Collateral effects of deletion of *nlpD* on *rpoS* and *rpoS*-dependent genes. Reply.

The authors reply: In their Letter to the Editor, Tsunoi et al. (1) discuss a very interesting and relevant aspect of our published work (2) and suggest topics for further investigation. This published study identifies the *E. coli* NlpD protein as a potent regulator of gene expression in human cells. This effect is attributed to the inhibition of RNA polymerase II phosphorylation (Pol II), mediated, in part, by effects of NlpD on the Pol II RPB1 subunit and PAF1C (2, 3). The discovery of the NlpD effect was made possible by the isolation of a loss-of-function mutant (SN25) from a human carrier of the parent strain *E. coli* 83972, and a mutation strategy was devised to introduce the *nlpD* point mutation from SN25 into the parent strain and to complement SN25 with a fully functional *nlpD* gene cluster. To address whether NlpD exerts its effects on host cells independently or in synergy with sigma38 (σ^{38}), the 5' *nlpD* coding sequence was deleted in *E. coli* 83972, while leaving the *rpoS* promoter intact. The partial *nlpD* deletion mutant lost the ability to inhibit PAF1C and Pol II phosphorylation.

In the *E. coli* genome, *nlpD* and *rpoS* form an operon. σ^{38} expression is regulated, in part, from a promoter located within *nlpD*. Elegant studies addressing the role of σ^{38} as a global regulator of bacterial gene expression are referenced in the letter by Tsunoi et al. (1). Global analyses of the *rpoS* regulon in *E. coli* K-12 have identified more than 1000 genes (4, 5). As pointed out by Dr. Iwase and colleagues, effects on σ^{38} are therefore likely to affect bacterial phenotypes, under a variety of conditions. A loss of σ^{38} expression changes metabolic activity or virulence in uropathogenic *E. coli* (6). Our preliminary comparison of *E. coli* 83972 and SN25 gene expression profiles indicates that typical σ^{38} -dependent stress response genes are downregulated in SN25 relative to the 83972 wild-type strain.

Importantly, the effects of NlpD on human cells were characterized using recombinant NlpD protein rather than whole bacteria. Therapeutic efficacy was demonstrated as inhibition of inflammation and accelerated bacterial clearance from infected tissues in a murine urinary tract infection model (Figure 1). In contrast to NlpD, recombinant σ^{38} displayed no affinity for RPB1 or PAF1C in pull-down experiments. Instead, the data suggest that, by inhibiting TBP binding, σ^{38} itself may act as an additional regulator of gene expression in infected hosts (2).

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Figure 1. Recombinant NlpD treatment accelerated bacterial clearance in CY17-infected C57BL/6 mice (A) and attenuated inflammation (B). Data are presented as mean $\pm$ SEM ($n = 5$ mice). Two-way ANOVA with Sidak's multiple comparison tests, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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Conflict of interest: IA and CS are inventors on patents related to the therapeutic use of NlpD (US 16/341,962, Europe 17797991.1, Australia 2017344453, India 20194718316, Singapore 11201903336U). IA and CS are shareholders of SelectImmune Pharma, a biotech startup company developing alternatives to antibiotics in UTI treatment, and CS is chairman of the board.

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