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THE SIGNIFICANCE FOR EPIDEMIOLOGICAL STUDIES ANTI-MEASLES ANTIBODY DETECTION EXAMINED BY ENZYME IMMUNOASSAY (EIA) AND PLAQUE REDUCTION NEUTRALIZATION TEST (PRNT)

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ABSTRACT

The paper discusses the role of anti-measles antibodies for protection and significance for epidemiological studies determination of antibodies by different serological methods. The comparison of anti-measles virus antibodies levels measured by enzyme immunoassay (EIA) and Plaque Reduction Neutralization Test (PRNT) was described. It was found that the 200 mIU/ml of anti-measles activity measured by PRNT (level protection against symptomatic disease) is equivalent of 636 mIU/ml measured by EIA (Enzygnost®Anti-Measles Virus/IgG, Simens).

Key words: *measles elimination programme*, *International Units, anti-MeV Abs,*

INTRODUCTION

Strategic plan for measles and congenital rubella infection for WHO European Region assumes their elimination by 2020 (1). The success of this plan depends on achieving and maintaining high levels of population immunity. Serological surveys are important tool to assess of population immunity, however in the view of the complexity of the immune response to measles virus infection, a correct interpretation of serological test results is important.

Although the anti-measles virus antibodies (anti-MeV Abs) level which gives protection against infection or illness is still under debate, based on an efficacy study during an outbreak of measles it was shown that PRNT (Plaque Reduction Neutralization Test) titers of <120 (corresponding to 200 mIU/ml) were not protective against measles, titers of >120 but <1 052 may protect against classic measles but not against mild clinical infections, and those of >1 052 (corresponding to 1 841 mIU/ml) indicate full protection (2,3,4). While the classic PRNT is widely accepted as the "gold standard" in measles serology, nevertheless, this test is not widely used because of labor-intensive, time-consuming and technically demanding. Attempts have been made to improve the PRNT test, such as fluorescence-based plaque reduction microneutralization assay (PRMN) using a recombinant measles virus engineered to express EGFP (enhanced green fluorescent protein) developed in microscope and automated version (5), or standardized neutralization enzyme immunoassay (Nt-EIA), which employed EIA (enzyme immunoassay) to detect the inhibition of growth of measles virus in Vero cells in the presence of anti-MeV Abs (6). Although these assays (PRMN, Nt-EIA) are not so time-consuming as classic PRNT, the commonly utilized laboratory method, suitable for routine clinical application as well as for epidemiological purposes, is the enzyme immunoassay (EIA). Determining the anti-MeV antibodies by EIA, certain aspects related to the type of measured Abs should be considered (the relationship between different sets of anti-MeV antibodies was graphically presented on figure 1): a) the EIA tests detect Abs against all viral proteins, while the PRNT detects only functional neutralizing antibodies (Nt-Abs) against specific proteins: hemagglutinin (H) and fusion protein (F); b) EIA measure a specific class of Abs (IgG or IgM or IgA) while the PRNT measures Nt-Abs that could belong to all classes of antibodies; c) the antibodies to the nucleocapsid (N) protein, which do not contribute directly to neutralization (and as consequence in protection), are the most abundant antibodies formed in response to infection and immunization and therefore the EIA predominantly detects antibodies to this antigen, because N protein is also the most abundant protein found in MeV-infected cells and such as, used for coating wells of EIA plate.

The aim of this study was to compare the results of the standardized samples examination for the anti-measles antibodies presence by enzyme immunoassay (EIA) and neutralization test (PRNT) and to define the protective level of anti-MeV Abs examined by a commercial kit used in the Laboratory of Virology NIPH-NIH.

MATERIAL AND METHODS

The test used in the present study was Enzygnost®Anti-Measles Virus/IgG (Simens, formerly DadeBehring, Germany), the kit routinely used in the Laboratory of the Department of Virology, National Institute of Public Health – National Institute of Hygiene (NIPH-NIH). The kits of two lots (41961, 42196) were used.

The test were performed according manufacturer's procedure, allowing quantification by measuring the optical density (OD) of a single serum dilution in antigen and control wells. The difference of these ODs (ΔOD) multiplied by a correction factor were used to evaluate the qualitative result according to the following cut-off values: results with ΔOD <0.100 were considered as negative, results with ΔOD in range 0.100-0.200 were considered as equivocal and results with ΔOD >0.200 were considered as positive. Quantitative values of anti-MeV IgG were calculated using formula: $\log_{10}$ mIU/ml = α*ΔODβ (where α and β are lot-dependent constants, as well as nominal value used for calculate of correction factor). The quantitative results were expressed in mIU/ml.

For calibration purpose the 3rd WHO International Standard for Anti-Measles, NIBSC code: 97/648 (7) containing 3 000 mIU/ml anti-measles activity measured by PRNT was used. Standard proceedings was consistent with the attached instruction. The freezed-dried residue was reconstituted in 1 ml of distilled water, aliquoted and stored at -70°C. A series (5-points starts from undiluted sample) of two-fold dilutions were prepared and the level of anti-MeV IgG by EIA Enzygnost®Anti-Measles Virus/IgG was determined. Based on the results obtained in four independent experiments, in which serial dilutions of the standard were tested in triplicate, the calibration curve was prepared (mIU-EIA *versus* mIU-PRNT)

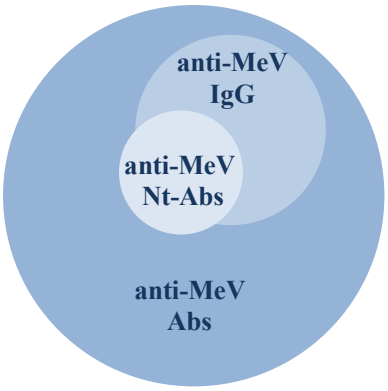

Fig.1. The relationship between different sets of antibodies against measles virus (MeV): all antibodies produced as answer to the MeV infection (anti--MeV Abs), neutralizing antibodies (anti-MeV Nt--Abs) measured by Plaque Reduction Neutralizing Test (PRNT) and a total pool of IgG (anti-MeV IgG) measured by enzyme immunoassay (EIA)

RESULTS

The preparation of two-fold dilution of the 3rd WHO International Standard results in obtaining a five samples with concentration of anti-MeV activity measured by PRNT of: 3 000, 1 500, 750, 375 and 187.5 mIU/ml respectively. The first four samples with the highest concentration were positive in EIA with ΔOD values (the mean and standard deviation for 12 examinations) of 0.955±0.28; 0.728±0.35; 0.568±0.21; 0.406±0.20 respectively. The sample with the lowest concentration (187.5 mIU/ml) was equivocal with ΔOD value of 0.198±0.11. The relationship between anti-MeV activity expressed in mIU/ml measured by EIA and PRNT was shown in figure 2. In model of linear regression the calibration curve was constructed (fig. 3). The analyzed relationship was describe by formula:

mIU/ml-EIA = 332.7 + 1.5 * mIU/ml-PRNT

(linear regression, r^2=60.9%, R=0.78, p=0.000).

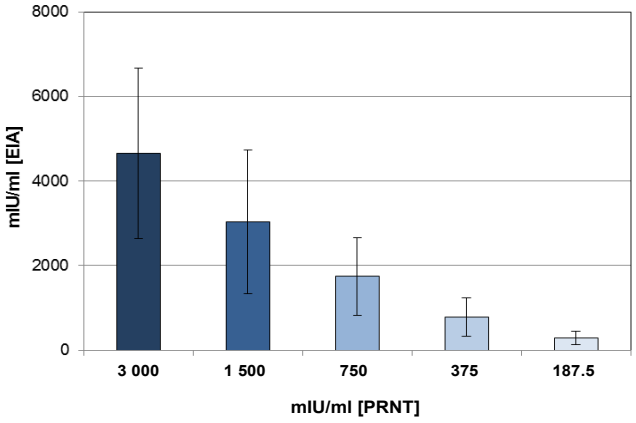

Fig.2. The level of anti-MeV IgG expressed in mIU/ml measured by EIA in five dilutions of the 3rd WHO International Standard for Anti-Measles, NIBSC code: 97/648 are shown as means ± SD (upper and lower bounds labelled) of 12 examinations

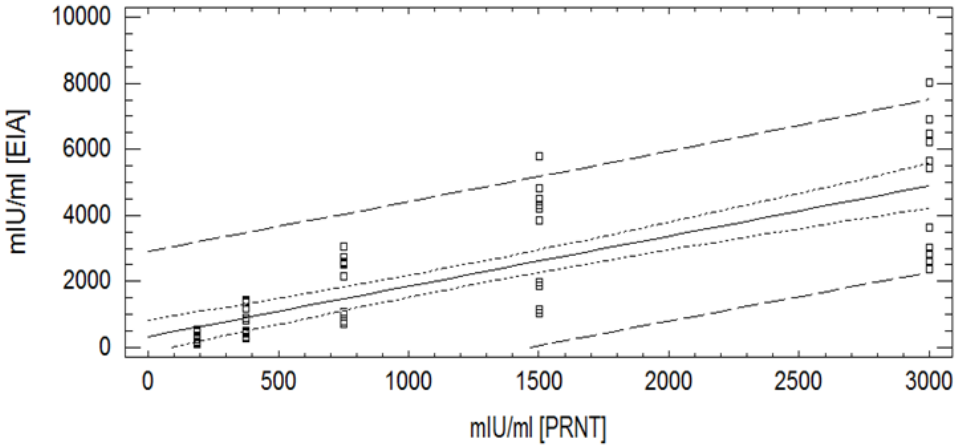

Fig.3. The calibration curve describing the relationship between anti-measles virus antibodies level measured in International Units (mIU/ml) by enzyme immunoassay (EIA) and Plaque Reduction Neutralization Test (PRNT)

DISCUSSION

Enzyme immunoassay, because to its advantage: low labor-intensive, low costs, low time-consuming, technically no-demanding is commonly utilized laboratory method for serology purpose. In this paper we presented results of comparison of anti-measles antibodies levels measured by Enzygnost EIA kit and Plaque Reduction Neutralization Test (PRNT). Analyzing the associations between antibody levels, as others, we found that EIA values were higher than neutralizing antibody values (5,8). In our study, the level corresponding to 200 mIU/ml measured by PRNT was equivalent of 636 mIU/ml measured by EIA, and this may be largely due to the different formats of methods: MeV antigens and antibodies detecting.

Although the question about the protective levels of antibodies against measles is still open, it is generally accepted that 200 mIU/ml of neutralizing antibodies protect against the classic measles (3). This level determined by EIA will be higher and as shown in ESEN study (European Sero-Epidemiology Network), varies depending on the kit used (10). In view of the fact that there are a variety of EIA kits, the calibration studies are strongly recommended. Among the commercially available tests, Enzygnost (Siemens, formerly DadeBehring) is characterized by the best parameters (9), and for this reason it is widely used in the WHO National Reference Laboratories of many countries (10). The results of the present study confirm the observations described by Janaszek et al. (11) who adopted a value of 500 mIU/ml (measured by ELISA-BehringwerkeTM, formerly DadeBehring, now Siemens) as the protective level, only slightly lower than that referred to 636 mIU/mL in this study.

The limitation of this study could include to use the 3rd International Standard for Anti-Measles, which is not recommended by WHO for tests ELISA (12). However it should be noted that this recommendation relates to the creation of unit in the measurement system (unitage). The value of 3rd IS is true with respect to the neutralization test, but not ELISA. So not about the use of the standard as such, but about the use of the its units this recommendation concerned, and in this sense results of described experiment confirms WHO recommendation, pointing to discrepancies in the results obtained by different methods.

Although antibodies are an important element of protection against measles, and their measurement allows to conclude on immunity, it should be emphasized that the mechanisms responsible for the resistance are very complex and not be “an all-or non-phenomenon” (2) as the independence between humoral and cellular measles-specific immune response was demonstrated in the recent studies (13,14).

CONCLUSION

The 200 mIU/ml of anti-measles activity measured by PRNT (level protection against symptomatic disease (3) is equivalent of 636 mIU/ml measured by EIA (Enzygnost®Anti-Measles Virus/IgG, Simens).

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