Evidence for a redox-sensitive protein tyrosine kinase in nuclear factor kappa B activation and interleukin 2 production in EL4.NOB1 cells.

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Nuclear factor kappa B (NFkB) is a transcription factor that is activated in many different cell types. In response to viruses, bacteria and stress factors as well as phorbol esters and inflammatory cytokines such as interleukin 1 (IL1), NFkB becomes activated [1]. It regulates the expression of various genes involved in the immune and inflammatory response, including the genes for cytokines, cell surface receptors, adhesion molecules and acute phase proteins. Although there is no clear consensus on the events leading to NFkB activation, a model has been proposed based on evidence to date. NFkB is located in the cytoplasm of resting cells in an inactive state complexed with the inhibitor protein, IxB. Upon stimulation the IxB protein becomes phosphorylated and dissociates from the complex. The now active NFkB complex can then translocate to the nucleus where it binds to the kB consensus sequence and causes a change in gene expression. Evidence for the involvement of protein kinases and protein phosphatases in this process comes from the inducing effect of the protein kinase C activator, phorbol myristate acetate (PMA) [2] and the protein phosphatase inhibitor, okadaic acid [3], and from the inhibiting effect of the tyrosine kinase inhibitor, herbimycin A [4].

IL1 and PMA both activate NFkB and induce IL2 production in the murine thymoma cell line, EL4.NOB1. In our study we probed the importance of phosphorylation in the signal transduction pathway leading to these responses using a range of protein kinase inhibitors. Following stimulation, cells were fractionated and nuclear extracts were prepared using the method as outlined [5]. These nuclear extracts were incubated with [32-P] labelled DNA containing the NFkB consensus sequence and analysed for NFkB binding activity by electromobility shift assay as described [2]. NFkB present in the nuclear extract binds to the labelled DNA probe causing it to retard in a 5% polyacrylamide gel, while unbound or free probe migrates to the bottom of the gel. Following electrophoresis, gels were dried and autoradiographed.

IL2 produced by EL4 cells in response to stimulus was measured using an enzyme-linked immunosorbant assay (ELISA).

Initially we looked at the effect of staurosporine, a potent PKC inhibitor, on NFkB activation and IL2 production. As expected, staurosporine inhibited both PMA activation of NFkB and PMA induction of IL2. Interestingly, while having no effect on IL1-induced NFkB staurosporine significantly enhanced IL1-stimulated IL2 production. Staurosporine had little effect on IL2 production on its own. Table 1 summarises the effect of the protein kinase inhibitors on IL1- and PMA-stimulated IL2 production respectively.

Table 1. Effect of inhibitors on IL2 production in EL4 cells

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration</th>
<th>C</th>
<th>+ IL1</th>
<th>+ PMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbimycin A</td>
<td>100nM</td>
<td>0.03 ± 0.08</td>
<td>0.11 ± 0.06</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Genistein</td>
<td>50nM</td>
<td>0.11 ± 0.06</td>
<td>0.28 ± 0.06</td>
<td>0.28 ± 0.06</td>
</tr>
<tr>
<td>Quercetin</td>
<td>5ng/ml</td>
<td>0.24 ± 0.08</td>
<td>0.20 ± 0.09</td>
<td>0.81 ± 0.09</td>
</tr>
</tbody>
</table>

Figure 1. Cells were pretreated for 1 hour with the following concentrations of herbimycin A: 0.02μM (lane 3), 0.2μM (lane 4), 2μM (lane 5), 0.04μM (lane 6), 0.4μM (lane 9). Following washing, the cells were treated with medium (lanes 1&6), IL1 (10ng/ml) for 15 minutes (lanes 7-9) or PMA (100ng/ml) for 24 hours (lanes 2-5). Retarded protein-DNA complexes are shown. A result representative of three experiments is shown.