G302 Cloning and expression of putative common epitope derived from structural regions of enteroviruses

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Enteroviruses are the major infectious materials of the aseptic meningitis through oral-fecal route. So oral administration of the vaccine is one of the most effective immunization system to protect chlidren from the diseases. For theses reasons, this study was focused on the developing vaccine candidates which can be used as oral administrable vaccnine against aseptic meningitis caused by various enteroviruses. To analyze antigenicity of structural proteins, putative common epitope region was cloned from the enteroviral RNA and its proteins expressed by bacterial system were examined by western blotting with convalescent serum. Enteroviral RNA was prepared from cultured viruses which were isolated and characterized by neutralizing test and RT-PCR was performed with synthetic primers which has been designed for amplification of group common linear epitopes region. Through pET30a/BL21(DE3) expression system, we could get 12 kDa protein after inducing with 0.1 mM of IPTG. To examine antigenicity of expressed protein, crude extracts prepared from bacteria were run on 12.5% of PAGE and western blotted. Expressed proteins were immunologically reacted with serum bled from several patients who were diagnosed aseptic meningitis caused from enterovirus infection. These results suggest that the expressed protein containing common linear epitopes has strong antigenicity against serum IgG specific for several enteroviruses which have been known to be a major agent of aseptic meningitis.

G303 PKC and Calcium Influx Mediate TGF-induced increase in IgA Production

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TGFβ-1 is well known to be a critical switch factor for IgA antibody which is one of the most important defense mechanisms against pathogens at the mucosal surface. However, little is known for the signal transducton mechanism by which TGF-β1 increases IgA production. The present study examined specifically the involvement of PKC and calcium influx in this matter. Normal mouse spleen B cells or B cell lymphoma (CH12.LX, μ^+) were activated with LPS and TGF-β1. At the same time, calphostin C, a PKC inhibitor, was added to culture and Ig synthesis was measured by ELISA. TGF-β1 induced 6590 ng/ml of IgA production in the culture of LPS-stimulated mouse spleen B cells. Under the same condition, 20 nM of Calphostin C reduced TGF-β1-induced IgA production by 80%. Similarly, Enhancement of IgG2b production by TGF-β1 was decreased 50% by the treatment of Calphostin C. No significant change was observed in IgM, IgG1 production. Further, EGTA (Ca^{++} scavenger) decreased TGF-β1-induced IgA production by 1.5 fold but it did not result in IgG2b reduction. In order to confirm the results with nomal B cells. Clonal B cell Lymphoma was tested, and calphostin C and EGTA individually abolished the TGF-β1-induced IgA synthesis as much as seen in the normal spleen B cell culture. The result from the present study indicate that TGF-β1 induced IgA production is mediated through PKC and Ca^{++}channel.