

Molecular Diagnostics and Evaluation of Specific T-cell Responses in Infants with Primary Herpesviral Infections

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ABSTRACT

Among the viral pathogens, members of the Herpesviridae play most important role in development of generalized infections in newborns. Several recent investigations showed that antigen-specific T helper cells play a key role in induction of an effective immune response during acute viral infections as well as in maintenance of effective immune regulation in chronic viral infections. Blood samples from the two groups of infants selected from the newborn intensive care unit with HSV (n=16) and CMV (n=14) infections were studied by molecular (consensus PCR) and immunological (ELISA and EliSpot) methods. It was shown that infants could produce specific T cell immune response comparable to adult patients. Cumulative frequencies of specific T cell responses against HSV and CMV antigens were higher in survived infants in comparison with deceased infants. Our results indicate to the influence of the strength of the specific T cellular immune response on the outcome of the primary HSV and CMV infections.

KEYWORDS: *herpes simplex virus, cytomegalovirus, molecular diagnostics, consensus PCR, specific T cell response*

Currently the infant mortality rate in Georgia is very high and varies from 15 to 25 deaths per 1000 live births [1]. One of the most important causes of the infant death is the development of generalized infections [2]. In the majority of such cases the causative agents are not being identified. As a rule, all newborns with a generalized infection with wide range of nonspecific symptoms; including hyperthermia or hypothermia, jaundice, bleeding with associated coagulopathy, respiratory insufficiency, vascular instability, hepatomegaly, splenomegaly; are diagnosed as "neonatal sepsis" (without identification of etiology) and treated with broad-spectrum antibiotics. Despite this "empiric" treatment, the mortality rate for neonates with generalized infections of unknown origin is high, estimated to be over 65%. In addition, the "neonatal sepsis" is frequently associated with infections of central nervous system - meningitis and encephalitis, which makes prognosis even poorer [2].

Currently, testing for viral infections in pediatric patients is ultimately rare, and includes only serological testing, leaving physicians to treat patients with limited clinical information. Yet, some patients with generalized infection likely have a viral infection, a subset of whom has a treatable viral infection. Early identification of preventable or treatable viral infections may be one key to reducing the low survival rate seen in this high-risk population.

Among the viral pathogens, members of the Herpesviridae play most important role in development of generalized infections in newborns. In general, from the family of herpesviruses eight human herpesviruses (herpesvirus 1 through 8) have major role in morbidity and mortality in humans. These are: herpes simplex virus (HSV), varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and recently identified human herpesvirus type 6 (HHV6), type 7 (HHV7) and type 8 (HHV8). In infants, along with other manifestations, herpesviruses may cause generalized infection and meningoencephalitis. Clinical peculiarities of the disease, as well as severity and outcome depend considerably on the particular types and subtypes of herpesviruses. This indicates the importance of typing of these viruses in clinical samples [3].

Several recent investigations showed that both CD4+ and CD8+ T helper cells play a key role in induction of an effective immune response during acute viral infections as well as in maintenance of effective immune regulation in chronic viral infections. Further investigation are needed to characterize the role of the cellular immune responses in the clinical course of persistent viral infections [4].

We have studied blood samples from the 2 groups of infants (0-6 weeks of age) selected from the newborn intensive care unit with diagnosed HSV (n=16) and CMV (n=14) infections.

Tests based on PCR have assumed an important role for the laboratory detection of these agents [5]. Even for viruses such as HSV, which can be readily isolated, diagnosis by PCR has become the "gold standard" for some diseases such as herpetic encephalitis [6]. Because infection with different or multiple species of herpesviruses can cause similar symptoms, PCR tests have been designed to detect more than one herpesvirus at a time. Methods used to achieve this have included parallel PCRs, multiplex PCRs with several primer pairs, and test with consensus primer pair targeting conserved genomic regions. Methods for subsequent confirmation of the species of the virus detected have included hybridization with a species-specific probe, nested PCR with species-specific primers, and restriction enzyme analysis [7-9]. In our study we have used PCR with two consensus primer pairs, followed by restriction enzyme analysis with BamHI and BstUI as previously described [5]. Alternatively the identification of PCR products was done by Chemicon Oligo Detect Assay.

Serology for HSV and CMV was done by ELISA method. Presence of anti-HSV and anti-CMV IgM antibodies or alternatively positive PCR in absence of virus-specific IgG antibodies was considered to correspond to the primary HSV and CMV infections correspondingly.

Specific T cell responses were assessed by EliSpot-interferon-gamma assay using CMV and HSV antigens. 96-well EliSpot plates were coated with anti-human IFN-gamma antibodies. After blocking with foetal calf serum, triplicate wells were filled with cell suspension containing 100 000 PBMCs. Plates were incubated at 37°C for 40 hours with antigens. 1 mkg/ml phytohemagglutinin and medium alone served as positive and negative controls

respectively. Wells were then washed and spots were detected after the addition of anti-human IFN-gamma-biotin detection antibodies, followed by streptavidine alkaline phosphatase and substrate incubated at room temperature until the appearance of blue spots [10].

We have found that infants could produce specific T cell immune response comparable to adult patients.

Cumulative frequencies of antigen-specific T cell responses against HSV and CMV antigens were higher in survived infants in comparison with deceased infants. Median value of Cumulative frequencies of antigen-specific T cell responses against CMV antigens was equal to 1245 spot-forming cells / 1 mln PBMCs in survived infants versus 835 spot-forming cells / 1 mln PBMCs in deceased ones ($p < 0.05$). For HSV infection these values

equaled correspondingly to 152 vs. 43 spot-forming cells / 100 000 PBMCs ($p < 0.05$).

Our results indicate to the influence of the strength of the specific T cellular immune response on the outcome of the primary HSV and CMV infections and confirms that antigen-specific T cell responses play a key role in the clinical course of persistent viral infections. These findings may contribute to our understanding of the mechanisms of regulation of herpesviruses replication by the immune system. These limited data indicate to the importance of further investigations of the association between the strength and pattern of specific immune responses and clinical course of persistent viral infections.

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Молекулярная диагностика и оценка специфического Т-клеточного иммунного ответа у новорожденных с первичной инфекцией герпесвирусами

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РЕЗЮМЕ

Герпесвирусы играют значительную роль в развитии генерализованной инфекции у новорожденных детей. Сила и тип антиген-специфического Т-клеточного иммунного ответа в значительной степени определяют способность организма регулировать течение как острой, так и хронической вирусной инфекции. Нами исследованы 2 группы новорожденных с первичной инфекцией вирусом простого герпеса (16 случаев) и цитомегаловирусом (14 случаев) с использованием молекулярных (консенсус ПЦР) и иммунологических (ELISA и EiiSpot) методов. Установлено, что новорожденные способны развить специфический Т-клеточный иммунный ответ такой же силы, как это наблюдается у взрослых. Кумулятивные частоты Т-клеточного ответа против антигенов вируса простого герпеса и цитомегаловируса выше у выживших новорожденных в сравнении умершими детьми.

Ключевые слова: вирус простого герпеса, цитомегаловирус, молекулярная диагностика, консенсус ПЦР, специфический Т-клеточный иммунный ответ