Epigallocatechin-3-Gallate Reduces Airway Inflammation in Mice through Binding to Proinflammatory Chemokines and Inhibiting Inflammatory Cell Recruitment

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Epigallocatechin-3-Gallate Reduces Airway Inflammation in Mice through Binding to Proinflammatory Chemokines and Inhibiting Inflammatory Cell Recruitment


One major activity of chemokines is the recruitment of immune cells to sites of infection and inflammation. CD4+ Th1 cells play critical roles in host defense against pathogens and in the pathogenesis of many immune-mediated diseases. It was reported that epigallocatechin-3-gallate (EGCG) exhibits anti-inflammatory properties, but the mechanisms have not been completely defined. In this study, we found that EGCG markedly decreased recruitment of murine OVA-specific Th1 cells and other inflammatory cells into the airways in a Th1 adoptive-transfer mouse model. In vitro analysis revealed that EGCG inhibited CXCR3 ligand-driven chemotaxis of murine and human cells. Surface plasmon resonance studies revealed that EGCG bound directly to chemokine receptors. These results indicated that one anti-inflammatory mechanism of EGCG is binding of proinflammatory chemokines and limiting their biological activities. These findings support further development of EGCG as a potent therapeutic for inflammatory diseases.

Chemokines are small chemoattractant cytokines involved in normal and pathological immune processes (1, 2). Proinflammatory chemokines, such as CXCL9–11, participate in inflammatory responses by recruiting immune cells to sites of infection and inflammation (3). For example, in individuals with allergic inflammation, CXCL10 is capable of worsening pre-existing asthmatic airway inflammation (4). Therapeutically inhibiting proinflammatory chemokine production and/or interaction with chemokine receptors could reduce Ag-specific inflammatory responses in the lungs and other tissues.

As a subset of effector CD4+ T cells that secrete IFN-γ, Th1 cells can influence a range of immunologic responses, and they participate in the pathogenesis of a variety of inflammatory disorders (5–7). It was reported that adoptive transfer of OVA-specific Th1 cells induces noneosinophilic airway inflammation upon allergen challenge (8). The abundant expression of CXCR3 on Th1 cells (9) implicates CXCR3 ligands CXCL9–11 as mediators of this inflammation. In addition, the migration of Ag-specific effector CD4+ T cells to the lung is significantly decreased in CXCR3-deficient mice during parainfluenza virus infection (10), suggesting that blocking CXCR3-directed T cell recruitment may limit immunopathology during respiratory virus infections. Therefore, therapeutically targeting CXCR3 ligands and/or CXCR3 also could reduce airway and lung inflammation driven by Ag-specific Th1 cells.

Polyphenols from green tea are receiving increasing attention for preventing cancers, as well as cardiovascular and degenerative diseases (11). Among these polyphenols, epigallocatechin-3-gallate (EGCG) is the most abundant green tea catechin (12). Despite evidence of EGCG’s anti-inflammatory properties in vitro and in vivo, its precise mechanisms of action have not been fully defined. A number of studies suggested that EGCG exhibits antioxidant and anti-inflammatory properties, including inhibition of expression of proinflammatory cytokines and chemokines (13–23). In vivo studies showed that EGCG markedly reduced the severity of pulmonary fibrosis, arthritis, and colitis in animal models (15, 21, 24, 25). In a small number of studies, EGCG was also shown to limit cellular migration, including neutrophil responses to CXCL8/IL-8 and THP-1 cell responses to bacterial formyl peptide (25–27). In this study, we examined more closely whether EGCG might exhibit anti-inflammatory properties through targeting chemokine and chemokine–receptor interactions, thereby inhibiting inflammatory cell migration. We found that EGCG binds directly to proinflammatory chemokines CXCL9–11 and inhibits the capacity of these chemokines to recruit CXCR3+ cells in vitro. In vivo, EGCG inhibited recruitment of Th1 and other inflammatory cells into airways and lung interstitium in a Th1 cell adoptive-transfer airway-inflammation model.

Materials and Methods

Mice

Recipient mice were 7–8 wk-old male SCID mice on the BALB/c background, and T cell donor mice were DO11.10 TCR-transgenic mice harboring an OVA-specific TCR. These strains were obtained from The

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Abbreviations used in this article: CAS, chemical abstract service; EC, epicatechin; EGCG, epigallocatechin gallate; GAG, glycosaminoglycan; GT, gallotannin; ISH, in situ hybridization; i.t., intratracheally; Rmax, maximum binding capacity; SPR, surface plasmon resonance.

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Jackson Laboratory (Bar Harbor, ME). All animals were housed in a pathogen-free environment and given food and water ad libitum. All experiments were approved by the Children’s Hospital of Pittsburgh Animal Research and Care Committee.

In vitro differentiation of Th1 cell subsets

Th1 cells were derived from DO11.10 mice on the BALB/c background cultured with wild-type BALB/c irradiated spleenocytes. CD4+CD62L+ naïve T cells were isolated from the spleens of DO11.10 TCR OVA-specific transgenic mice and were enriched by negative selection to remove non-CD4+ cells, followed by positive selection for CD62L+ cells using Ab-coated magnetic beads (Miltenyi Biotec, Auburn, CA). Naïve T cells were then cultured for 6 d with irradiated APCs (7 × 10^6 cells; whole-spleen homogenate from BALB/c mice) pulsed with OVA peptide 323–339 (5 μM) under T cell-polarizing conditions (20 U/ml IL-2, 5 ng/ml IL-12, and 10 μg/ml anti-IL-4; R&D Systems, Minneapolis, MN). Following polarization, Th1 cells were restimulated with anti-CD3/anti-CD28 beads (Invitrogen, Carlsbad, CA) plus IL-2 (4 U/ml) for 48 h; cytokine production was confirmed by Lincoplex assay (Millipore, Billerica, MA).

Th1 cell adoptive-transfer airway-inflammation model

BALB/c SCID mice were treated with OVA (50 μg at 1 mg/ml in PBS) by intratracheal (i.t.) challenge on day 0; the following day, mice received 1 × 10^6 Th1 cells by i.v. transfer. Transfer mice were challenged i.t. for another three consecutive days (days 2–4) with OVA (50 μg at 1 mg/ml in PBS). Twenty-four hours later (day 5), airway disease was assessed as indicated below. On days 1–4, mice received EGCG (10 mg/kg) or PBS vehicle by i.p. injection. Mice were sacrificed on day 5 of the study, and lungs were harvested, preserved with PBS and processed. Inflammatory cells were measured in the bronchoalveolar lavage by differential cell counts (total cell counts by Coulter counter, differentials done by hand counting of H&E-stained Cytospin slides), and immunomodulatory proteins were measured by Lincoplex analyses. One lobe of lung was fixed in 10% formalin in PBS for paraffin embedding and histologic sectioning. Histology was examined and assessed by H&E staining. The remaining lung was homogenized in PBS for cytokine and chemokine analysis by Lincoplex measurements, according to the manufacturer’s recommendations. This adoptive-transfer experiment was performed and then repeated, each time with a total of eight mice: two receiving OVA i.t. and not adoptively transfected with DO11.10 Th1 cells; three receiving OVA i.t., adoptively transfected with DO11.10 Th1 cells, and receiving PBS i.p.; and three receiving OVA i.t., adoptively transfected with DO11.10 Th1 cells, and receiving EGCG i.p.

In situ hybridization and immunohistochemistry

In situ hybridization (ISH) for CXCL10 and TCR β mRNA was performed as described (28). Autoradiographic exposure times were 14 d following ISH with antisense and control sense 35S-labeled probes.

Immunohistochemistry for F4/80 and CD3 was performed as described (29). Briefly, paraffin-embedded lung tissue sections were deparaffinized in xylene, washed with absolute ethanol, and processed as Target retrieval solution (Dako, Carpenteria, CA) or 0.01 M sodium citrate (pH 6) for 20 min in a rice steamer. Tissue sections were incubated with rat anti-F4/80 and mouse anti CD3 (Dako) for 1 h at room temperature and detected using the SuperPicTure Kit (Zymed Laboratories, South San Francisco, CA), according to the manufacturer’s recommendations. Counterstaining was performed with hematoxilin (Fisher Chemicals, Fairlawn, NJ).

Chemotaxis-inhibition assays

Cell transfections were described as performed previously (29). Briefly, the L1.2 mouse pre-B cell line was electroporated with the pcDNA3.1 (Invitrogen) plasmid vector expressing murine CXCR3, and stably transfected cells were obtained after selection with 1 mg/ml G418 (Sigma). Chemotaxis was performed toward murine CXCL9–11 (100 μM; PeproTech, Rocky Hill, NJ), and CXCL10 (1–100 μM; chemical abstract service [CAS] No. 989-51-5, Sigma) epicathechin gallate (ECG) (100 μM; CAS No. 1257-08-5, Sigma) or epicatechin (EC) (100 μM; CAS No. 490-46-0, Sigma), or gallotannin (GT; 50 μM; 1401-55-4, Sigma). Structure images of the EGCG-like polyphenols in Fig. 5 were obtained from the Sigma-Aldrich Web site and are reproduced in this article with permission. The 96-well ChemToX chemotaxis system (NeuroProbe, 5-μm pore, 96-well, 10 mm; NeuroProbe,大山, CA) was used in these chemotaxis assays as described (29). CXCR3 stably transfected L1.2 cells (2 × 10^5) in 20 μl RPMI 1640/0.1% BSA were loaded above the membrane. After incubation for 3 h at 37°C, 5% CO2, the cells on top of the membrane were removed with a scraper, and the migrated cells in the bottom wells were counted using a hemacytometer. Chemotaxis with primary murine Th1 cells was performed in the same way, but with a 2-h incubation during migration.

Human CD4+ cells were isolated from archived, cryopreserved PBMCs by positive selection for CD4 (Miltenyi Biotec) and then rested overnight in complete RPMI 1640 medium. The purity of CD4+ cells was >95%, as measured by flow cytometry. Chemotaxis with primary human CD4+ cells and EGCG was performed as already described using human CXCL9, CXCL10, and CXCL11 (PeproTech, Rocky Hill, NJ) and with a 2-h incubation during migration.

Surface plasmon resonance analyses of EGCG-binding chemokines

Binding analyses of chemokines to EGCG and related polyphenols were performed on a Biacore 3000 instrument (Biacore Life Sciences, GE Healthcare, Piscatway, NJ). Individual chemokines (CXCL9, 10, and 11; PeproTech, Rocky Hill, NJ) were immobilized onto three separate flow cells of a CM5 chip by standard amine coupling chemistries to covalently bind the chemokine to the dextran matrix of the chip. Two-fold serial dilutions of purified EGCG (starting at 25 μM) in HBS-EP buffer (0.01 M HEPES [pH 7.4], 0.15 M NaCl, 0.005% surfactant P20, GE Healthcare) were flowed over the surfaces at a rate of 80 μl/min for 3 min. Dissociation was allowed to occur for 10 min in buffer only. Upon completion of each association and dissociation cycle, surfaces were pulsed with 0.01 M NaOH to regenerate the surface. All measurements were double referenced by subtracting out the response to the reference flow cell surface, as well as a buffer-only injection. Sensogram results were analyzed using BIAevaluation 4.1 software (Biacore Life Sciences). Absolute binding analyses of EGCG and related polyphenols (GT, epigallocatechin [EGC], ECG, and EC) to immobilized chemokine were performed in the same manner, at a single concentration of 100 μM analyte. All response curves were normalized to the calculated maximum binding capacity in resonance units (Rmax) of the captured ligands to account for minor surface differences.

Statistical analyses

All statistical analyses were performed using the Minitab software package (State College, PA). In vivo study data were analyzed using the two-sample t test to compare differences between the control and EGCG-treated animals. Paired t tests were used to examine in vitro EGCG chemotaxis inhibition data. A p value <0.05 was considered significant.

Results

EGCG reduces Ag-driven airway inflammation

We used a Th1 cell adoptive-transfer airway-inflammation model to determine whether EGCG has an impact on Ag-specific Th cell-mediated pulmonary inflammation. This model has been used for studies on the trafficking and function of Th1 cells in allergic airway inflammation (8, 30). In this model, adoptive transfer of OVA-specific Th1 cells induces non eosinophilic airway inflammation following allergen challenge (8). To provide the opportunity to examine the anti-inflammatory properties of EGCG in the context of Ag-specific T cell responses, OVA was administered i.t. to SCID mice; on the following day, OVA-specific D011.10 Th1 cells were adoptively transferred and OVA was administered i.t. and EGCG (or saline) was administered i.p. on each of the next 4 d, with mice sacrificed on the next (and final day) of the study.

As a gross measure of airway and pulmonary inflammation, lung tissues were sectioned and stained with H&E. Microscopic examination revealed that control SCID mice receiving OVA but no Th1 cells had normal lung histology (Fig. 1A). Adoptive transfer of OVA-specific Th1 cells followed by daily i.p. injection of saline resulted in peribronchiolar and perivascular infiltrates, as well as alveolar inflammation and congestion (Fig. 1B). These results were similar to those reported in previous studies with allergen-specific Th1 cells (8). Compared with animals receiving saline, there was a clear reduction in airway inflammation in mice treated i.p. with EGCG after adoptive transfer of OVA-specific Th1 cells.
A reduction in peribronchiolar and perivascular infiltration was observed, as was reduction in interstitial infiltration. To identify the subsets of inflammatory cell types, we performed immunohistochemistry to detect CD3+ lymphocytes and F4/80+ macrophages in lung tissues. We found that the numbers of CD3+ cells and F4/80+ cells were decreased in EGCG-treated mice (Fig. 1F, I) compared with those treated with saline (Fig. 1E, H). In many areas, EGCG potently reduced the accumulation of T cells and macrophages in basal layers of conducting airways that was found in saline-treated control mice (e.g., Fig. 1E, H). In addition, in bronchoalveolar lavage fluid, we found an increase in total leukocyte count, from $1.9 \times 10^5$ cells/ml in mice receiving no adoptively transferred Th1 cells to $6.0 \times 10^5$ cells/ml in mice receiving Th1 cells. This decreased to $4.0 \times 10^5$ cells/ml in mice receiving Th1 cells plus EGCG, but this difference was not statistically significant. In summary, EGCG significantly reduced Th1-driven, Ag-specific airway inflammation in a murine adoptive-transfer model.

**EGCG reduces TCRβ⁺ cell levels in lung tissues following adoptive transfer of Th1 cells**

We used ISH to detect TCR-β mRNA to examine further the extent to which the adoptively transferred T cells were components of the cellular infiltrates in lungs of the adoptive-transfer–recipient SCID mice. As shown in Fig. 2, TCR-β⁺ cells were absent from the lung tissues of the naïve SCID mice (Fig. 2A) but were abundant at the sites of cellular infiltration in mice receiving the OVA-specific Th1 cells followed by daily saline treatment (Fig. 2B). These T cells were located primarily within peribronchiolar and perivascular sites of infiltration. When animals received EGCG daily following adoptive transfer of OVA-specific Th1 cells, the numbers of TCR-β⁺ cells in lung tissues were reduced overall and within the inflammatory foci (Fig. 2C). These results demonstrated that EGCG inhibited recruitment of OVA-specific T cells into lung tissues exposed to OVA. These data also indicated that the CD3⁺ cells unexpectedly observed in the lung tissues from control SCID mice (Fig. 1D) were likely not conventional T cells and might be NKT cells.

**EGCG reduces local CXCL10 levels in lung tissues**

We performed ISH to detect CXCL10 mRNA to determine whether EGCG affected levels of the IFN-inducible, Th1-recruiting chemokine CXCL10 in the lung tissues of recipient mice. Expression of CXCL10 mRNA was minimal in control mice (Fig. 3A), but it was upregulated dramatically in mice receiving OVA-specific Th1
cells and treated with PBS (Fig. 3B). CXCL10 mRNA^+ cells were localized to peribronchial, perivascular, and interstitial infiltrates (Fig. 3B). In contrast, animals receiving OVA-specific Th1 cells, followed by EGCG treatment, had greatly reduced CXCL10 mRNA-expression levels (Fig. 3C). Consistent with the ISH findings, CXCL10 protein levels in lung tissues were decreased significantly in the mice treated with EGCG after transfer of OVA-specific Th1 cells compared with those treated with PBS after Th1 cell transfer (Fig. 3D). These data indicated that EGCG decreased the expression of the proinflammatory type I chemokine, CXCL10, in murine lung tissues during an Ag-specific inflammatory response.

**EGCG inhibits CXCR3 ligand-mediated chemotaxis**

Having shown that EGCG can inhibit Th1 and other inflammatory cell recruitment to airways and lung tissues in vivo, we next examined whether EGCG could directly inhibit CXCR3^+ cell migration toward proinflammatory chemokines CXCL9–11 in vitro. Indeed, migration of OVA-specific murine Th1 cells toward CXCL9–11 was inhibited by EGCG in a dose-dependent manner (Fig. 4A), with 30–50% inhibition at 1 μM. EGCG also inhibited migration of murine cells engineered to express murine CXCR3 (Fig. 4B). The migration of primary human T cells toward CXCR3 ligands also was inhibited by EGCG in a dose-dependent manner (Fig. 4C). EGCG was not toxic after 24 h of incubation, with these responder cells maintaining >90% viability, even in 100 μM EGCG (data not shown).

To examine whether the repeating gallate (trihydroxy benzene) structure on EGCG might contribute to its activity against CXCR3 ligands, we performed in vitro chemotaxis inhibition analyses with additional, related natural polyphenols. Although EGCG inhibited chemotaxis in this assay by 99% (Fig. 5A), the epicatechins EGC and EC, which have one or no gallate groups, respectively, inhibited chemotaxis driven by CXCL9 (Fig. 5A) by only 20–30%. Another related polyphenol, ECG, which differs from EGCG by a single hydroxyl group, inhibited chemotaxis by 65% (Fig. 5A). In contrast, GT (also called tannic acid), which contains five gallate groups, inhibited chemotaxis driven by CXCL9 by 98% (Fig. 5B). Similar findings were obtained with murine CXCL10 and CXCL11 (data not shown). Overall, these data indicated that EGCG inhibits murine Th1 and CXCR3^+ cell migration toward CXCL9–11, due in large part to the gallate moiety on the polyphenol, providing one mechanism explaining EGCG reduction of inflammation driven by OVA-specific Th1 cells in OVA-treated murine lungs.

**EGCG binds directly to murine CXCL9, CXCL10, and CXCL11**

We performed surface plasmon resonance (SPR)-binding analyses between EGCG and murine CXCR3-specific chemokines to explore further the potential mechanisms of EGCG inhibition of Th1 cell migration. All sensogram curves were normalized to the computed Rmax of the immobilized chemokine chip surface to refine comparisons of the binding characteristics of the individual chemokines. EGCG bound to all three murine chemokines, CXCL9–11 (Fig. 6). As demonstrated in the representative sensogram in Fig. 6A, the binding was dose responsive. Discriminating examinations of binding dynamics between the chemokines were determined at the lowest EGCG dilution of 3.12 μM (Fig. 6B). CXCL10 and 11 had similar binding characteristics that varied from those observed with CXCL9. The relative on- and off-rates of CXCL10 and 11 were higher than those observed for CXCL9, and they both bound a higher level of EGCG. However, the CXCL9 curve exhibited a lower relative off-rate, as evidenced by a more stable binding of EGCG during the dissociation phase (i.e., CXCL9 retained a higher percentage of bound molecules). Additionally, CXCL10 and 11 binding presented as a more biphasic curve, whereas CXCL9 more closely represented a 1:1 Langmuir binding curve.

Having shown that related polyphenols differ in their strength of inhibition of chemotaxis (Fig. 5), we next examined whether they also differed in their binding of CXCR3 ligands. These polyphenols were used at the same concentration (100 μM), chosen to...
be high so as to maximize the potential for observing binding. All sensogram curves were normalized to the computed $R_{\text{max}}$ of the immobilized chemokine chip surface to refine comparisons of the binding characteristics. In addition to EGCG, GT and ECG bound to CXCL9 (Fig. 6C) and CXCL10 and CXCL11 (data not shown). GT bound at a higher level than did EGCG and ECG, and it demonstrated a faster on-rate and a slower off-rate compared with the smaller polyphenols. Similar to EGCG, binding by GT and ECG was dose responsive (data not shown). In contrast, EGC and EC, both of which lack a gallate group present on EGCG and

![FIGURE 4. EGCG inhibits CXCR3-mediated chemotaxis. Chemotaxis was performed against CXCL9–11, with or without coincubation of EGCG (1, 10, or 100 μM) on the bottom side of the membrane separating the indicated responder cells and chemokines. These data were obtained from three independent experiments (A, B) or three donors (C), and the mean values are indicated by the line in each set. Paired $t$-test analyses were performed to compare migration in the EGCG-containing cultures with migration in the absence of EGCG (0 μM) and for all chemokines and for all responder cell populations, $p < 0.05$ with 10 or 100 μM EGCG.](http://www.jimmunol.org/)

![FIGURE 5. Inhibition of CXCL9-mediated chemotaxis by EGCG-related polyphenols. Chemotaxis was performed against CXCL9 with or without coincubation of EC, EGC, ECG, or EGCG (100 μM; A) or GT (50 μM; B) on the bottom side of the membrane separating chemokines from L1.2 cells engineered to express murine CXCR3. These data were obtained from three independent experiments, each performed in triplicate, and the mean values are shown by the line in each set. Paired $t$-test analyses were performed on log$_{10}$-transformed data values in relation to the data obtained with chemokine alone. The structures for EGCG and related green tea polyphenols are shown on the bottom (structure images used with permission from Sigma-Aldrich).](http://www.jimmunol.org/)
ECG, demonstrated minimal binding to the chemokines (Fig. 6C). In dose-response analyses, the low level of EGC and EC binding was less than that observed with buffer alone. Differences in the binding properties of these related polyphenols correlated strongly with the extent to which they inhibited CXCR3-driven chemotaxis (Fig. 6D). In summary, EGC and structurally related polyphenols bound directly to CXCR3 ligands, and this binding correlated with their ability to inhibit chemotaxis.

Discussion

The green tea polyphenol, EGC, is a remarkable molecule with multiple demonstrated health benefits and minimal toxicity (12, 16, 19, 31, 32). EGC has multiple effects that can vary with the system of study, but they usually are considered anti-inflammatory, such as via inhibition of NF-κB and other pathways (33, 34), and typically antioxidant, although pro-oxidant effects have been described at high concentrations (35). With the goal of defining potential mechanisms by which EGC is anti-inflammatory, we showed in this study that EGC markedly decreased Ag-specific airway inflammation in a murine Th1 cell adoptive-transfer model. In addition, we showed that EGC binds directly to the proinflammatory chemokines CXCL9, CXCL10, and CXCL11 and dramatically inhibits their chemotactic abilities.

Inflammation plays a major role in the pathogenesis of pulmonary and other diseases through the recruitment of inflammatory cells. In turn, these inflammatory cells can contribute to local production of proinflammatory chemokines, such as CXCL9–11, which further augments inflammatory responses. Chemokines CXCL9–11 are potent chemoattractants for T cells, NK cells, plasmacytoid dendritic cells, and macrophages (9, 36–38). Th1 cells can contribute to local, effector site inflammation, and adoptive transfer of OVA-specific Th1 cells induces noneosinophilic airway inflammation in mice following allergen challenge (8). Therefore, inhibition of excessive Th1 cell recruitment to sites of Ag exposure could reduce harmful inflammation. Chemokines are critical molecules involved in host defense against infection, but they are also contributors to pathological inflammatory processes (2). Therefore, the development of inhibitors for chemokines or their receptors remains an attractive approach for treating inflammation.

In this study, in vivo analyses revealed that EGC reduced Th1 cell and other inflammatory cell recruitment, as well as the overall inflammatory response in airways and lung tissues. EGC was shown to exhibit anti-inflammatory effects (13–16), although the mechanism(s) by which EGC is anti-inflammatory have not been completely defined and are likely to be multifaceted. EGC inhibits rat neutrophil migration toward chemokine CINC-1 in vitro in a dose-dependent manner, and it reduces neutrophil infiltration into inflammatory sites in FITC-OVA-induced allergic inflammation in rats (26). EGC also inhibits the migration of CD8+ T cells by binding to CD11b (39); improves atopic dermatitis-like lesions in mice by suppressing macrophage migration inhibitory factor, TNF-α, and IFN-γ (40); and alleviates the severity of collagen-induced arthritis (21). EGC also can protect mice from binding (3.125 μM) to the three CXCR3 ligands, examined individually. C, EGC and related polyphenols (100 μM) were injected over immobilized CXCL9; representative binding curves are shown. D, A Pearson’s correlation analysis was performed to examine the relationship between the percentage of chemotaxis inhibition shown in Fig. 5 and the mean RU values for each polyphenol in the 10 s prior to the end of the binding phase of the curves in C.
Con A-induced hepatitis due to decreased expression of cytokines (TNF-α and IFN-γ) and chemokines (CXCL10 and CCL3) in liver (41). Furthermore, it was reported recently that EGCG inhibits CXCL10 production from oncostatin M-stimulated human gingival fibroblasts (42). In this study, we found that CXCL10 mRNA and protein expression levels were decreased in the lung tissues of EGCG-treated mice (Fig. 3), which likely explains, in part, the reduction in inflammatory cells in the lungs of EGCG-treated mice.

Importantly, we identified a new mechanism by which EGCG inhibits inflammation, which is via binding to the proinflammatory CXCR3 ligands (Fig. 6). Therefore, the anti-inflammatory effects of EGCG in Ag-specific responses might be attributed, in part, to its capacity to bind and attenuate the activity of proinflammatory chemokines. Although we have not identified the EGCG-binding sites on CXCR3 ligands, it is conceivable that the glycosaminoglycan (GAG)-binding domain is the target for EGCG. Chemokines bind to GAGs on the extracellular matrix and establish haptotactic gradients (43). The two gallate groups on EGCG, each composed of a trihydroxyl benzene moiety, arguably resemble the heavily hydroxylated hyaluronan and chondroitin sulfate GAGs (44). Therefore, EGCG might bind to the GAG-binding domains of CXCR3 ligands and decrease their chemotactic availability.

The importance of the gallate group in this inhibition was supported by our findings that EGC and EC, which contain one or no gallate groups, respectively, do not exhibit nearly the same CXCR3 ligand-inhibitory or -binding properties as do EGCG and ECG. EGC differs from EGCG by the absence of a single hydroxyl group. Furthermore, GT, which contains five gallate groups, is highly inhibitory to CXCR3-mediated chemotaxis and binds strongly to CXCR3 ligands. The correlation between binding and chemotaxis inhibition revealed that none of these polyphenols will bind to CXCR3 ligands without inhibiting them. GT was also shown to inhibit CXCL12/CXCR4-driven chemotaxis, although binding to CXCL12 was not examined (45). We did not determine whether binding of chemokines by EGCG sterically impedes interaction with CXCR3 or whether there are more allostatic effects through EGCG-driven structural alterations to the bound chemokine. Further analyses are required to identify the determinants on these and possibly other chemokines for EGCG binding and whether these binding sites are also GAG-binding domains.

Inhibition of CXCR3 ligand function would be expected to be of therapeutic value in the context of severe acute and chronic inflammation. One outcome from inhibition of Th1-driven, CXCR3-mediated inflammation would be the potential to skew the local immune response away from a type 1 profile and possibly toward a type 2 profile. Although this could be beneficial to the individual, creating a milieu skewed away from an IFN-γ-dominated profile could be detrimental, depending on the Ag or infectious agent at the center of the response. Therefore, it will be important to examine the effects of EGCG on different inflammatory conditions beyond the type 1 environment examined in this study. In addition, there are other contexts in which it might not be desirable to inhibit chemokine-driven inflammation. For example, if EGCG has broad chemokine inhibitory effects, its action during immune invasive events immediately following vaccination or infection might reduce the resulting adaptive immune response. Additional studies are needed to determine whether chemotactic and functional responses of innate immune cells might also be impacted by EGCG. The extensive consumption of green tea and its widely supported health benefits suggest that the benefits of EGCG occur within a therapeutic window that is below a deleterious threshold.

Overall, these results provided biochemical and immunopathological insights into the beneficial properties of EGCG and support its further development as a potent and safe anti-inflammatory compound with therapeutic potential for inflammatory and infectious diseases. Combinatorial chemistry and therapeutic studies based on the core structure of EGCG could inform the design of novel and target-specific anti-inflammatory drugs.

Disclosures
The authors have no financial conflicts of interest.

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