cells decreased with time. After 24 h, the G_1 peak completely disappeared (Fig. 2). In the case of EL4 cells, flow cytometric analysis detected accumulation of hypodiploid cells only under thiol excess (1000 μM 2-mercaptoethanol).

DNA fragmentation analysis by agarose gel electrophoresis showed that 38C13 cells after 16 h of incubation under thiol deprivation (2-mercaptoethanol withdrawal) and also under thiol excess (1000 μM 2-mercaptoethanol) produced a ladder typical for apoptosis. EL4 cells produced the ladder only under thiol excess (Fig. 3). Thus, flow cytometric analysis and DNA fragmentation analysis proved that thiol deprivation induced apoptosis only in 38C13 cells, while thiol excess induced apoptosis in both 38C13 and EL4 cells as well as in other cells (Raji, HeLa).

Correlation of thiol effects with the presence of an SH group

All tested compounds with a free SH group such as 2-mercaptoethanol (50 μM), L-cysteine (500 μM) or dithiothreitol (500 μM) supported survival and growth of 38C13 cells during 24 h of incubation. While L-cysteine supported survival and growth of the cells, L-cystine was without any supportive effect. The incubation with extra 500 μM L-cystine (approximately 200 μM L-cystine is a regular component of the culture medium) resulted in the death of 38C13 cells similar to the control incubation without 2-mercaptoethanol, i.e. without any compound with a free SH group (Fig. 4A). Compounds with a free SH group, i.e. 2-mercaptoethanol (50 μM), L-cysteine (500 μM) or dithiothreitol (500 μM), did not produce any ladder during 22 h of incubation either. On the other

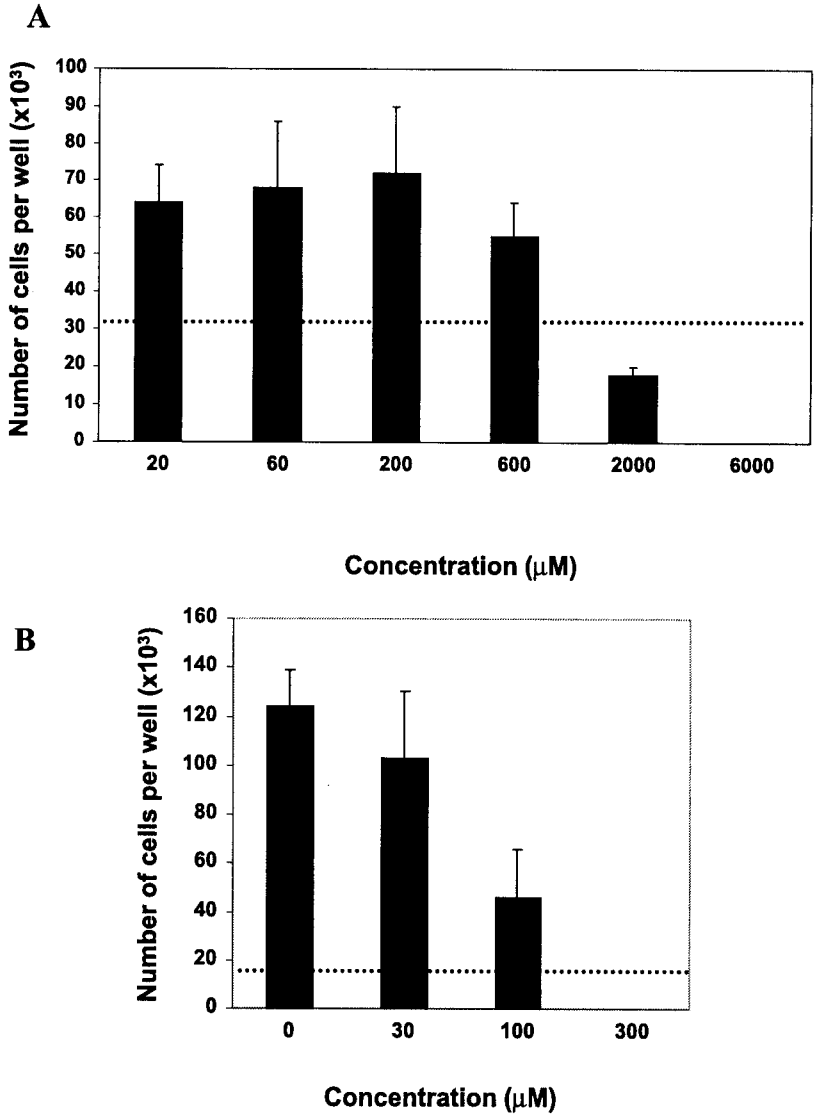

Fig. 5. Effect of (A) ascorbic acid (20–6000 μM) and (B) diamide (30–300 μM) on the growth and survival of 38C13 cells in the presence of 50 μM 2-mercaptoethanol. Control cells were incubated (A) with 20 μM ascorbic acid because the basic medium itself contains 20 μM ascorbic acid, or (B) without diamide. Cells were seeded at 40 x 10^3 cells/100 μl of medium in the well. The number of cells in the inoculum is shown as a dotted line. The number of living cells was determined after 24-h incubation. Each column represents the mean of at least four separate cultures ± SEM.

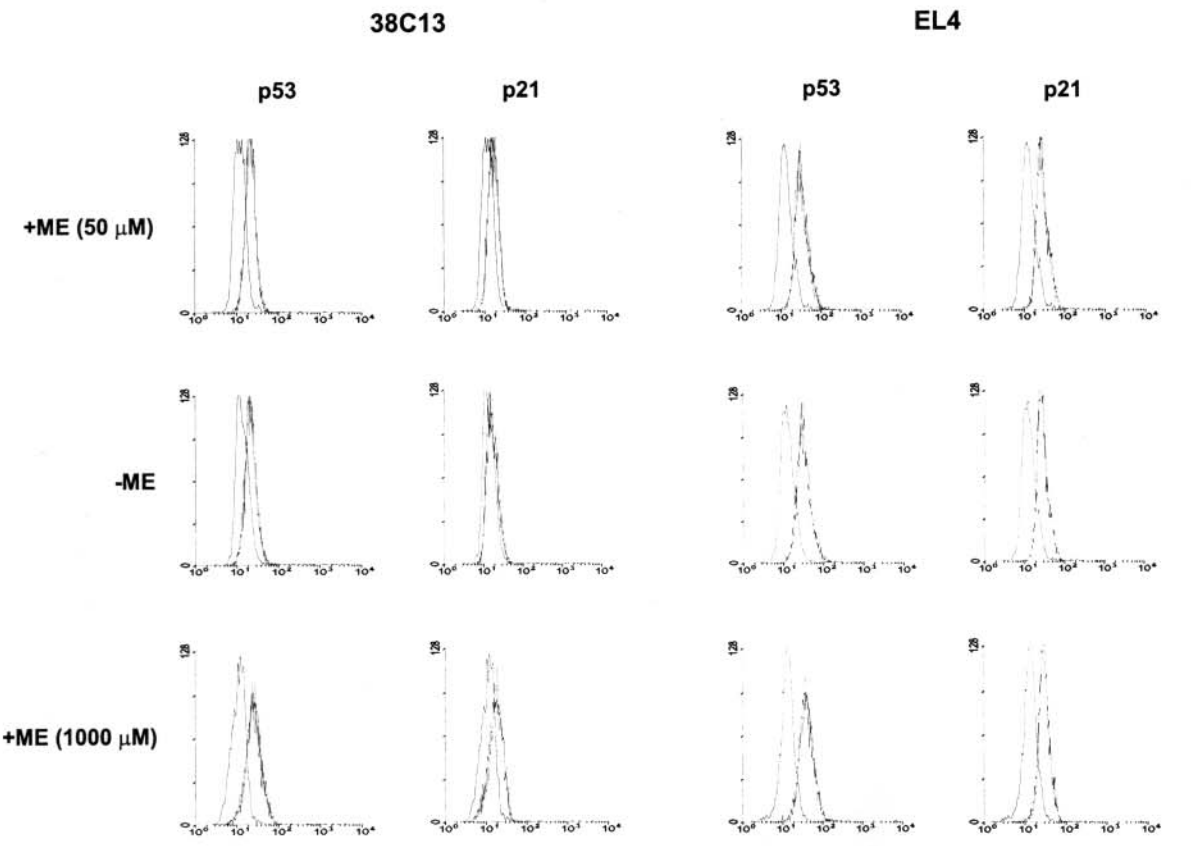

Fig. 6. Effect of availability of thiols, represented by 2-mercaptoethanol (ME), on the expression of p53 and $p21^{CIP1/WAF1}$ by 38C13 and EL4 cells. Control conditions (basic medium with 50 μM 2-mercaptoethanol), thiol deprivation (without 2-mercaptoethanol), and thiol excess (with 1000 μM 2-mercaptoethanol) were tested. After 12 h of incubation the cells were stained with specific antibody (mouse monoclonal IgG antibody PAb 240 specific for mouse p53, mouse monoclonal IgG antibody HZ 52 specific for mouse p21), as well as with relevant control non-specific immunoglobulin (non-specific mouse 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.

hand, the incubation with extra L-cystine (500 μM), like in the control incubation without 2-mercaptoethanol, produced a typical ladder (Fig. 4B).

We also tested the effect of non-thiol antioxidant ascorbic acid in the range of concentrations 20–6000 μM. Ascorbic acid alone was unable to support survival of 38C13 cells at any concentration used during 24 h of incubation (data not shown). When ascorbic acid was applied together with 50 μM 2-mercaptoethanol, concentrations higher than 600 μM resulted in cell death. The cells grew in the presence of 600 μM and lower concentrations of ascorbic acid (Fig. 5A). Thiol cross-linking compound diamide at concentrations higher than 100 μM completely abrogated the survival-supporting effect of 50 μM 2-mercaptoethanol (Fig. 5B).

The findings showed that the presence of compounds with a free SH group was required for the survival-supporting and growth-supporting effects. However, the death-inducing effect of high thiol concentrations did not seem to be specifically related to the presence of a free SH group.

p53 and $p21^{CIP1/WAF1}$ expression under thiol deprivation and thiol excess

In order to acquire a better understanding of mechanisms involved in apoptosis induction by thiol deprivation and thiol excess in the studied cells, we assessed the expression of p53 and p21 under thiol deprivation and thiol excess. Repeated experiments employing indirect immunofluorescence showed that thiol deprivation (2-mercaptoethanol withdrawal) and also thiol excess (1000 μM 2-mercaptoethanol) did not significantly change the p53 level in 38C13 as well as in EL4 cells during 12-h incubation. Concerning the p21 level, there also was no change for both 38C13 and EL4 cells under thiol deprivation as well as under thiol excess. Data for a 12-h incubation period are shown in Fig. 6. Similar data were obtained with Raji and HeLa cells. In the case of Raji cells, even a certain decrease in the p21 level was seen for thiol excess after 24-h incubation (data not shown).

No change in the p53 level could be seen for apoptosis induction by thiol deprivation and thiol excess in 38C13 and EL4 cells. Apoptosis induction in both 38C13 and EL4 cells was not associated with any change in the p21