Sequential asymptomatic enterovirus infections in a patient with

MHC class II primary immunodeficiency

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Patients with primary immunodeficiencies are usually susceptible to enterovirus infections and have higher risks to develop severe clinical forms. We report a unique description of a boy with MHC-ClassII deficiency infected by 9 different enterovirus serotypes during a 2-years-period, with very mild clinical symptoms; probably due to the immunoglobulin therapy he was receiving.
CASE REPORT

The case-patient is the older child of Tunisian consanguineous healthy parents. He was born in 2002 with, apparently, no neonatal pathology. Breastfed for 20 months, he had a history of multiple episodes of upper respiratory infections and diarrhea with failure to thrive (-2 Standard Deviations in weight and size). At the age of 3 months, he developed a chronic diarrhea (6 to 7 stools per day) which required hospitalization. He was hospitalized again at 4 years of age for diarrhea and dehydration; the diagnoses of mucovicidiosis and of coeliac disease were ruled out based on the negativity of the sweat testing and the results of duodenal fibroscopy and biopsy, respectively. At 5 years of age, because of multiple respiratory infections, a thoracic scannography was performed and concluded to a diffuse bronchiectasis. His vaccination history against poliomyelitis included four doses of trivalent oral poliovirus vaccine (OPV) administered at 3, 4, 5 and 18 months of age; he received another dose of OPV at 4 years of age during a subnational polio mass vaccination campaign. In December 2007, Major Histocompatibility (MHC) Class II deficiency was diagnosed based on the defect of expression of MHC Class II molecules on the surface of resting peripheral blood mononuclear cells and confirmed by expression study on phytohemagglutinin-activated blasts, as assessed by flow cytometry. A severe CD4 lymphopenia (6%) was found along with subnormal immunoglobulin titers: 644 mg/dL for IgG (normal: 929±228 mg/dL), 74 mg/dL for IgA (normal: 56±18 mg/dL), and 132 mg/dL for IgM (normal: 93±27 mg/dL) (1). Since, he was treated on monthly basis with substitutive intravenous immunoglobulins and was regularly followed by the expert physicians and nurses of the Bone Marrow Transplantation Center in Tunisia, specialized in managing and providing care for these patients. Bone marrow transplantation could not be performed due to the unavailability of a compatible donor. The molecular analysis did not show the presence of the recurrent 752delG26/RFXANK gene.
mutation, observed in most MHC Class II deficient Maghrebian patients (2). At each visit and
prior to immunoglobulin transfusion, a blood sample was collected for residual immunoglobulin
titration. A complete medical visit was performed; all clinical symptoms present at the day of
the visit and those that occurred during the previous month were recorded and appropriate
treatment prescriptions were provided.

In July 2009, when he was 7-years old, a WHO collaborative study aiming the search for
chronic poliovirus excretors among patients with immunodeficiencies was initiated and he was
enrolled in the study in October 2009. The study protocol was approved by the Ethical Review
Board of Pasteur Institute of Tunis and the Ethical Review Board of WHO-Geneva (4). Written
informed consent was obtained from the patient parents. Part of the clinical history and the
virological findings for the patient (data up to Day 454, approximately 15-months of follow-up)
was previously published in a paper summarizing the study results for the whole cohort (3). This
patient was specifically selected because we continued to identify new episodes of enterovirus
infections with additional serotypes. Herein, we report detailed clinical data and the results of
virological investigations up to Day 1270, thus, approximately 42 months of follow-up. The
initial stool samples were collected at the patient’s home residence and the subsequent samples
were obtained during the monthly follow-up medical visits that the patient had for his
substitutive immunoglobulin therapy. A total of 30 samples were collected. Stool extracts were
inoculated onto three cell lines: RD (human rhabdomyosarcoma cell lines), L20B (transgenic
mouse cell line expressing the gene of human cellular receptor for poliovirus) and HEp-2C
(human larynx epidermoid carcinoma cell lines). Inoculated cells were followed-up daily for
cytopathic effect (CPE) up to 10 days. Polioviruses were identified by the presence of a CPE on
L20Bs then serotyped and intratyped by real-time PCR using poliovirus serotype-specific and
vaccine-specific primers. Non-polio-enteroviruses were identified by the presence of a CPE on
RD and/or HEP2-C cells and the absence of CPE on L20B cells. The type was determined by
partial sequencing of a 356-nucleotide fragment in the VP1 region of the enterovirus genome. Briefly, RNA extraction and PCR amplifications were carried out as described previously (5). PCR products were sequenced in both directions, the sequence of each isolate was deduced by aligning the respective forward and reverse sequences and the serotype was determined by comparison to the sequences of EV prototype strains of the different EV serotypes as described previously (9).

The enterovirus serotypes detected in the immunodeficient patient during the whole study period are indicated in Figure 1. A vaccine-related poliovirus type 1 was isolated in the initial sample (D1) and the patient excreted the same serotype during more than 4 months, up to D132. Vaccine-related polioviruses type 2 and type 3 were then detected in the specimens collected at D249 and D277, respectively. The patient was then re-infected successively by 6 different enterovirus serotypes: Echovirus type 30 during 118 days (D368-D486), Coxsackievirus type B2 during 48 days (D507-D555), Echovirus type 30 detected at Day 616, Echovirus type 6 during 99 days (D657-D756), Coxsackievirus type B2 at Day 796, Echovirus type 25 at Day 856, Coxsackievirus type B4 during 30 days (D887-D917) and Echovirus type 20 during 53 days (D1052-D1104). Comparison of the partial VP1 sequences of isolates from same serotypes revealed identical sequences for Echovirus type 6 and Coxsackievirus B4 and very few mutations for the other serotypes: a maximum of two nucleotide differences for Echovirus type 20 and Coxsackievirus type B2 and three nucleotide differences for Echovirus type 30.

During the whole study period, the patient residual serum IgG titres were persistently satisfactory with a minimal value of 681mg/dL. The clinical features were marked by multiple episodes of upper respiratory infections of bacterial origin including *Haemophilus influenzae* and *Streptococcus pneumoniae*, isolated repetitively in the respiratory tract specimens collected at D448, D486, D756 and D1104 (Fig. 1). Few episodes of rash, diarrhea and fever were also
noted during the study period but with no serious disease that could be related to enteroviral infections such as meningitis, encephalitis or paralysis.

This study reports unusual sequential and very frequent enterovirus infections in a patient with MHC class II deficiency. Interestingly, no severe clinical symptoms could be clearly related to the enterovirus infections. MHC class II deficiency is a relatively common primary immunodeficiency which affects infants and young children. The affected T-cell and B-cell responses expose the patient to repeated microbial infections, including enteroviral infections, and severe forms, especially with neurological presentations (encephalitis, meningitis, paralysis) were frequently reported in patients with immune deficiencies (6, 7, 8). Our patient was receiving substitutive immunoglobulin therapy regularly since 5-years of age and during the study period with adequate residual IgG serum levels. This may suggest the possible role of this treatment in preventing severe forms of enterovirus infections; although it seems that this treatment does not protect against the infection itself. In fact, the very high susceptibility of our patient to enteroviral infections is remarkable especially if we consider the relatively low endemicity of enterovirus infection in Tunisia; according to the national enterovirus surveillance data, regularly conducted since 1991, enterovirus shedding was found in only 4-5% of acute flaccid paralysis cases and their healthy contacts during the last decade (3, 9). We have previously noted the high susceptibility to enterovirus infections in a cohort of patients with various types of primary immunodeficiencies (PIDs): 13.4% (11 out of 82) of all patients and 20.7% (11 out of 53) of those with humoral or combined immunodeficiency were found enterovirus positives (3). However, the case patient was particularly prone to such infections as he was sequentially infected by 9 different enterovirus serotypes during a period of 25 months. In contrast, no enterovirus was detected in his stool specimens starting from December 2012 and up to April 2013. We could not identify, at the clinical level, any change or
particular event that may explain this unusual high susceptibility to infection during the 2-years period and the absence of infection subsequently.

Regardless of the clinical presentation, enterovirus infection in patients with primary immunodeficiencies was frequently reported as chronic infection (3, 7, 10). In our case-patient, the first detected virus was a Sabin-like poliovirus type1; it was excreted during a period exceeding 4 months, from D1 to D134. It was well established that immunodeficient patients exposed to OPV may excrete poliovirus strains for many months or years (11-14) and these patients may constitute a reservoir for potentially neurovirulent polioviruses after eradication of wild polioviruses. These patients may receive the live-attenuated vaccine before their immune deficiency is diagnosed; they may also be infected with vaccine strains excreted by OPV-vaccinated contacts. Screening for poliovirus excretors among patients with PIDs is now highly recommended. The identified poliovirus excretors should then be followed until poliovirus excretion stops, antiviral therapy may even be proposed to accelerate the clearance of the excreted viruses. Our case patient received OPV during the two first years of life, before his immune deficiency was diagnosed but, he received it again at school entry, few months before sampling despite the fact that his disease was already known and he should have not be given any live vaccine. Fortunately, he did not become a chronic poliovirus excretion although the mechanism by which the excretion stopped at D134 remained unclear. Polioviruses type 2 and type 3 were then detected in his stool specimens at Day249 and Day277, respectively. Family data indicated that his newborn brother was given the first and the second dose of OPV in the same period which also did not had to happen as no live vaccine should be given to the household contacts of immunodeficient patients. After the three poliovirus serotypes, an Echovirus type 30 was detected over 4 months; it was replaced by a Coxsackievirus type B2 and than re-appeared. The same is for the Coxsackievirus type B2 which was replaced by Echovirus type 30 and type 6 during few months and than reappeared. A re-infection with the same
serotype is possible but a latent carrier state during which the virus is not detected can not be excluded. Only part of the VP1 genomic region was sequenced for typing purpose and the identified sequences of isolates from the same serotype were very close to each other; thus, only more advanced molecular investigations may help to differentiate between the two hypotheses. For all the remaining enterovirus serotypes, the excretion period was relatively time-limited and did not exceed 3-4 months. In fact, it was previously established that enterovirus excretion may last up to 3 months or slightly more in apparently immunocompetent individuals (15). Although it is well established that patients with PIDs are highly susceptible to viral infections, the novel finding from this case report is the occurrence of such diversified and numerous infections in a single patient and in a short period of time. It provides further evidence on the extreme susceptibility of patients with primary immunodeficiency to enterovirus infections. Since these patients can become chronically infected by enteroviruses and in particular polioviruses, they can be seen as potential reservoirs for pathogenic enteroviruses. They are also at risk for severe forms of enterovirus infection but, interestingly, no severe clinical consequence was associated to the sequential presence of several enterovirus serotypes in this patient receiving substitutive immunoglobulins regularly. Adequate surveillance, clinical management and follow-up for these patients is essential to prevent the occurrence of severe forms for the patient and the propagation of virus strains that may be of concern for the global polio eradication initiative.
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References


FIG. 1. Enteroviruses excretion and clinical features during the study period

PV1: Poliovirus type 1; PV2: Poliovirus type 2; PV3: Poliovirus type 3; E30: Echovirus type 30; CB2: Coxscackievirus B type 2; E6: Echovirus type 6; E25: Echovirus type 25; CB4: Coxscackievirus B type 4; E20: Echovirus type 20; N: Negative
IgG: Immunoglobulin G

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IgG: Immunoglobulin G