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Original article

Markers of a recent bocavirus infection in children with Kawasaki disease: "A year prospective study"

Marqueurs d'une infection récente à bocavirus de l'enfant au début d'une maladie de Kawasaki : étude prospective

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ABSTRACT

Background. – Retrospective studies and case-reports have suggested the possible role of various viruses in the pathogenesis of the Kawasaki disease.
Objectives. – To determine prospectively the incidence of Kawasaki diseases associated with a recent bocavirus infection in the course of a year.
Study design. – Thirty-two children with Kawasaki disease were enrolled in a 13 months prospective study to assess the frequency of human bocavirus type 1 infections. Seasonal shedding of virus, markers of recent infection such as viraemia, viral load, and serum interferon alpha were analyzed.
Results. – Three of 32 (9%) children had HBoV-DNA in the serum suggesting a recent infection. HBoV-DNA was detected in naso-pharyngeal aspiration of 7/32 (21.8%) children with Kawasaki Disease and six of them (18%) had an increased viral load. No common respiratory viruses were isolated from the 32 patients with the exception of one adenovirus. The seven bocaviruses were identified during the winter-spring season. In addition, 4 of 7 of Kawasaki disease patients shedding bocavirus had detectable interferon alpha in the blood, indicating a possible active or recent viral infection.
Conclusions. – This study shows that a recent bocavirus infection is concomitant with the onset of some cases of Kawasaki disease. Bocavirus may be a cofactor in the pathogenesis of this disease as previously reported for other infectious agents.

Mots clés :
Bocavirus
Vascularite
Maladie de Kawasaki
Interféron
Virémie

RÉSUMÉ

Plusieurs études suggèrent le rôle de différents virus dans la pathogenèse de la maladie de Kawasaki.
Buts de l'étude. – Déterminer de façon prospective l'incidence d'une infection récente à bocavirus associée à l'apparition d'une maladie de Kawasaki.
Patients et méthodes. – Sur une période de 13 mois, 32 enfants développant une maladie de Kawasaki ont été recrutés dans une étude prospective à la recherche d'une infection récente à bocavirus. Le portage du virus en fonction de la saison, les marqueurs d'une infection récente : charge virale, virémie, interferonémie ont été analysés.
Résultats. – L'ADN du bocavirus a été détecté dans 3 sérums sur 32 (9 %) des enfants suggérant ainsi une infection récente. Sept patients sont porteurs d'ADN viral dans les aspirations naso-pharyngées mais avec une charge virale à des valeurs faibles ou modérées. Excepté un adénovirus isolé chez un 8e patient, les cultures virales et les tests par immunofluoresence sont restés négatifs. Sept bocavirus ont été identifiés dans la période hiver–printemps et aucun parmi les 6 cas de maladies de Kawasaki observés

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dans la période été automne. De plus, 4/7 patients porteurs de bocavirus avaient une interferonémie positive qui est en faveur d'une infection virale récente.
Conclusion. – Cette étude démontre qu'au moins 9 % des maladies de Kawasaki se déclare au cours ou à la suite d'une infection récente à bocavirus, Ces virus pourraient donc être un cofacteur dans la pathogénie de cette maladie au même titre que d'autres agents infectieux déjà rapportés.

1. Background

Kawasaki disease (KD) (MIM 611775) is a vasculitis predominantly affecting young children and is a frequent common cause of acquired heart disease in the pediatric age [1]. To date, various pathogens have been shown to be associated to KD [2]. The most likely pathogenic hypothesis is consistent with an abnormal response to various infectious agents in genetically susceptible individuals [3]. In a first retrospective single center study, we showed that human bocavirus (HBoV) might be one of the triggering agents [4] (Fig. 1). Two KD patients were also mentioned in an epidemiology study performed in Hong Kong children [5] and a bocavirus-associated new KD case has been recently reported [6].

The objectives of the present study are:

- to evaluate prospectively the frequency of a recent bocavirus infection in children affected by a KD in measuring the load of HBoV-DNA in blood;
- to determine the season influence on the frequency of bocavirus shedding in KD.

2. Study design

We performed a prospective study in the cardiologic unit in Necker Hospital (Paris). Thirty-two children with KD, 20 males, 12 females, aged from 6 months to 9 years, median: 24 months, mean age: 25.4 months, were hospitalized between the 1st December 2006 to the 31st December 2007 for typical KD as defined by the American Heart Association criteria [7] and were prospectively included. Twelve children on 32 (37.5%) had developed coronary dilatation or an aneurysm.

3. Collection of samples and viral studies

Blood samples were collected, between 5 and 15 days after the onset of fever and before intravenous immunoglobulins (IVIG) administration. Naso-pharyngeal aspirates (NPA) were taken also for each KD patient during the 1st–2nd week after the onset of fever. Then blood and NPA were tested for the presence of HBoV-DNA type 1 with the real time bocavirus type 1 PCR assay as previously described [3]. The 2 primers and the Taqman probe were designed in the NS1 gene (forward primer: 5′ AAA AAA CTA CCA CGC AAC CCT AGA 3′; reverse primer: 5′ AAA AAA CTA CCA CGC AAC CCT AGA 3′) (Invitrogen). The probe bocavirus 6: FAM AAC GAA GTC ATT CCA GGG CCT CAA ACA AT (Applied Biosystems); Viral loads were measured in comparison with a plasmid containing a 799-bp subgenomic fragment of HBoV as previously described [7].

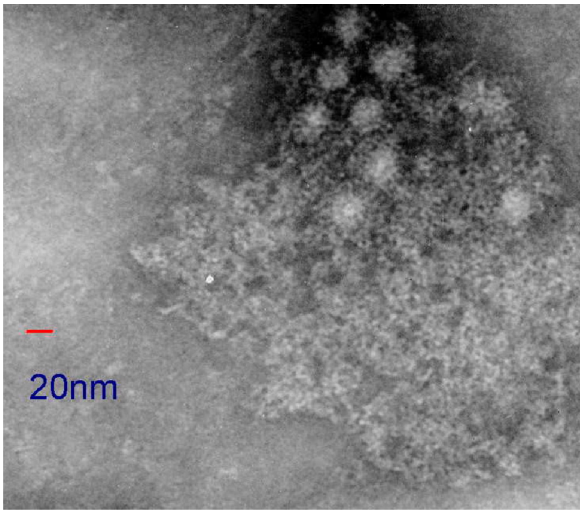

Fig. 1. Agglutinated bocavirus by immunoglobulins.

NPA were also tested for common viral respiratory pathogens except metapneumovirus by direct immunofluorescence assays with monoclonal antibodies to RSV, Influenza A and B viruses, Adenovirus, and Parainfluenza type 1, 2, 3 viruses (Argene, France). All NPA were inoculated into HUH7 [8] and A549 cell lines monolayers, which are very sensitive for virus isolation. NPA specimens were stored frozen at −80 °C.

Serum alpha-interferon was measured using a biological assay as previously described [9]. The detection threshold of the test is 1–2 IU/ml. A titer < 2 IU/ml was considered negative.

The study was approved by the institutional review board of the Comité de Protection des Personnes, Île-de-France 3. For ethical reasons, the blood of controls children could not be taken and tested.

4. Results

Thirty-two children affected with KD were included during the study period.

HBoV-DNA was detected in the serum of 3/32 (9.3%) patients who had also viral DNA in their NPA. Four patients had viral DNA only in the NPA; finally 7 out of the 32 KD patients (21.8%) sheared DNA bocavirus. One adenovirus (3.1%) was isolated but neither VRS, nor influenza A and B, para-influenza type 1, 2, 3 viruses were found in the 32 KD patients with cell cultures and immunofluorescence assays.

The seven KD patients with HBoV-DNA out of 26 (27%) patients were observed during the annual peak of viral epidemics in winter and spring (Table 1). No bocavirus was detected in the 6 KD cases during the summer–autumn period.

The viral load in the 3 sera of KD patients ranged between 3×10^2 and 10^4 equivalent genome/ml and was associated with a positivity of the NPA (Table 1). The viral load in NPA of the KD patients (samples taken 8th–21st day) ranged from 3×10^2 to 3×10^8 copies/ml. In one patient (No. 2), the number of viral copies was low: 3×10^2 copies/ml although the sample was taken early at the 8th day of the disease.

Interferon alpha was detected in the serum of 11/30 KD patients (36%) (mean titer 6, 4 IU/ml) and in 4/7 KD patients shedding bocaviruses. There was no evidence of correlation between the presence of interferon in the serum and cardiac complications (Table 1).

5. Discussion

Kawasaki disease is an acute vasculitis which affects small to medium-size vessels, leading to coronary aneurysms in 25% of patients. The immunopathologic mechanism involved in the