Immunodominant domains of the *Measles virus* hemagglutinin protein eliciting a neutralizing human B cell response

O. T. Ertl$^{1,2}$, D. C. Wenz$^{1,2}$, F. B. Bouche$^1$, G. A. M. Berbers$^3$, and C. P. Muller$^{1,2}$

$^1$Department of Immunology and WHO Collaborating Center for Measles, Laboratoire National de Santé, Luxembourg
$^2$Medizinische Fakultät, Universität Tübingen, Tübingen, Germany
$^3$LTR, Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven, The Netherlands

Summary. The most important neutralizing and protective antibodies against *Measles virus* (MeV) are directed against the hemagglutinin protein (MeV-H). To define the MeV binding domains recognized by human antibodies a set of 10 non-redundant MeV-H-specific monoclonal antibodies (mabs) was used to block their binding in a competition ELISA. Sera from both naturally infected and vaccinated individuals showed similar competition patterns. Two distinct domains were identified as the main target of human antibodies. One domain corresponded to the region of the previously described hemagglutinin noose epitope (HNE, aa 380–400) [35], which is recognized by hemagglutination-inhibiting, neutralizing and protective mabs. The second region is defined by a mab with strong neutralizing but weak hemagglutination-inhibiting activity. Mabs with a strong neutralizing capacity with respect to wild-type viruses seemed to displace more human antibodies than those with a weaker neutralizing activity. Human antibodies seem to react more weakly with the hemagglutinin regions that bind the CD46 and the fusion protein and more strongly with the putative CD150 binding site and the top loops of β-sheet 2 and 3 of the hemagglutinin.

Introduction

Despite of efficient vaccines, measles continues to be a serious threat for children, particularly in the Third World. After natural infection or vaccination a vigorous and long lasting immune response protects against reinfection. The role of antibodies is highlighted by the fact that newborns and infants are protected by maternal antibodies in the absence of a cellular immune response [2]. Furthermore, measles can be prevented by passive administration of specific immunoglobulins
Even in overt disease, patients benefit from γ-globulin infusion [1]. Although the cell-mediated response is important for virus clearance, antibodies fully protect against recurrent disease [34].

Protective antibodies are mainly directed against the hemagglutinin and, to a lesser extent, against the fusion protein (for review see [8]). Epitopes [13, 24, 35] or antigenic domains [11, 16, 17, 22, 32, 33] have been identified with mouse monoclonal antibodies (mabs; [8]). However, little is known about the nature and the distribution of antigenic sites against which the human antibody response is directed. Attempts have been made to map antigenic determinants recognized by human sera in pepscan [23, 26, 29, 30]. However, our experience with a large set of neutralizing and protective MeV-H specific mabs suggests that most neutralizing antibodies are directed against conformational epitopes and cannot easily be mimicked by peptides [8, 13, 35]. In this study we investigated the antigenic domains that are targeted by late convalescents and vaccinees operationally defined by ten non-redundant mabs using recombinant MeV-H protein.

**Materials and methods**

**Human sera**

The serum panel was collected from 30 donors including late convalescents from Luxembourg (n = 7, age 25 to 67, median 36 years), and Providence, RI, USA (n = 7, age 25 to 90, median 52 years) as well as from Luxembourgian early convalescent children who had measles 15 to 19 months earlier (n = 7, age 6 to 13, median 11 years). In addition, samples were obtained from school children (n = 7, age 12 years) vaccinated at the age of 10 to 26 months.

**Serological assays**

Maxisorp® microtiter plates (NUNC, Roskilde, Denmark) were coated with 150 ng per well of partially purified recombinant MeV-H protein produced in BHK-21 cells and subsequently washed and blocked as described before [7]. Mock-transfected BHK cells served as negative antigen control. These plates were used for titrating mabs and for the competition ELISAs described below.

Saturating concentrations of each mab were determined over a wide range (1:10² to 1:2.8 × 10⁷) by serial dilution in dilution buffer [7]. 50 µl of each dilution was added into MeV-H-coated plates and incubated for 2 h. After a washing step, 50 µl of negative human serum (dilution 1:250) supplemented with 0.1% naive mouse serum was then added (as in the competition ELISA). Binding of mabs was detected with 50 µl of alkaline phosphatase.

---

**Fig. 1.** Binding of a late convalescent serum (dilutions 1:100–1:625; HS3077.4) to antigen-positive (MeV-H; panel A, F) and antigen-negative (mock transfected BHK cell preparation; panel B, G) microtiter plates preincubated with 2-, 10- and 50-fold saturating concentrations of mab BH26 (A–E) or BH99 (F–J). Panel C and H: net signal. Panel D and I: % reduction of net signal of sab in comparison to sab binding in absence of mabs. Panel E and J: binding of mab BH26 or BH99 after addition of diluted serum (1:100–1:625). Mab dilutions in panel A–C, F–H: 1:200, ⋄; 1:1000, ×; 1:5000, □; no mab, ●. Mab dilutions in panel D, E, I, J: 1:200, closed bar; 1:1000, grey bar; 1:5000, open bar