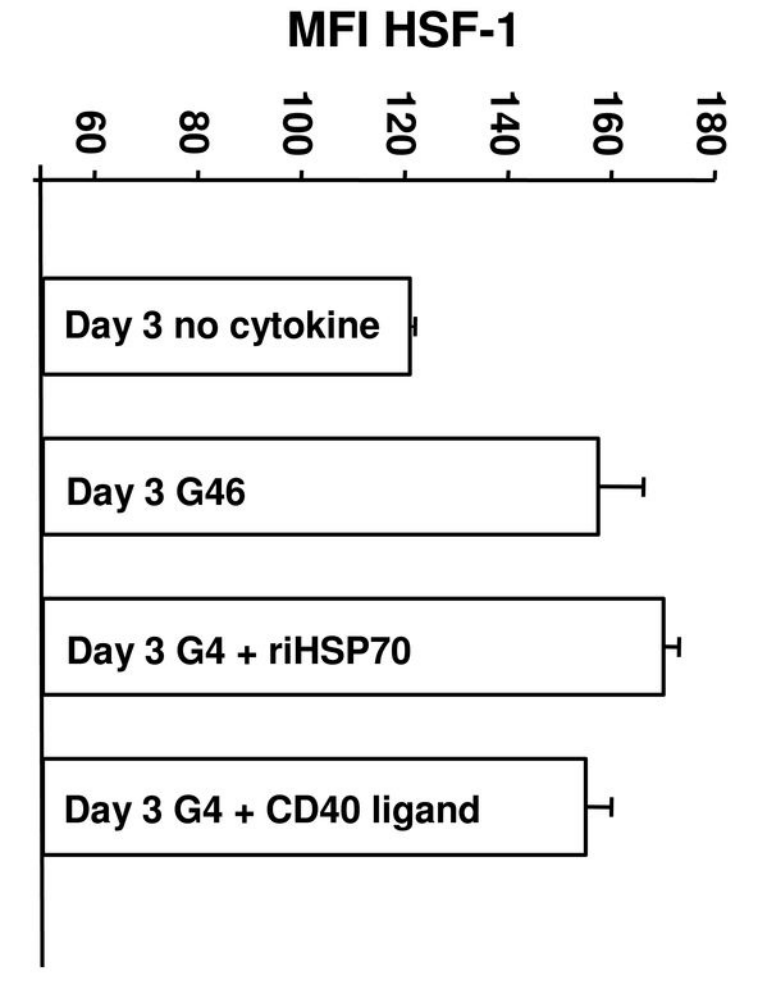
Supplemental Figure Legends

Supplementary Figure 1. Increases in the levels of HSF-1 substantiate cytokine triggered iHSP70 activity during the monocyte to DC transition. As measured by flow cytometry in permeabilized cells on day 3, the total level of the iHSP70 transcription factor HSF-1 increased with GM-CSF(G), IL-4 (4) and IL-6 (6) treatment versus no cytokine controls. Increased expression of HSF-1 also occurred with riHSP70+GM-CSF+IL-4 and CD40 ligand GM-CSF+IL-4. Results represent the mean ± SE; N-2-3 for each condition.

Supplementary Figure 2 Fluorescence microscopic analysis revealing translocation of HSF-1 from the cytoplasm to the nucleus with GM-CSF+IL-4+IL-6 on day 3. Consistent with active transcription of iHSP70, intense nuclear staining for HSF-1 occurred in cells exhibiting typical DC morphology. Inset represents cells stained with isotype control. Nucleus was counterstained with propidium iodide; original magnification = 60X.

Supplementary Figure 3. Proposed endogenous/autocrine iHSP70 adjuvant activity promoting dendritic cell growth during the monocyte to DC transition. Monocyte-DC precursors, but not mature DC, monocyte/macrophages generated with M-CSF, or lymphocytes, respond to certain DC/pro-inflammatory cytokines (GM-CSF, IL-4, IL-13, and IL-6) to produce a concurrent heat shock protein associated response which includes induction of intracellular iHSP70, surface iHSP70 expression, maintenance of HSP receptors such as CD91 and CD40, and release of extra-cellular iHSP70 from viable DC. In certain physiological settings such as the hyperthermic rheumatoid joint heat stress may cooperate with cytokines to further enhance the cytokine triggered response. Extracellular iHSP70 and, in identical fashion, CD40 ligand were powerful enhancers of BCL-xL expression. Thus, it is conceivable that engagement of CD40 on the DC surface by endogenously produced extra-cellular iHSP70 signals increased cytoprotection via BCL-xL. As previously shown by others, signaling through CD40 would also contribute to DC growth through the induction of cytokines (IL-12) via NFk-B signaling. This model identifies GM-CSF/IL-4(IL-13)/IL-6 and heat stress as cooperative elements for promoting HSP/DC activity in normal and abnormal immunity. Our model is consistent with the well-recognized anti-apoptotic effects of iHSP70 in other cell types and previous descriptions that protection from apoptosis via BCL-2 family members is required for the selective expansion of the myeloid DC lineage. Finally, our model lends new insight into how DC may respond to "danger" without succumbing to the potentially lethal factors present in the inflammatory environment.


MFI HSF-1
60
80
100
120
140
160
180
Day 3 no cytokine
Day 3 G46
Day 3 G4 + riHSP70
Day 3 G4 + CD40 ligand