Reviewer's report

Title: Respiratory Syncytial Virus and TNFalpha Induce Chemokine Gene Expression Through Distinct NF-kappaB Pathways

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Reviewer: Dr Richard Rippe

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Accept after revision, which I do not need to see

The manuscript, "Respiratory Syncytial Virus and TNFalpha Induce Chemokine Gene Expression Through Distinct NF-kappaB Pathways" by Carpenter et al. describes studies showing that RSV increases chemokine gene expression (IL-8, MCP-1, and RANTES) that is mediated by a redox sensitive NF-kappaB signaling pathway distinct from TNFalpha-mediated NF-kappaB activation. Chemokine expression induced by TNFalpha and RSV infection was also differently inhibited by NF-kappaB inhibitors N-acetyl-L-cysteine (NAC) and dexamethasone (DEX) suggesting chemokine gene expression is induced differently by NF-kappaB. RSV and TNFalpha also were reported to have different kinetics of NF-kappaB complex formation. NAC and DEX were reported to have different effects of NF-kappaB binding activity, which the authors conclude represent different signaling pathways leading to NF-kappaB activation. This is an interesting study which begins to understand the molecular mechanism responsible for RSV mediated chemokine gene expression. Several issues are raised in this study that should be addressed by the authors.

Major issue:
1. This is a descriptive study with respect to NF-kappaB activation mediated by RSV
infection and TNFa; however, the study falls short of actually demonstrating that different signaling pathways are utilized by RSV infection and TNFa treatment to activate NF-kB. Do these different stimulate share portions of the classical signaling pathway to activate NF-kB, for example NIK, IKK or are there convergent signaling signal leading to NF-kB activation? Does RSV utilize a kinase-dependent mediated signaling pathway like TNFa does? This information would greatly improve the manuscript.

Minor issues:
1. In addition to NF-kB, AP-1 has also been considered to be a redox induced transcription factor that controls chemokine gene expression. Why didn’t the authors investigate AP-1 binding activity in these studies?
2. In Figure 2 the authors indicate that MCP-1 and IL-8 expression levels continue to increase at 24 hrs; however, it actually appears from the figure that RSV infection results in a decrease in MCP-1 and IL-8 expression at 24 hrs and not an increase, even when considering the levels of GAPDH. Secondly, which band(s) are represented by the GAPDH signal? If all four bands at the bottom of the gel represent GAPDH then why does the GAPDH RPA generate multiple bands?
3. In Figures 4 and 5, the quality of the NF-kB gel shifts is rather poor. It is difficult at best for the reader to accurately assess the intensities of the different complexes formed. These gel shifts should be repeated to more clearly demonstrate complex formation. Supershift assays should be shown to demonstrate the identity of each complex. In addition, what do the different arrows and brackets represent in both figures? What do "sc" (Figures 4 and 5) and "nc" (Figure 5) represent? In the Materials and Methods section the authors state "a specific or non-specific oligonucleotide as indicated in the figure legends..."; however, no such indication is noted in the figure legends. The sequence of the NF-kB oligonucleotide and that of the non-specific oligonucleotides should be provided.

Competing interests:

None declared.