POSTER: WEST NILE VIRUS: CHARACTERIZATION OF MONOCLONAL ANTIBODIES AND POTENTIAL APPLICATION IN LABORATORY DIAGNOSIS

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Introduction
West Nile virus (WNV) is a single-stranded RNA virus, member of the Japanese encephalitis virus (JEV) serocomplex in the genus Flavivirus. It is transmitted in natural cycles between mosquitoes and birds, whereas humans and horses are considered dead-end hosts.
The purpose of this work was to produce monoclonal antibodies (MAbs) against WNV and to characterize them for the subsequent use in laboratory diagnosis.
Methods
For MAbs production mice were immunised against the WNV reference strain E 101 and against the WNV isolate 203204/08, originated from magpie (Pica pica) during WN epidemic occurred in northern Italy in 2008.
Hybridomas were screened by indirect ELISA against the homologous virus and by immunofluorescence (IF) in infected Vero cells. Immunoperoxidase (IPMA) was used to evaluate the reactivity profile of MAbs with different WNV strains belonging to lineages 1 and 2 and Usutu virus. Capability of MAbs to neutralize virus infectivity was investigated by a virus-neutralization test (VNT) performed with 100 TCDI50 of the homologous virus in Vero cells. Some MAbs were tested in indirect ELISA at Pasteur Institute to assess the possible cross-reaction with members of JEV serocomplex, such as DEN1, DEN2, DEN3, DEN4, YF and JE. Each MAb was also examined in Western blotting (WB) and indirect ELISA with a recombinant E protein DIII (E DIII) produced in E.coli.
Six MAbs (three neutralizing and three non-neutralizing) were conjugated with peroxidase and evaluated in all possible combinations as capture and tracer antibodies, to develop sandwich ELISA assays for WNV antigen detection. Competitive ELISA assays were designed to evaluate the competition between MAbs and sera of experimentally infected SPF chickens and of horses immunized with inactivated vaccine. MAbs reciprocal competition was also studied in order to determine whether they bind to overlapping epitopes.
Results
During the screening phase 37 MAbs (raised against WNV E 101) were selected; among them 29 resulted specific and reactive with all WNV strains tested. The remaining eight MAbs showed cross-reaction between the JEV serocomplex, with MAbs 2A8 and 4G9 exhibiting high reactivity also against Usutu virus. Thirteen MAbs showed neutralizing activity: 12 of these recognized the recombinant viral protein E DIII and competed with each other. All MAbs were negative in WB, suggesting the conformational nature of target epitopes.
MAbs 3B2 and 3D6 provided the best performance when used in ELISA assays for antigen or antibody detection. The characterization of MAbs raised against the WNV 203204/08 isolate is still ongoing; results will be presented at the congress.
Conclusions
MAbs produced offer wide applicability in various analytical methods. MAbs 3B2 and 3D6 (neutralizing and reactive against WNV E DIII) can be used in sandwich ELISA, IF or IPMA for WNV identification and in competitive ELISA to reveal anti-WNV neutralizing antibodies in equine and avian sera. Moreover, conjugated with peroxidase they can be used as tracer in an IgM-capture ELISA for detection of early antibodies and diagnosis of recent infection in horses. Since 3B2 and 3D6 showed no cross-reactivity with JEV serocomplex, they are useful for the development of antigen and antibody detection tools for WNV surveillance in areas as the Mediterranean basin, where Usutu virus is present.