

GATAGTCCACTGAATG-3', and 5'-ACAAGTC-  
ACTGTGCACAGATA-3'. The VCAM-1 cDNA  
probe was prepared according to the products of  
VCAM-1 cDNA and is fully described in our previ-  
ous report.<sup>18</sup> For Northern blotting, 20 µg of total  
RNA was separated by electrophoresis on a 1.2%  
agarose gel containing 6.7% formaldehyde and then  
was transferred to a Hybond-N nylon membrane  
(Amersham). After baking at 80 °C for 2 h, mem-  
branes were prehybridized for 4 h at 42 °C in hybrid-  
ization buffer (50% formamide, 10 × Denhart's so-  
lution, 10 mM EDTA, 0.1% SDS, and 100 µg/mL  
single-stranded salmon sperm DNA). Hybridization  
was carried out for 16 h at 42 °C with a VCAM-1  
cDNA probe (3×10<sup>6</sup> cpm/mL). Then, the mem-  
branes were washed and autoradiographed with  
x-ray film (Kodak XAR-5) using an intensifying  
screen at -80 °C.

### Transfection

HUVECs were seeded in 6-well plates. When  
cells were confluent, medium was replaced the se-  
rum-free Opti-MEM (GIBCO). Then the cells were  
transfected with the pNFκB-Luc reporter plasmid and  
phRL-TK control plasmid using LipofectAMINE™  
2000 (GIBCO). After transfection, cells were treated  
with interleukin-1β (IL-1β, 2 ng/mL) and/or tested  
compounds for 6 h. Each well of the cell lysate was  
used to assay luciferase activity with a FireLite kit  
(Packard Instrument, Meriden, CT) as described.<sup>18</sup>  
Luciferase activities of reporter plasmids were nor-  
malized to luciferase activities of the internal control  
plasmid.

### IKK Kinase Activity Assay

Equal amounts of total cellular protein (200 µg)  
were immunoprecipitated with an IKK-α specific an-  
tibody (Santa Cruz Biotechnology) and protein  
A/G-PLUS agarose, and the kinase assay was carried  
out as described in our previous report.<sup>18</sup>

## RESULTS AND DISCUSSION

First we examined the inhibitory effects of various  
tea polyphenols on the expressions of VCAM-1 and

ICAM-1 induced by IL-1β in HUVECs. As shown in  
Fig. 1A, IL-1β significantly induced the expressions  
of VCAM-1 and ICAM-1, and 10 µM of ECG, EGCG,  
or TF-3 markedly inhibited the expressions of  
VCAM-1 and ICAM-1 induced by IL-1β. TF-3 was  
the most effective tea polyphenol in inhibiting the ex-  
pressions of VCAM-1 and ICAM-1. ECG, EGCG,  
and TF-3 inhibited the expression of VCAM-1 in-  
duced by IL-1β in dose-dependent manners (Fig. 1B).  
The inhibition of VCAM-1 and ICAM-1 by ECG,  
EGCG, and TF-3 also resulted in inhibiting the adhe-  
sion of HL-60 cells to HUVECs (Fig. 2). To examine  
whether the inhibition of VCAM-1 expression was me-  
diated by a pre-translational mechanism, we inves-  
tigated the mRNA level of VCAM-1 by Northern blot

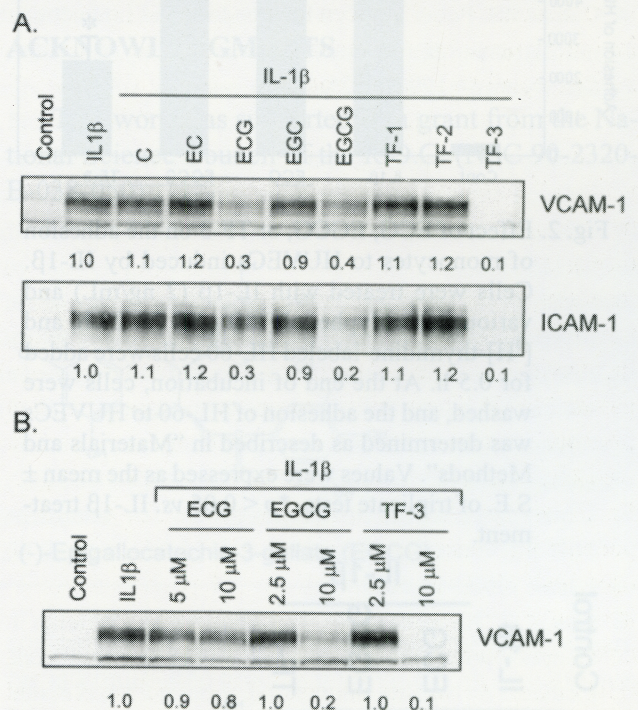


Fig. 1. Effect of various tea polyphenols on the expres-  
sion of VCAM-1 and ICAM-1 induced by IL-1β in  
HUVECs. (A) Cells were treated with IL-1β (2  
ng/mL) and various tea polyphenols (10 µM) for  
24 h, and VCAM-1 and ICAM-1 were detected by  
Western blot analysis. (B) Cells were treated  
with different concentrations of ECG, EGCG, or  
TF-3 and IL-1β (2 ng/mL) for 24 h, and  
VCAM-1 and ICAM-1 were detected by West-  
ern blot analysis. Band intensities were quanti-  
fied by densitometry, and the relative amounts  
are presented at the bottom of the figure.