

analysis. As shown in Fig. 3, 10 μ M of ECG inhibited the mRNA level of VCAM-1 by 60%, however EGCG and TF-3 completely inhibited the mRNA expression of VCAM-1 induced by IL-1 β . These results suggest that ECG, EGCG, and TF-3 inhibited VCAM-1 expression at the transcription level. Because activation of NF κ B is critical for induction of VCAM-1 by IL-1 β or other inflammatory cytokines,^{7,8} we determined

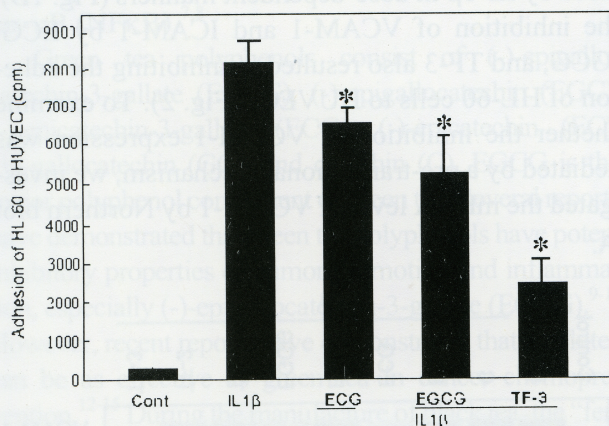


Fig. 2. Effect of ECG, EGCG, or TF-3 on the adhesion of monocytes to HUVECs induced by IL-1 β . Cells were treated with IL-1 β (2 ng/mL) and various tea polyphenols (10 μ M) for 24 h, and [³H]-thymidine-labeled HL-60 cells were added for 0.5 h. At the end of incubation, cells were washed, and the adhesion of HL-60 to HUVECs was determined as described in "Materials and Methods". Values were expressed as the mean $\pm$ S.E. of triplicate tests. * p < 0.05 vs. IL-1 β treatment.

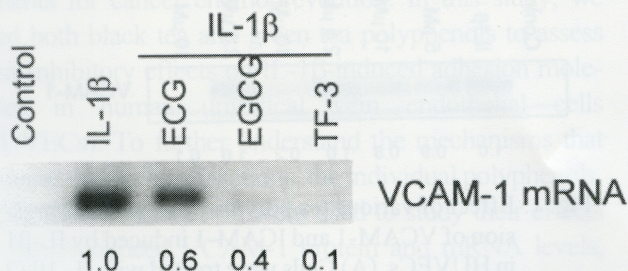


Fig. 3. Effect of ECG, EGCG, or TF-3 on the mRNA expression of VCAM-1 induced by IL-1 β in HUVECs. Cells were treated with 10 μ M of ECG, EGCG, or TF-3 and IL-1 β (2 ng/mL) for 5 h, and the mRNA level of VCAM-1 was detected by Northern blot analysis. Band intensities were quantified by densitometry, and the relative amounts are presented at the bottom of the figure.

whether these 3 polyphenols might suppress NF κ B activation in IL-1 β -induced HUVECs. Transition transfection with a NF κ B-dependent luciferase reporter plasmid was performed to examine whether these 3 polyphenols inhibited NF κ B binding activity in IL-1 β -induced HUVECs. As shown in Fig. 4A, 10 μ M

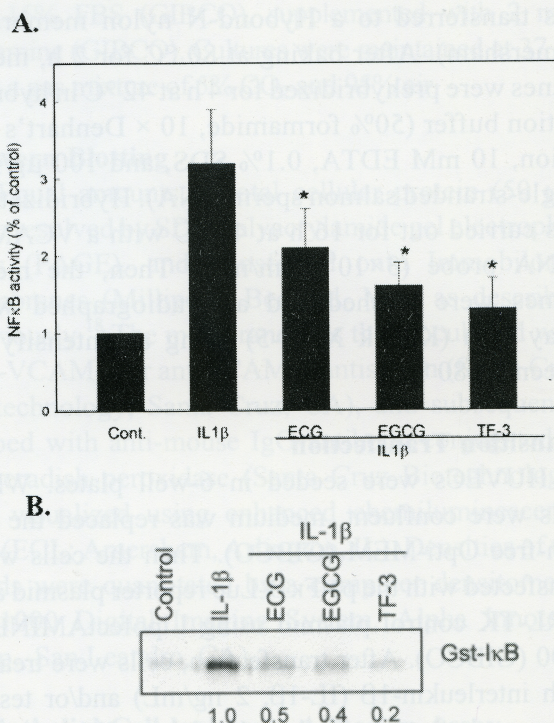


Fig. 4. Effect of ECG, EGCG, or TF-3 on I κ B kinase and NF κ B activities induced by IL-1 β in HUVECs. (A) Cells were transfected with the pNF κ B-Luc reporter plasmid and phRL-TK control plasmid. After transfection, cells were treated with 10 μ M of ECG, EGCG, or TF-3 and IL-1 β (2 ng/mL) for 6 h. Cells were harvested, and the levels of luciferase activities were determined as described in "Materials and Methods". Values are expressed as the mean $\pm$ S.E. of triplicate tests. * p < 0.05 vs. IL-1 β treatment. (B) Cells were treated with 10 μ M of ECG, EGCG, or TF-3 and IL-1 β (2 ng/mL) for 30 min. Total cell lysates (200 μ g) were used for immunoprecipitation, and the I κ B kinase activities were assayed with Gst-I κ B as substrates. The experiment was performed as described in "Materials and Methods", and ³²P-labeled Gst-I κ B is shown. Band intensities were quantified by densitometry, and the relative amounts are presented at the bottom of the figure.