers. Antibody content in the sera was evaluated using Dade Behring enzyme immunoassay kits recommended by WHO for standard detection of specific anti-measles antibodies. These diagnostic kits are

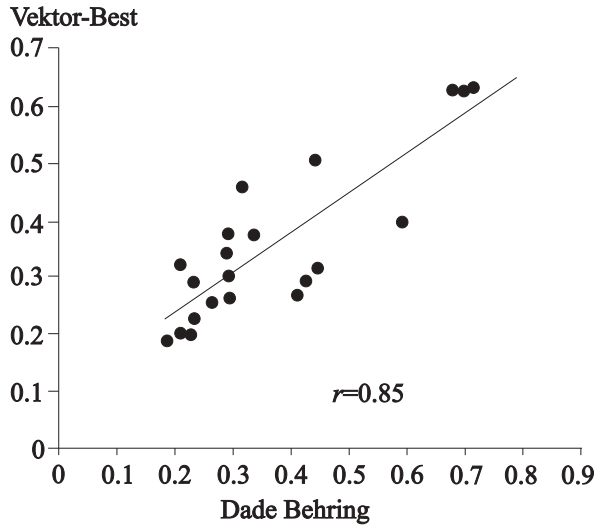

Fig. 1. Correlation of the results of titration of sera containing anti-measles IgG antibodies by two diagnostic kits. The results are presented in optical density units.

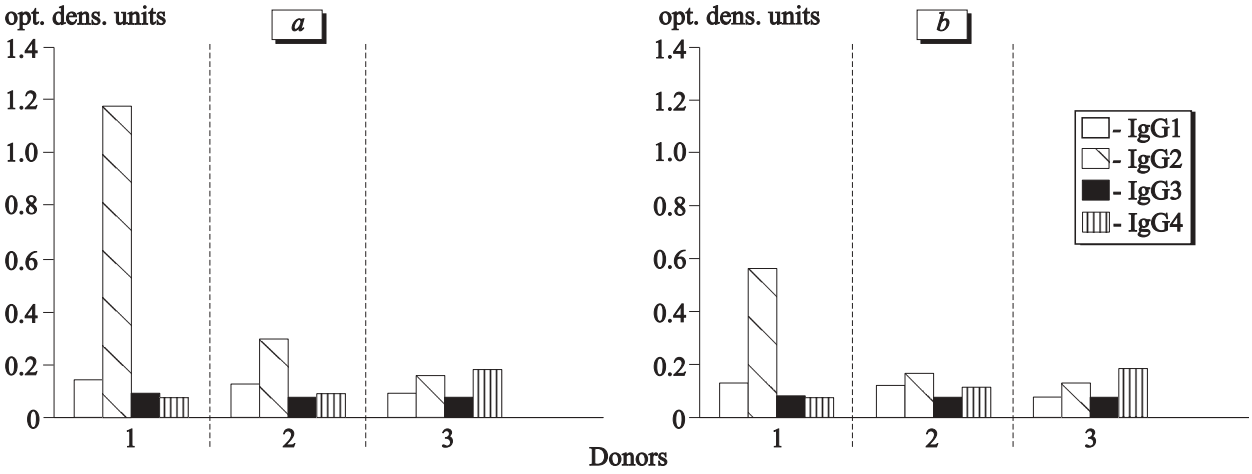

Fig. 2. Distribution of anti-measles IgG antibodies by subclasses in three immune donors. *a*) sera diluted 1:50; *b*) 1:100 dilution.

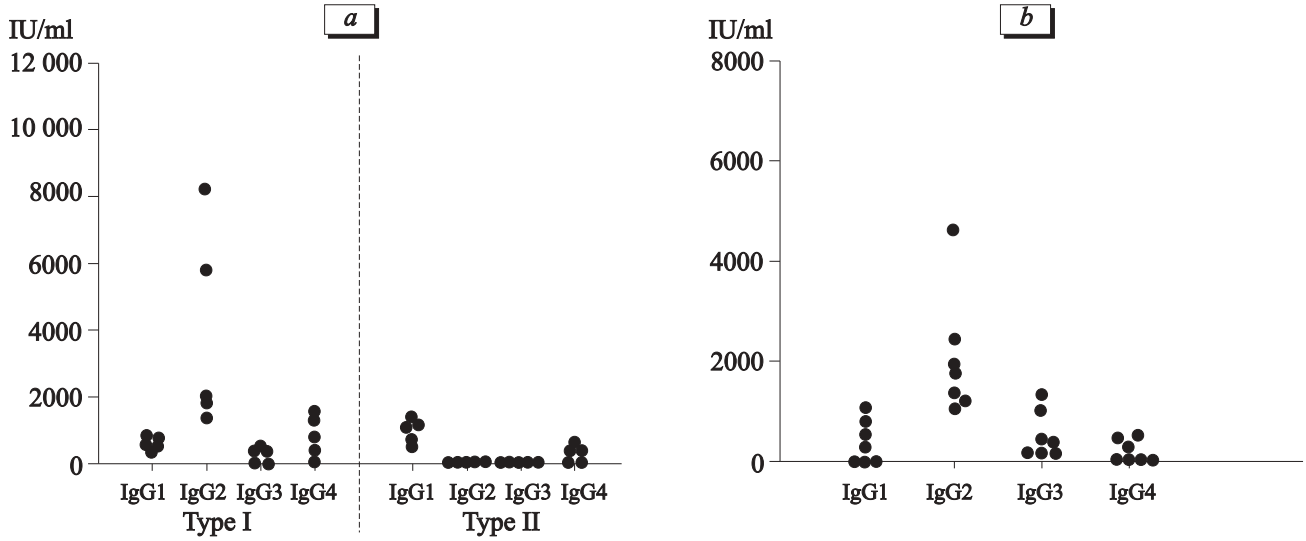

Fig. 3. Distribution of anti-measles IgG antibodies by subclasses in donors with a history of the diseases more than 10 years ago (*a*) and in patients with measles during the acute period of the disease (*b*).

expensive, and we therefore used a similar test system offered by Russian company Vektor-Best (by almost an order of magnitude cheaper). However, two diagnostic kits were compared for correct quantitative evaluation of the results. The comparison showed high correlation between the results obtained using the two kits (Fig. 1).

Sera with high, medium, and low levels of anti-measles antibodies (8800, 5600, and 3900 IU/ml, respectively) were selected from the collection of immune sera. The use of these sera in different dilutions (1:50 and 1:100) showed that 1:50 dilution is optimal, as with this dilution nonspecific reactions do not surpass the baseline level and both high and low antibody titers are clearly seen (Fig. 2). The serum of a highly immune donor titered in the Dade Behring kit was selected as the reference serum.

The sera from subjects who had measles more than 10 years before the study were analyzed. By the spectra of specific antibodies belonging to different IgG subclasses, all examinees can be divided into 2 groups: with predominant content of IgG2 antibodies and without this subclass of antibodies (Fig. 3, *a*). On the other hand, analysis of sera from 7 patients with typical clinical picture of measles showed type I distribution in all of them (IgG2 predominated, Fig. 3, *b*).

Hence, we developed a simple semiquantitative method demonstrating the content of anti-measles IgG subclasses. During the acute phase of measles specific antibodies are presented mainly by IgG2 antibodies, while subjects with a history of measles more than 10 years before testing can be divided into 2 subgroups: with predominance of IgG2 specific anti-measles antibodies (group 1) and IgG1 antibodies (group 2).

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