72 h post-infection and there was no adverse off-target silencing effect. Gene silencing by 29-mer shRNAs targeted at the $3D^{pol}$ region (sh-3D) was the most effective, achieving 91% viral inhibition. Further evaluation found that no enhanced inhibitory effects were observed when sh-3D was cotransfected with each of the other two candidates. This study showed an improvement in triggering RNAi using the more potent 29-mer shRNAs, indicating its therapeutic potential against EV71.

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## Crosstalk Between Scavenger Receptors (SR-A) and Toll-like Receptors (TLR) Results in Rapid Pro-Inflammatory Cytokine Differentiation in Monocytes Exposed to Cytomegalovirus (CMV)

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**Introduction:** Disease from CMV occurs rarely in immunocompetent hosts. Monocytes are an important first defense against CMV disease due to multiple pattern recognition receptors including SR-A and TLRs. However, SR-A and TLR receptor interactions that contribute to pro-inflammatory cytokine induction immediately after monocyte exposure to HCMV are not defined. A better understanding of cellular mechanisms and signaling cascades during innate immune and pro-inflammatory responses will be important in studying CMV pathogenesis. To test this hypothesis, we used the human monocytic THP-1 cell line to investigate the interacting roles of these receptors shortly after contact with CMV and their involvement in inducing pro-inflammatory cytokines.

**Methods:** THP-1 cells were incubated for 1, 5 and 10 min with low passage wild-type CMV isolated from a congenitally infected infant. After incubation, total RNA was extracted from cells and treated with DNase. RNA was subjected to reverse transcription. cDNA was analyzed by semi-quantitative PCR for gene expression.

**Results:** At 1 min post CMV exposure, mRNA levels were elevated for SR-A and Lyn, an SR-A associated cytoplasmic tyrosine kinase. This suggests SR-A and Lyn are activated simultaneously immediately after THP-1 exposure to CMV. Surprisingly, at 10 min, there was a dramatic elevation of TLR2, implying SR-A is acting upstream of TLR2. However, mRNA levels of TLR3 and TLR9 were inhibited 1, 5 and 10 min after THP-1 exposure to CMV. Marked elevation of TNF-alpha over baseline at 10 min suggests that SR-A and TLR crosstalk plays a role in TNF-alpha activation. Similar elevation was seen for IL-12 while IFN-beta levels were unaffected, suggesting IFN-beta is not responsible for earliest inflammatory responses in CMV-exposed THP-1.

**Conclusion:** Our findings establish for the first time a novel paradigm in CMV-exposed monocytes between SR-A and TLR receptor signaling pathways that are important for induction of pro-inflammatory cytokine expression. Such a relationship in CMV-exposed monocytes between these two receptor families that induce pro-inflammatory cytokines could be important in CMV infection outcome.

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## Solid-phase synthesis of the anti-HBV dinucleotide SB 9000—Microwave-assisted Functionalization of Solid Supports

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SB 9000 is being developed as a new class of antiviral agent against HBV. For the in vitro and in vivo studies of SB 9000, we needed access to large amounts of SB 9000, which is most conveniently prepared by solid-phase synthesis using phosphoramidite chemistry.

For the large-scale manufacture of SB 9000, availability of nucleoside-loaded solid support is critical. Recently, we have developed ultra fast methods for functionalization of controlled-pore glass (CPG) using microwave-assisted procedures. This technology provides rapid access to amino-functionalized and succinylated-CPG in large-scale. Thus, following a 10-minute, microwave exposure of a slurry of native CPG in aminopropyl-(triethoxy)silane, amino-propyl CPG was obtained. Further reaction of the amino-support with succinic anhydride in the presence of coupling reagent and catalyst, under microwave conditions for 5 min, resulted in succinylated CPG. These methods were applicable to a range of supports including LCAA-CPG, Primer support®, and so on. Concurrently, we have also developed efficient procedures for the loading of nucleosides on functionalized CPG using LOTUS®, a versatile multi-purpose chemical synthesis reactor. We have obtained loadings ranging from 80 to 220 μmol/g depending upon the solid support used.

5′-O-dimethoxytrityl-2′-deoxy-adenosine-loaded CPG, obtained following these protocols, was used for the synthesis of SB-9000. The sequence of synthetic steps involving detritylation, coupling, sulfurization, were all carried out in an inert atmosphere using LOTUS® reactor. Following the synthesis, cleavage and removal of protecting groups was achieved by treatment with 28% NH4OH to give crude SB 9000. HPLC purification, followed by desalting, endotoxin-removal, and lyophilization gave SB 9000 in >95% purity. The Rp, Sp-SB 9000 was fully characterized by spectral methods, and was employed for all in vitro and in vivo studies.

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