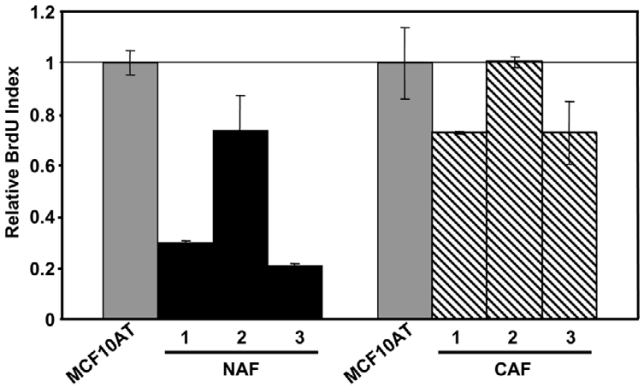
Table 2

5-Bromo-2'-deoxyuridine (BrdU) labeling* of MCF10AT cells grown in monoculture and in co-cultures with normal breast-associated fibroblasts (NAF) and carcinoma-associated fibroblasts (CAF)

Culture	BrdU-labeling indices of MCF10AT cells (mean ± standard error of the mean)	BrdU-labeling indices of MCF10AT cells (group mean ± standard error of the mean)	Comparison of BrdU-labeling indices of MCF10AT cells between groups (linear regression)	
MCF10AT ($n = 6$)	27.7 ± 7.2	27.7 ± 7.2	MCF10AT vs MCF10AT + NAF ($P = 0.013$)	MCF10AT + NAF vs MCF10AT + CAF ($P < 0.001$)
NAF-1+ MCF10AT ($n = 7$)	17.1 ± 2.8	14.1 ± 1.4 ($n = 20$)		
NAF-2 + MCF10AT ($n = 6$)	13.8 ± 1.9			
NAF-3 + MCF10AT ($n = 7$)	11.4 ± 1.9			
CAF-1 + MCF10AT ($n = 8$)	25.9 ± 4.9	25.5 ± 2.8 ($n = 22$)	MCF10AT vs MCF10AT + CAF ($P = 0.935$)	
CAF-2 + MCF10AT ($n = 8$)	26.6 ± 3.2			
CAF-3 + MCF10AT ($n = 6$)	23.5 ± 7.4			

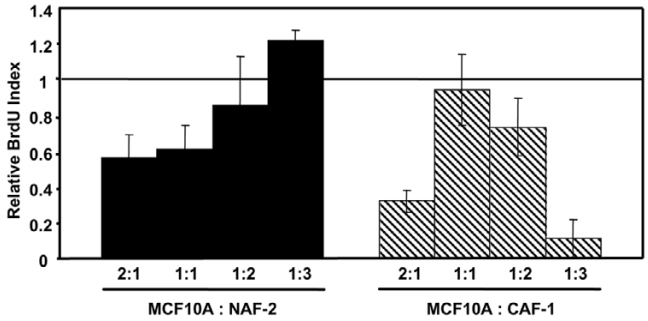
*Assessed by immunocytochemistry

Figure 5



5-Bromo-2'-deoxyuridine (BrdU) labeling, assessed by flow cytometry, of MCF10AT monocultures and co-cultures with normal breast-associated fibroblasts (NAF) and carcinoma-associated fibroblasts (CAF). These data are representative of replicate experiments indicating that NAF suppress proliferation of MCF10AT cells to a greater extent than do CAF. Again some variability in extent of suppression is present among individual NAF cultures and individual CAF cultures.

Figure 6



Relative 5-bromo-2'-deoxyuridine (BrdU) indices of MCF10A cells in co-culture with varying quantities of normal breast-associated fibroblast NAF-2 and carcinoma-associated fibroblast CAF-1. With increasing numbers of NAF-2, the mean rate of proliferation of co-cultured MCF10A cells increased, with a significant difference in BrdU-labeling index observed between a ratio of epithelial cells to fibroblasts (E:F) of 2:1 versus an E:F of 1:3 ($P < 0.05$). With increasing numbers of CAF-1, the mean rate of proliferation was highest at an E:F of 1:1. The rate of proliferation at an E:F of 1:1 was significantly higher than that at an E:F of 2:1 ($P < 0.05$). At an E:F of 1:3, CAF-1 caused a decreased proliferation of and enhanced cell death of MCF10A cells.

of 1:3, however, CAF-1 caused a decrease in proliferation of MCF10A cells and enhanced cell death, as assessed by microscopic morphology. At an E:F of 1:3, the total number of viable MCF10A cells was reduced in co-culture with both NAF-2 and CAF-1; however, this reduction was more marked with CAF-1.

Neither NAF nor CAF had a significant effect on the rate of apoptosis of MCF10A cells or MCF10AT cells when grown at an E:F of 2:1 after 2 weeks of co-culture, as assessed by TUNEL assay (Fig. 7).

Quantities of IGF II are no different in NAF versus CAF

As an initial attempt to identify differences between NAF and CAF that explain our observed results, expression of IGF II in NAF and in CAF was assessed. A higher level of expression of IGF II in CAF than in NAF may provide an explanation for the higher rate of proliferation of MCF10AT cells allowed by CAF in comparison with NAF.

ELISA performed on cell lysates of NAF and CAF cultures demonstrated variability in expression of IGF II among cultures, but no significant difference was observed in the mean IGF II quantity between NAF and CAF in monolayer cultures (Table 3) or in 3D monocultures (Table 4). Although in monolayer cultures more CAF than NAF had