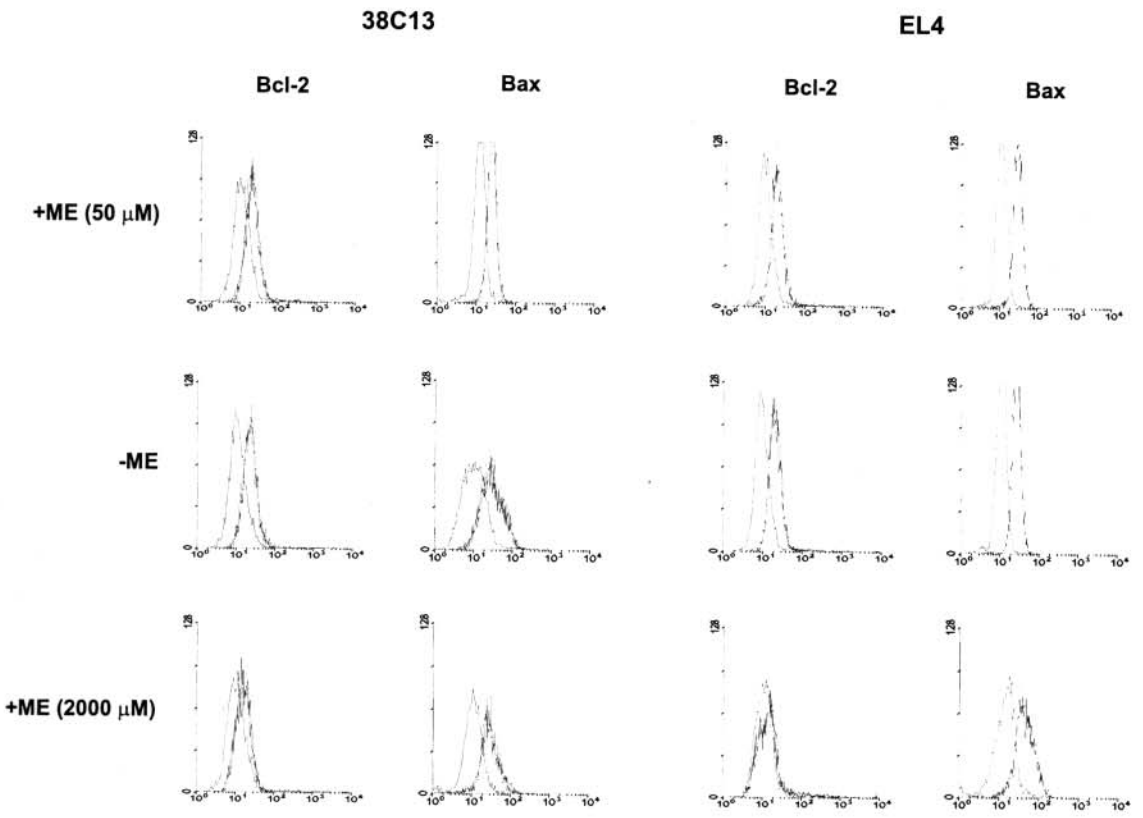

Fig. 7. Effect of availability of thiols, represented by 2-mercaptoethanol (ME), on the expression of Bcl-2 and Bax by 38C13 and EL4 cells. Control conditions (basic medium with 50 μM 2-mercaptoethanol), thiol deprivation (without 2-mercaptoethanol), and thiol excess (with 2000 μM 2-mercaptoethanol) were tested. After 12 h of incubation the cells were stained with specific antibody (hamster monoclonal IgG antibody 3F11 specific for mouse Bcl-2, rabbit polyclonal IgG antibody Sc-562 specific for mouse Bax), as well as with relevant control non-specific immunoglobulin (non-specific hamster IgG, non-specific rabbit IgG), and analysed by flow cytometry. The bold line represents staining with specific antibody and the fine line represents staining with control non-specific immunoglobulin. The data shown were obtained in one representative experiment of three independent experiments.

level, either. Thus, it seems that apoptosis induction by thiol excess and also apoptosis induction by thiol deprivation are not directly related to a specific significant change in the p53 level and particularly in p53 activation (detected by an increase in the p21 level).

Bcl-2 and Bax expression under thiol deprivation and thiol excess

We also assessed the expression of antiapoptotic Bcl-2 and proapototic Bax. Repeated experiments employing indirect immunofluorescence showed that apoptosis induction by thiol excess in 38C13 cells and also in EL4 cells correlated with a decrease in the Bcl-2 level during 16 h of incubation under thiol excess. On the other hand, thiol deprivation in both 38C13 and EL4 cells did not significantly change the Bcl-2 level. Data for a 12-h incubation period are shown in Fig. 7 and Table 1. Apoptosis induction by thiol excess and by thiol deprivation in 38C13 cells was not associated with a significant change in the Bax level during 16 h of incubation. Simi-

Table 1. Effect of availability of thiols, represented by 2-mercaptoethanol (ME), on the expression of Bcl-2 by 38C13 and EL4 cells[a]

Cells	Bcl-2 expression[b]		
	+ ME (50 μM)	- ME	+ ME (2000 μM)
38C13	22.2 (11.8)	23.7 (11.5)	17.0 (11.1)
EL4	21.1 (10.5)	21.8 (10.1)	15.0 (12.2)

[a]Control conditions (basic medium with 50 mM 2-mercaptoethanol), thiol deprivation (without 2-mercaptoethanol), and thiol excess (with 2000 μM 2-mercaptoethanol) were tested. After 12 h of incubation the cells were stained with specific antibody (hamster monoclonal IgG antibody 3F11 specific for mouse Bcl-2) as well as with relevant control non-specific immunoglobulin (non-specific hamster IgG) and analysed by flow cytometry.

[b]Mean fluorescence intensities were obtained in one representative experiment of three independent experiments. Values in brackets represent control staining with non-specific immunoglobulin.

larly, apoptosis induction by thiol excess in EL4 cells was not associated with a significant change in the level of the Bax protein. Data for a 12-h incubation period are shown in Fig. 7.

Apoptosis induction by thiol excess in both 38C13 and EL4 cells seemed to correspond with the decreased level of antiapoptotic Bcl-2. The level of proapoptotic Bax was not changed. However, apoptosis induction by thiol deprivation in 38C13 cells was not associated with any change in the level of antiapoptotic Bcl-2 or in the level of proapoptotic Bax.

Discussion

We found that thiol deprivation induced apoptosis in mouse B-cell lymphoma 38C13 but not in mouse T-cell lymphoma EL4 and that thiol excess induced apoptosis in 38C13 as well as in EL4 and also in other lymphoid cells. Thus, we demonstrate that not only thiol deprivation can induce apoptosis in sensitive lymphoma cells, as it was supposed for several cell types previously (Sato et al., 1995; Castro-Obregon and Covarrubias, 1996; Neumann et al., 1998; Aoshiba et al., 1999), but also that thiol excess induces apoptosis in lymphomas. Actually, apoptosis induction by thiol excess is not a surprising finding (Takagi et al., 1974; Held and Melder, 1987; Held and Biaglow, 1994). However, it has not been demonstrated explicitly until now. Interestingly, 38C13 cells, which are highly sensitive to thiol deprivation, were found previously to also be highly sensitive to iron deprivation (Kovar et al., 1997). On the other hand, EL4 cells, which are resistant to thiol deprivation, are also resistant to iron deprivation. However, the sensitivity to thiol deprivation does not correlate with the sensitivity to iron deprivation for all cell types tested (our unpublished data).

It has been demonstrated that only compounds with a free SH group are able to prevent apoptosis in sensitive cells and thus only deprivation of such compounds, i.e. thiols, results in apoptosis. There are several lines of supportive evidence: (i) we and others (Sato et al., 1995; Marchetti t al., 1997) show that the thiol cross-linking agent diamide, which mimics disulphide bridge formation, abrogates the thiol prevention of apoptosis. (ii) We show that while L-cysteine, in a manner similar to other thiols, prevents apoptosis in sensitive cells, L-cystine, with a disulphide bridge, is without any effect. (iii) We also show that ascorbic acid, a non-thiol antioxidant, is unable to substitute for thiols in preventing apoptosis. Similarly, other non-thiol antioxidants have been shown to be unable to substitute for thiols (Delneste et al., 1996; Deas et al., 1997). However, there are some contradictory data showing that the antioxidants ascorbic acid, catalase and vitamin E can substitute for thiols and prevent apoptosis (Aoshiba et al., 1999; Ikeda et al., 1999). 2-mercaptoethanol seems to be the most potent thiol tested. Differences in efficient concentrations probably reflect differing chemical reactivity of the thiols involved.

As discussed above, the mechanism of apoptosis induction by thiol deprivation is very likely related to a specific function of thiols, i.e. compounds containing a free SH group, in preventing apoptosis. This thiol function probably concerns the redox status of the cell (Sato et al., 1995; Castro-Obregon and Covarrubias, 1996; Marchetti et al., 1997; Falk et al., 1998; Aoshiba et al., 1999; Hall, 1999; Cai et al., 2000; Yang et al., 2000). It was speculated that the redox state of mitochondrial thiols could regulate opening of mitochondrial permeability transition (PT) pores and thus switch on the apoptotic programme of the cell (Marchetti et al., 1997). Recently it has been shown that oxidation of a thiol residue of the adenine nucleotide translocator (ANT), one of the proteins of the permeability transition pore complex (PTPC), leads to mitochondrial membrane permeabilization (Constantini et al., 2000). Another considered mechanism of apoptosis induction by thiol deprivation is upregulation of Fas receptor expression resulting from the change in the redox state of the cell due to thiol depletion. However, the relation between the control of Fas receptor expression and redox changes remains unclear. Autocrine apoptosis induction by the Fas ligand can be involved here (Delneste et al., 1996; Deas et al., 1997; Neumann et al., 1998). On the other hand, Furuke and coworkers (Furuke et all., 1999) showed that thiol deprivation suppressed the expression of the Fas ligand in activated NK cells. Several other mechanisms of apoptosis induction by thiol deprivation, such as thiol inhibition of NF-kappa B activation (Lin et al., 1995) or thiol regulation of the uptake of indispensable cystine by its reduction to cysteine (Falk et al., 1998), are also considered.

The mechanism of apoptosis induction by thiol excess, demonstrated in this study, can be related to the generation of H_2O_2. Thiol cytotoxicity resulting from the generation of H_2O_2 has been described previously (Takagi et al., 1974; Held and Melder, 1987; Held and Biaglow, 1994). However, our data concerning particularly the cell death induced by relevant high concentrations of non-thiol reductant ascorbic acid show that the mechanism is not necessarily related to the function of compounds with a free SH group and point at the possibility that it could be related to the effect of excess of any reductant.

Our data concerning the expression of p53 and $p21^{CIP1/WAF1}$ support the suggestion that thiol availability controls apoptosis independently of the p53 control system. Similarly, our data concerning the expression of Bcl-2 and Bax correspond with the suggestion that apoptosis induction by thiol deprivation