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Was Induction of HIV-1 Through TLR9?

Equils et al. (1) reported implication of simultaneous activation of Toll-like receptors (TLRs) on HIV replication. When spleen cells from HIV-1 transgenic mice were exposed to agonists of TLR2, TLR4, and TLR9, p24 production was induced. The agonist of TLR9 used in this study was bacterial CpG DNA.

In retrospect, these experimental observations could explain the consistent virus load increases found in plasma of HIV-1 infected individuals in Phase 1 dose-escalation trials conducted with GEM91 a few years ago.

GEM91, an antisense oligodeoxynucleotide phosphorothioate (5’-CTC TCG CAC CCA TCT CTC TTC T-3’) complementary to gag gene of HIV-1, showed potent inhibition of HIV-1 replication in cell cultures (2). In clinical trials, GEM91 was dosed by continuous i.v. infusion for 8 days in a blinded dose-escalation study. Plasma HIV-1 virus load measurements, expressed as mean log_{10} copies per milliliter by the bDNA method, were compared to pretreatment values for the six GEM91-treated subjects in each of the four highest dose groups. Results for the three placebo-treated subjects in each group were pooled. Plasma bDNA for placebo-treated subjects remained within 0.13 log_{10} of pretreatment baseline while mean plasma bDNA increased at 4 and 8 days in all four treated groups. By 6 days after the end of GEM91 infusions, the bDNA was again similar in GEM91 and placebo-treated subjects (Fig. 1).

At the time, the seemingly paradoxical increases in plasma virus load were puzzling. In GEM91 sequence, there is presence of CpG motif. In the last few years, it has been shown in many reports that phosphorothioate linked oligodeoxynucleotides containing CpG motif mimic properties of bacterial CpG DNA, and activate immune response through TLR9. It is possible, based on the present evidence, that an increase in HIV-1 copy numbers following administration of GEM91 was due to TLR9 activation. However, this induction was temporary as by day 14, bDNA levels returned to pretreatment levels. Doses below 1.2 mg/kg/day did not have an effect on bDNA results. Development of GEM91 was ceased as thrombocytopenia and serum transaminase elevations occurred in some patients, thereby limiting continuous treatments and increase in viral load.

A second-generation antisense oligonucleotide (GEM92) was built on a truncated GEM91 sequence and the CpG motif was modified to neutralize its immunostimulatory activity. Presently several antisense phosphorothioate oligodeoxynucleotides containing CpG motif are in human clinical trials for various diseases and indications.

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The Authors Respond

The Toll-like receptor (TLR) family mediates innate immune responses to pathogen-associated molecular patterns (PAMP) that are conserved molecular patterns produced by pathogens but not by their host. Bacterial DNA acts as a PAMP by virtue of a 20-fold greater frequency of unmethylated CG dinucleotides found in microbial DNA versus vertebrate DNA (1). In addition, synthetic oligodeoxynucleo-

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tides (ODN) containing the proper CpG-DNA motif mimic the immunostimulatory effects of bacterial DNA (2).

Genetic complementation studies using 293 cells and experiments using TLR9-deficient mice show that TLR9 is the receptor for bacterial CpG DNA (3, 4). TLR9 response is CpG motif-dependent as the methylation of CpG DNA or inversion of the CpG ablates the responsiveness (4). TLR9 also confers species-specific CpG response, and human PBMC respond best to GTCGTT motifs.

In the correspondence above, Drs. Agarwal and Martin show that GEM91, an antisense oligodeoxynucleotide phosphorothioate (5’-CTC TCG CAC CCA TCT CTC TTC T-3’) complementary to gag gene of HIV-1 has similarities to CpG DNA, and possibly activates the cells through TLR9 to induce HIV replication. Aside from the presence of TCG, this sequence is quite pyrimidine rich, and would be effective through the TLR9 pathway at activating mouse and human immune cells (A. M. Krieg (University of Iowa, Iowa City, IA), personal communication).

Because of the ability of CpG-DNA to activate human dendritic cells and B cells (5, 6) and induce Th1 response (7) it is included in the design of newly developed vaccines. Our observation that CpG DNA induces HIV replication through TLR9 (8), and Drs. Agarwal and Martin’s results suggest that CpG-DNA-like motifs included in the newly developed vaccines may have deleterious effects and in fact induce HIV replication. However the biological significance of these transient increases in HIV replication is yet to be determined.

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References