JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Rebuttal on Inhibitory Effect of (−)-Epigallocatechin 3-Gallate, a Polyphenol of Green Tea, on Neutrophil Chemotaxis in Vitro and in Vivo

Sir: I thank Dr. Kawai for his interest in our previous work about the effect of (−)-epigallocatechin 3-gallate (EGCG), which is the major component in green tea, on neutrophil chemotaxis (*1*).

Recently, Dr. Kawai (*2*) found that EGCG showed an inhibitory effect on the migration of a subset of T lymphocytes, CD11b(+)CD8(+) T cells, which play a key role in immune responses, and its inhibitory effect is independent of the kind of chemoattractant tested in the investigation. Moreover, he indicated that down-regulation of the expression of CD11b, a component of Mac-1 (CD11b/CD18), in CD11b(+)CD8(+) T cells, by EGCG led to inhibition of the migration of these cells. On that basis, in his comment to our previous paper, he pointed out that the concept could be applied to the mechanism of inhibition of the migration of neutrophils by EGCG (*1*). Additional experiments could not be done for certain reasons, although he proposed additional chemotaxis assays by using extracellular matrix. Therefore, I examine his proposal in detail below, as I largely agree with his concept.

First is whether EGCG could exert direct influence on neutrophils. As described in the discussion of our previous paper (*1*), we speculated that EGCG has its site of action on neutrophils, not on the chemoattractant. It is unlikely that EGCG could bind directly to cytokine-induced neutrophil chemoattractants (CINCs), rat potent ELR(+)-CXC chemokines, from our preliminary data. Consistent with our presumption, Hayakawa et al. have reported that EGCG directly binds to the cell surface of U937 monocytes (*3*). In addition, the inhibitory effect of EGCG regardless of chemoattractants or stimulants might also support the speculation that EGCG has the site of action on neutrophils (leukocytes).

Second, neutrophils are considered to express Mac-1 regardless of species. In addition, there is no doubt that Mac-1 is involved in the adhesion and chemotaxis of neutrophils regardless of chemoattractant and/or stimulant (*4*, *5*). Mac-1 plays a critical role in the infiltration of leukocytes into the inflammatory site, as noted by Dr. Kawai.

Third is that EGCG could suppress the expression of CD11b in neutrophils. Dr. Kawai showed that EGCG directly binds to CD11b expressed on CD11b(+)CD8(+) T cells, and its binding is competitively inhibited by anti-CD11b mAb. In addition, he showed that EGCG could suppress the expression of CD11b in these cells but did not affect the expression of α-integrins, CD11a and CD11c, and β1-integrin, CD29. These results suggest that suppression of the expression of CD11b by EGCG depends on one of its anti-inflammatory activities, but not on its toxicity, although one more concern about EGCG is its influence on cell viability.

Focusing attention on CD11b(+) cells (activated macrophages and neutrophils), Katiyar et al. have shown pretreatment with EGCG suppressed the infiltration of CD11b(+) cells in UVB-induced inflammation in mice (*6*), but they did not investigate the influence of EGCG on the expression of CD11b in CD11b(+) cells. Perhaps, there is no other finding on the influence of EGCG on the expression of CD11b in CD11b(+) cells such as neutrophils.

Also as suggested in the comment of Dr. Kawai, an independent type of inhibition on the expression of Mac-1 could be detected in our chemotaxis assay, because a polycarbonate filter coating without anything else was used. On the other hand, Hofbauer et al. have shown that EGCG reduced fMLP-induced neutrophil chemotaxis through the monolayer of endothelial cells (*7*). Pretreatment of the endothelial monolayer with EGCG also resulted in the reduction of fMLP-induced neutrophil chemotaxis. In that paper, in addition, it was shown that pretreatment of neutrophils, endothelial monolayers, or both cells with EGCG reduced adhesion of neutrophils to endothelial cell monolayers.

These results suggest that both types of mechanisms, dependent and independent of the expression of CD11b, might be involved in the inhibition of neutrophil chemotaxis. At present, it is unclear which type of mechanism is important in the inhibition of neutrophil infiltration in vivo, because the expression of CD11b as Mac-1 is a necessary event in the early phase of neutrophil infiltration in vivo.

Taken together, I largely agree with the comment of Dr. Kawai. Thus, if EGCG down-regulates the expression of CD11b in neutrophils, it might result in the inhibition of CINC-1-induced neutrophil chemotaxis.

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10.1021/jf048294l CCC: $30.25
Published on Web 01/13/2005

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Received for review October 14, 2004.

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