Conclusions: Our data suggests that BAY 41-2272 acts synergistically with NO in vessels from normotensive and hypertensive animals. Down-regulation of the enzymes involved in the NO/cGMP cascade account for decreased BAY 41-2272 relaxations in rings from SHR.

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P062. NO and GSNO stimulation of recombinant soluble guanylate cyclase from *Manduca sexta*

Xiaohui Hu, William R. Montfort
Department of Biochemistry and Molecular Biophysics, University of Arizona, USA

The best-defined in vivo target for nitric oxide (NO) is soluble guanylate cyclase (sGC), a heterodimeric protein with a regulatory heme domain for binding NO and a catalytic domain involving both subunits to perform enzymatic function. *S*-Nitrosoglutathione (GSNO) is a nitric oxide containing tri-peptide that exists in vivo and may play an important additional role in NO signaling. Here we show that both NO and GSNO stimulate recombinant *Manduca sexta* (tobacco hornworm) sGC, and that nucleotides ATP and GTP, as well as nucleotide analog YC-1, modulate its activity. The regulatory domains of *Manduca* sGC were expressed and purified from *Escherichia coli* as a functional heterodimer. Stopped-flow kinetic studies of NO binding to the fragment reveal a complicated proximal histidine release process: at lower NO concentrations (1–30 μM), the release rate is independent of NO concentration, while at higher levels, the rate increases with greater NO. Thus, neither a simple two-step model nor a NO-catalyzed model can fully satisfy our observations. GTP and YC-1 facilitate NO-heme binding while ATP inhibits this process, indicating that a regulatory nucleotide-binding site resides in the regulatory domain of the enzyme, away from the catalytic domain. We also co-expressed full-length *Manduca* sGC in *E. coli* as an active dimeric protein. Partially purified enzyme is able to synthesis cGMP from GTP and its enzymatic activity is augmented dramatically in the presence of nitric oxide. Consistent with the kinetic study of fragment dimer, ATP inhibits the enzyme activity while YC-1 stimulates it. GSNO can activate *Manduca* sGC as efficiently as authentic NO. Surprisingly, activation by GSNO appears not to require release of NO to the heme iron of the protein, suggesting that GSNO activates sGC by a mechanism other than Fe-NO bond formation. *Manduca* sGC has numerous free sulfhydryls and at least one of these is readily transnitrosated by GSNO in vitro. Taken together, these data suggest that sGC activity might be regulated by S-nitrosation in vivo.

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P063. Signaling cascade for novel anti-inflammatories based on apolipoprotein-E

Michael Vitek [a], Nobutaka Ohkubo [b], Dale Christensen [c], Carol Colton [b]
[a] *Cognosci/Duke*
[b] *Duke University Medical Center*
[c] *Cognosci*

Apolipoprotein E (apoE) affects immune responses and suppresses inflammation in an isoform specific manner (Brown et al. 2002). COG133 is a peptide corresponding to residues 133–149 of holo-apoE that possesses the immunomodulatory properties of holo-apoE (Laskowitz et al. 2001, Lynch et al. 2003, 2005). In response to lipopolysaccharide (LPS), COG133 significantly down-regulates the release of nitric oxide (NO), IL-6 and TNFa in BV2 microglial cells and in C57Bl/6 mice. These results suggested that we could employ the apoE-peptide, COG133, as a tool to understand how apoE suppresses inflammatory responses.

LPS binding to Toll-Like Receptor-4 (TLR4) activates an intracellular signal transduction cascade, which includes phosphorylation of MAP kinases and transcription factors, that eventually participate in stimulating NO and TNFa release. LPS induced phosphorylation of IKK and levels of phospho-IKK were reduced in the presence of COG133. Phosphorylation of IKK is needed to activate NFkB DNA-binding activity and COG133 reduced phospho-IKK levels resulting in decreased NFκB DNA-binding activity as shown with gel-shift methods. LPS also induced the levels of phosphorylated p38-MAP kinase, ERK and JNK, but the levels of these phospho-proteins were significantly reduced in the presence of COG133 plus LPS suggesting another site of apoE-peptide action on this inflammatory signaling cascade.

apoE peptides corresponding to COG133 were found to bind to cell-surface receptors that bind holo-apoE (Croy et al. 2004). We examined the role of these receptors in COG133 function by LPS-stimulation of macrophages from LDL-receptor containing or LDL-receptor lacking mice. Independent of the presence or absence of LDL receptors, LPS plus COG133 treated macrophages released significantly less NO than their LPS treated counterparts. We are currently employing siRNAs to reduce other apoE receptor levels and will discuss their roles in COG133-mediated inhibition of inflammation.

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P064. Macrophage nitric oxide (NO) stimulation by an immunomodulator: P-MAPA

Natália Bromberg [a], Giselle Z. Justo [b], Amedea B. Seabra [a], Nelson Durán [c,d]
[a] *Chemistry Institute—Universidade Estadual de Campinas SP, Brazil*
[b] *Biology Institute—Universidade Estadual de Campinas SP, Brazil*
[c] *IQ—Universidade Estadual de Campinas SP, Brazil*
[d] *NCA Universidade de Mogi das Cruzes (UMC), Brazil*
E-mail addresses: duran@iqm.unicamp.br, duran@umc.br (N. Durán)

We have earlier reported that the therapeutic effect of the immunomodulator P-MAPA, an aggregated polymeric form of protein magnesium ammonium phospholinoleate-palmitoleate anhydride, against tumors and bacteria may be partly produced by the number of macrophages available and the extent to which their functional activities are modulated. Recently, P-MAPA has been shown to modulate cell-mediated cytotoxicity and cytokine production, including IFN-g secretion. NO appears to be a major macrophage mediator of bacteria and tumor cell killing. Thus, this study assessed the ability of P-MAPA to modulate NO production by macrophages purified from the peritoneal cavity of thioglycolate-treated mice. Cells were stimulated with P-MAPA or lipopolysaccharides (LPS), and NO was determined by measuring nitrite. Maximal amounts of NO were produced after stimulation with less than 0.5 μg/mL P-MAPA. Comparable levels of NO were detected in LPS-stimulated cultures at the same dose range. LPS induction to the secretion of TNF, and the co-operation of IFN-g with TNF for NO secretion is known to mediate macrophage cytotoxicity, suggesting that these cytokines may play an important role in the mechanism by which P-MAPA, like LPS, induces NO production. Finally, the ability of P-MAPA to stimulate NO release may be of relevance to the effector functions of activated macrophages in infectious and cancer diseases.

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