The specific antimeasles IgG1, IgG2, IgG3, and IgG4 subclass response was measured with immunofluorescent method by binding of IgG antibodies with Vero cells infected by the measles virus vaccine strain (8) or with an enzyme-linked immunosorbent assay (10). The first allows us to receive qualitative results only. On the other hand, standardization of solid-phase methods for determination of subclass composition of antiviral antibodies, including antimeasles antibodies, has been difficult because properly standardized isotype-specific reagents and a standard serum with assigned weight-based units of different subclasses have been missing. In this work we succeeded partly in resolving this problem using a collection of commercially available peroxidase-linked monoclonal antibodies against various IgG subclasses. A single standard serum was provided to compare the data obtained in different experiments.

The specific antimeasles IgG1, IgG2, IgG3, and IgG4 subclass response patterns elicited after vaccination or during natural infection.

MATERIALS AND METHODS

Study population. Serum samples were collected from 30 children (12 girls and 18 boys; median age, 1.39 years; range, 1 to 3 years) and 10 children (five girls and five boys; median age, 4.78 years; range, 4 to 6 years) before and 30 days after vaccination, respectively, with a trivalent live attenuated measles, mumps, and rubella vaccine, Priorix (GlaxoSmithKline, Belgium).

Serum samples were also collected from 51 late-convalescent adults (more than 10 years after measles infection) and seven adults with natural measles infection at the 12th day after the onset of rash. The serum collection was randomized. Serum samples were stored at −20°C and used within 100 days. All children were seronegative before vaccination. Seroconversion (appearance of specific IgM and IgG antibodies) was reached in 97.2% cases within 1 month. IgM measles antibodies have been found in the sera of all adults with early infection. The adult volunteers also demonstrated IgG measles antibodies. Informed consent was obtained from the parents and volunteers. The study was
Measles IgM and IgG antibodies were tested by enzyme-linked immunosorbent techniques with a commercial kit (Human, Germany). Specific IgG subclasses were measured by an enzyme-linked immunosorbent assay (ELISA) standardized according to the general principles of solid-phase immunoassay; the detailed protocol has been described elsewhere (16). Briefly, we used measles antigen-coated 96-well microplates from the ELISA test for the detection of IgG antibodies to measles virus in human serum REF 51206 (Human, Germany). Serum samples were added at a dilution of 1:50 and then incubated for 30 min at 37°C. During preliminary experiments we used various serum dilutions (1:20; 1:50; 1:100; and 1:500). The dilution of 1:50 showed a lack of nonspecific reactions and at the same time revealed both low and high IgG concentrations in the sample. After washing we added peroxidase-linked anti-IgG1 (clone 10G/2C11), IgG2 (clone 23G/3C7), IgG3 (clone 22G/5G12), or IgG4 (clone 20G/5C7) monoclonal antibodies (Polygnost, St. Petersburg, Russia) at a concentration of 1 μg/ml according to the recommendations instead of anti-IgG conjugate belonging to the commercial kit. The plates were incubated at 37°C for 30 min. After washing, color reagent solution (tetramethylbenzidine) was added. Plates were finally read at 450 nm on a microELISA reader. For comparing the results of different experiments, a standard serum containing four IgG subclasses of antimeasles antibodies has been used. A negative and positive control sera from the commercially kit for the detection of anti-measles IgG antibodies have been used as an additional control.

Statistical analysis. The differences in the frequency of specific subclasses were analyzed by the χ2 test. The t test was employed to compare the means of two groups. P values of <0.05 were considered significant.

RESULTS

In the group of children younger than 3 years, the predominant subclass was IgG3. It was present in 100% of serum samples and contributed, on average, 63.3% of the total IgG response. The frequency and contributions of specific IgG1 (72.4% and 19.9%, respectively) and IgG4 (65.5% and 16.8%, respectively) to the total IgG antimeasles response were low, whereas IgG2 was not found (Fig. 1).

In contrast, in older children (4 years and older) the predominant subclass was IgG2. It was present in 90% of serum samples and contributed 42.6% of total IgG response. Other subclasses were also present but their contribution was much lower and averaged for IgG1, IgG3, and IgG4 it was 12.0, 22.1, and 23.3%, respectively (Fig. 1). Significant difference was observed in antimeasles IgG subclass distribution between the two groups of children (P < 0.001).

The subclass profile of antimeasles IgG antibodies of adult volunteers with measles history is presented in Fig. 2. In these subjects as in vaccinated children older than 3 years, IgG2 was the predominant subclass of total IgG. Thus, in early convalescence, IgG2 contributed 62% of the total IgG response, whereas in late convalescence, the contribution was lower (41.4%). Other subclasses were also present. There were no significant differences between various IgG subclasses revealed in early and late measles convalescence.

DISCUSSION

In the present study, the distribution of measles-specific antibodies among the four IgG subclasses was investigated in vaccinated children and adults with natural infection (convalescent phase) or with long-lasting humoral immunity to measles. A predominant subclass in adult subjects and in vaccinated children aged 4 years or older was IgG2. On the contrary, in vaccinated children younger than 3 years there were no antibodies belonging to the IgG2 subclass. It is very difficult to compare our data with the results of previous investigations due to differences in the experimental design and/or methodology. Nevertheless, it may establish the fact that all four isotypes are present in the sera of measles patients during the acute and memory phases of the immune response (see Fig. 2). Hence, there is no reason to consider that the antibody response to viral antigens is restricted to any IgG subclass. Such a conclusion is in accordance with the results obtained in other investigations (3, 6, 7, 13).

In our opinion, the fact that IgG2 antibodies were lacking in vaccinated children younger than 3 years is important but not surprising. Similar results were obtained in children vaccinated against hepatitis B virus. Thus, the children below 5 years of age responded mainly with IgG1 and IgG3 subclasses, whereas in older children anti-HBs antibodies belonging to all four IgG
subclasses have been revealed (6). The data correspond with widespread opinions about the much slower maturation of the IgG2 subclass in human (4, 11, 17). In particular, it has been shown that IgG1 and IgG3 levels increase rapidly, reaching the mean adult values by 1.5 to 2 years of age, whereas the amount of IgG2 remains lower than 50% of the adult value, having not yet reached the normal concentration at the age of 9 to 12 years.

In conclusion, we failed to demonstrate visible differences in IgG subclass composition between subjects with natural infection and vaccinated children except those below 3 years of age. It may be postulated that the IgG subclass profile in measles-vaccinated subjects depends on age. The humoral immune response of children younger than 3 years is immature, and the IgG2 subclass of virus-specific antibodies has not been revealed in the sera.

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