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Letter to the Editor

Opposing actions of STAT-1 and STAT-3 on the Bcl-2 and Bcl-x promoters

Dear Editor,

The Bcl-2 family of proteins contains at least 15 members which can have pro- or anti-apoptotic effects. All share at least one of four Bcl-2 homology domains (BH1 to BH4), and inclusion of different BH domains in individual Bcl-2 family members is generally responsible for pro- or anti-apoptotic activity (reviewed in[1]). Pro- and anti-apoptotic members can form heterodimers, and their ratio may determine whether a death or survival signal is delivered.[2] There is strong evidence that the actions of Bcl-2 proteins are exerted at the level of the mitochondrial membrane (reviewed in[3]). Thus many Bcl-2 members are anchored in the mitochondrial outer membrane, and anti-apoptotic family members such as Bcl-2 and Bcl-x_L form ion channels which increase the rate of proton extrusion from mitochondria.

Some studies have begun to address the transcriptional regulation of anti-apoptotic *Bcl-2* genes. In leukaemias, for example, a hybrid transcription factor formed by chromosomal rearrangement increases Bcl-2 expression,[4] and overexpression of the CCAAT enhancer binding protein, GADD153, reduces Bcl-2 expression with an accompany-

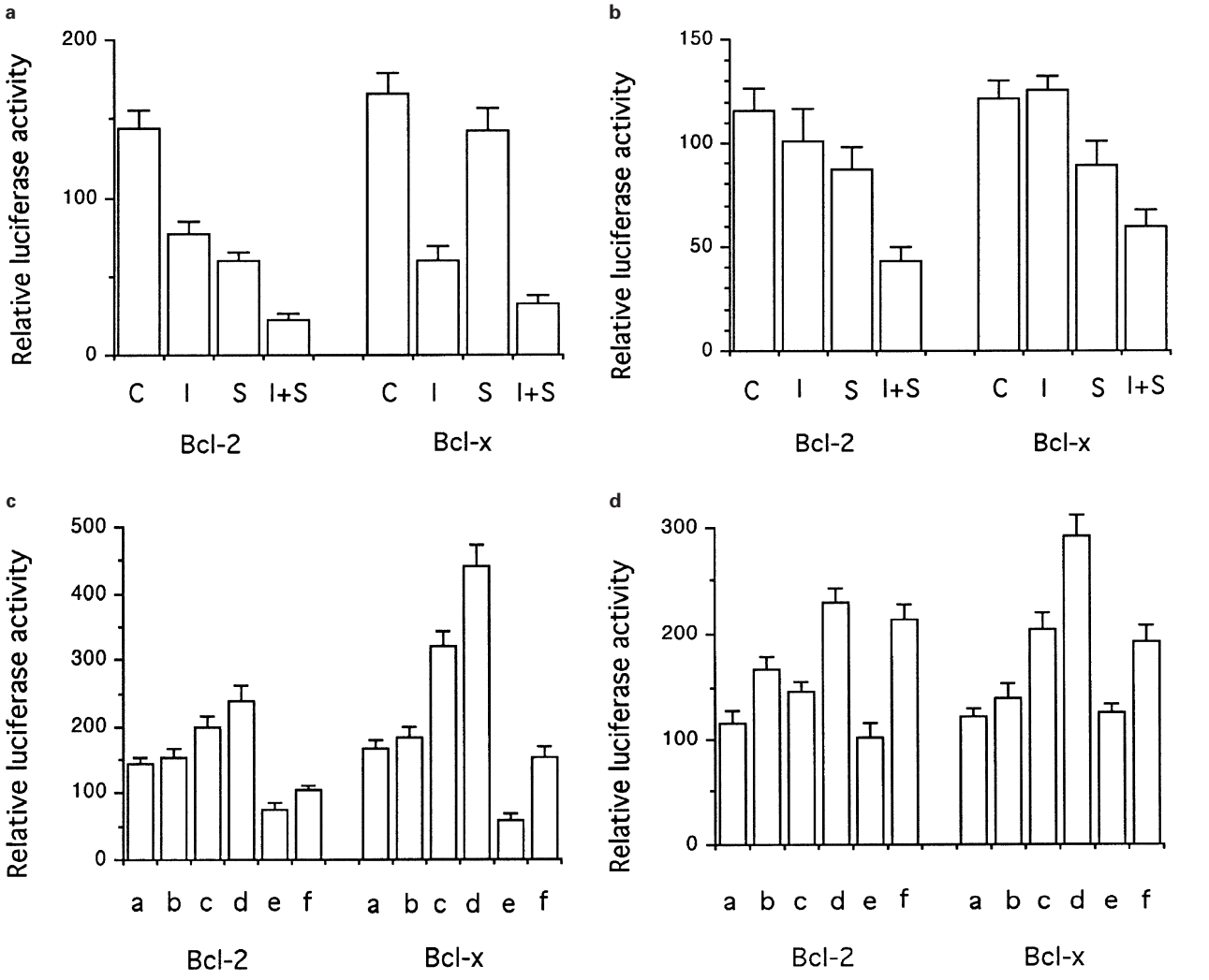

Figure 1 Effects of IFNγ (I), STAT-1 (S) and both IFNγ and STAT-1 (I+S) on control (C) basal activity of the Bcl-2 and Bcl-x promoters in STAT-1 expressing (**a**) and STAT-1 deficient (**b**) U3A cells. Effects of CT-1 and STAT-3 on basal activity of the Bcl-2 and Bcl-x promoters in STAT-1 expressing (**c**) and STAT-1 deficient (**d**) U3A cells. a: control basal activity; b: STAT-3; c: CT-1, d: CT-1+STAT-3; e: IFNγ; f: IFNγ+CT-1

ing increase in apoptosis.[5] In neuronal cells, transactivation of Bcl-2 by the POU factor, Brn-3a, is inhibited by p53, although p53 itself has little effect on basal Bcl-2 expression.[6] NF-κB-dependent enhancement of Bcl-x_L expression has been reported to mediate survival signals transduced through CD40 in B cells,[7] and the transcriptional repressor, Btf, which induces apoptosis, is normally retained in the cytoplasm by Bcl-2 and Bcl-x_L.[8]

In the heart, there is also evidence that apoptosis following ischaemia/reperfusion (IR) injury is associated with modulation of Bcl-2 and Bcl-x_L expression. For example, in the isolated perfused rat heart, IR reduces Bcl-2 expression, in parallel with increased AP-1 and reduced NF-κB.[9] Moreover, the reduction in apoptosis in IR rabbit hearts produced by phenylephrine preconditioning is associated with an increased Bcl-x/Bax ratio.[10] Since we have observed that activated Signal Transducer and Activator of Transcription (STAT)-1 is an important transactivator in the apoptosis induced by IR injury,[11] and others have shown that STAT-3 is involved in the cardioprotective actions of cardiotrophin-1 (CT-1),[12] we have studied whether these pro- and anti-apoptotic effects of STATs-1 and 3 are mediated by regulation of the expression of the Bcl-2 and Bcl-x_L genes.

In U3A-ST1 cells constitutively expressing STAT-1 (Figure 1a), IFNγ, a known activator of STAT-1, reduces basal expression of the Bcl-2 promoter. Transfection with STAT-1 by itself also reduces Bcl-2 promoter activity and overexpression of STAT-1 together with IFNγ treatment produces a reduction significantly greater than by either STAT-1 or IFNγ alone. This presumably reflects phosphorylation of transfected STAT-1 by Janus kinases activated by ligation of the IFNγ receptor. Figure 1a also shows that IFNγ reduces basal activity of the Bcl-x promoter; although the reduction in Bcl-x promoter activity produced by STAT-1 overexpression alone is less pronounced than with the Bcl-2 promoter, it is still significant, and IFNγ treatment of STAT-1 overexpressing cells again results in more profound reduction in Bcl-x promoter activity than IFNγ or STAT-1 alone. The reduction by IFNγ in Bcl-2 and Bcl-x promoter activity is not seen in the STAT-1 deficient cell line U3A, indicating that it is dependent on activation of STAT-1. Moreover, expressing STAT-1 alone or together with IFNγ treatment does reduce activity of both promoters, indicating that the effect of IFNγ can be restored by expressing STAT-1 in the STAT-1 deficient cells (Figure 1b).

Figure 1c shows that CT-1, a STAT-3 activator, can enhance Bcl-2 and Bcl-x activity. Moreover, CT-1 reverses the suppressive effect of IFNγ on the Bcl-2 and Bcl-x promoters (Figure 1c, bars e and f), most markedly in the STAT-1 deficient cell line (Figure 1d). In the STAT-1 deficient cells, both STAT-3 and CT-1 individually enhance Bcl-2 and Bcl-x promoter activity, and CT-1 also has this effect in STAT-1 expressing cells. The combined effects of STAT-3 and CT-1 are again greater than either factor alone.

The data suggest that one mechanism by which STAT-1 mediates IR-induced apoptosis is by reducing expression of anti-apoptotic Bcl-2 and Bcl-x genes. In addition, the cardioprotective effects of CT-1 may be mediated by STAT-3 induced increases in expression of these genes. STAT-1 and STAT-3 homodimers activate distinct sets of genes. In addition, STAT-1 and STAT-3 can also form heterodimers.[13] The results reported here suggest that, in the heart, the relative proportions of STAT-1 and STAT-3 may affect the transcriptional activity of two anti-apoptotic genes. It is unclear whether STAT-1 and STAT-3 act at a single site to regulate the Bcl-2 and Bcl-x promoters, or whether interaction between separate binding regions is involved. Promoter mapping studies are in progress to resolve this question.

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