Binding of Neutralizing Monoclonal Antibodies to Empty Capsids of Poliovirus Can be Blocked by Monospecific Antisera to Structural Polypeptides VP1 and VP2

Brief Report

By

H. UHLIG, Frauke HAARDT*, and R. DERNICK

Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Hamburg, Federal Republic of Germany

With 1 Figure

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Summary

Binding of two neutralizing monoclonal antibodies (Nt-mAbs) to natural empty capsids (NEC) of poliovirus, type 1, was blocked to the extent of 83 per cent to 98 per cent by monospecific rabbit antisera directed against the structural polypeptides VP1 and VP2. Monospecific antisera against VP3 or VP4, however, did not show this blocking effect. It is therefore assumed that VP1 and VP2 are located close together at the antigenic sites for the two mAbs.

The majority of neutralizing monoclonal antibodies (Nt-mAb) directed against poliovirus recognize natural empty capsids as well as infectious virus (ICENOGL E et al., 1981; EMINI et al., 1982; ROMBAUT et al., 1982). In some cases Nt-mAbs have also been reported to react with heated (56°C, 30 min) virus (BLONDIEI ET AL., 1983; TANIGUCHI ET AL., 1983; UHLIG ET AL., 1983). However, with the exception of one Nt-mAb directed against VP1 (BLONDIEI ET AL., 1983) they do not recognize isolated individual structural polypeptides. Both, natural empty capsids (NEC), also termed naturally occurring top component (SHARFF and LEVINTOW, 1963) and infectious virus can be isolated from cytoplasmic extracts of poliovirus infected cells. A structural similarity between virus and NEC was not only demonstrated.

* Present address: Physiologisch-chemisches Institut, Universität Hamburg, Martinistrasse 52, D-2000 Hamburg 20, Federal Republic of Germany.
with monoclonal antibodies. Both types of particles have almost identical isoelectric points, are resistant to proteolytic cleavage by trypsin, and expose primarily VP1 on their surfaces (Putnak and Phillips, 1981, 1982). However, the antigenic similarity between virus and NEC seems to be only partial and limited to certain areas of the surface. Indeed, immunodominant structures of infectious virus are different from those of NEC, since both particles can be distinguished by conventional antisera (Hummeler et al., 1962).

Recently, monospecific antisera against all four isolated structural polypeptides of poliovirus have been raised in our laboratory. Most of these reacted adequately with NEC but only very weakly with virus (Wiegert and Dernick, 1983), indicating that antigenic differences between virus and NEC existed on all structural polypeptides. Since both our monospecific rabbit antisera and some of our Nt-mAbs react strongly with NEC of poliovirus, we attempted to localize the neutralization epitopes of the two Nt-mAbs on NEC with the aid of a binding inhibition assay. Therefore we investigated whether or not these polypeptide-specific antisera were able to block binding of Nt-mAbs to NEC, and, if binding of the latter could be blocked, which of the antisera could do so. This type of binding inhibition assay has been applied successfully to topographical studies of other viruses (Stone and Nowinski, 1980; Massey and Schachterman, 1981).

Unlike these authors, who used radioactively labelled mAbs, we chose an enzyme-linked immunosorbent assay as test system (Uhlig et al., 1984). We performed our studies with two different Nt-mAbs to find out whether they recognized the same type of polypeptide.

Poliovirus, type 1, strain Mahoney, was grown in HeLa S3 cells, separated into infectious (standard) virus particles (SP) and naturally occurring empty capsids (NEC) as described (Drzeniek and Bilello, 1974; Yamaguchi-Koll et al., 1975) and stored at −20° C in 3M CsCl. Quantitative determination of SP and NEC was done spectrophotometrically, using extinction coefficients for SP of $A_{260 \text{ nm}} = 74$ and for NEC of $A_{280 \text{ nm}} = 16.2$ according to Heukeshoven and Dernick (1981).

Monoclonal antibodies were produced according to Kennett et al. (1978) and tested as described (Uhlig et al., 1983). The supernatants of hybridomas were used in the experiments described below. The indirect solid-phase ELISA of Katz and Crowell (1980) was applied with minor modifications. Fifty μl well of antigen solution in PBS were used; incubations of culture supernatants of peroxidase-labelled rabbit anti-mouse Ig (Dako, Copenhagen, Denmark) were carried out at 37° C for 2 hours in a humid atmosphere. Results were read...