

Polyphenolic Antioxidants Enhance IgE Production

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ABSTRACT

Epidemiological data showed that total IgE and IL-4 levels in cigarette smokers were elevated, comparable to those in the asthmatics. The etiological agent(s) elevating IgE production are not clear. We evaluate whether tobacco polyphenols potentiate IgE production in a rodent model. Mice were fed with rutin or CGA in drinking water during antigen sensitization, followed by antigenic challenge i.p. in alum. CGA and rutin were also delivered in a bolus intraperitoneally or intranasally along with antigens during immunization. Antigen-specific IgE and IgG responses were measured. Enhancement of total IgE responses via i.p. and drinking routes can be achieved at concentrations as low as 0.1% CGA. Furthermore, IgG1 responses but not IgG2a and IgG2b were augmented, indicating a Th2 type of response by CGA. Moreover, both antigen-specific and serum IgE production can be achieved when CGA and antigenic challenges were delivered intranasally in the absence of alum. In contrast, nicotine does not enhance antigen-specific IgE production, and only marginally affects serum IgE levels. The more polarized Th2 development in

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CGA-treated mice may account for enhancement of both antigen-specific and total IgE responses. High levels of IL-4 but not IFN- γ or IL-12, were observed in antigen-challenged mesenteric lymph nodes (MLN) cultures from CGA-treated mice. In contrast, significant levels of IL-4, IL-12, and IFN- γ were observed in antigen-challenged cultures from nicotine-treated mice. This study shows that tobacco polyphenols, CGA and rutin potentiated IgE production in vivo. Polyphenolic antioxidants enhance Th2 development. We propose that IgE production and T cell dichotomy may be critically influenced by the redox microenvironment. Enhanced Th2 development and IgE production henceforth may counteract more severe Th1-mediated tissue damage triggered by environmental oxidative stress.

Key Words: IgE; Polyphenol; Antioxidant; Th2 dichotomy.

INTRODUCTION

Oxidant-induced cancers, lung and heart inflammatory diseases account for a majority of smoking-related mortality (Wald and Hackshaw, 1996; Wynder and Graham, 1985). Numerous oxidant tar components, including polyaromatic hydrocarbons (PAH), quinonic compounds, and decomposed simple polyphenols, are pyro-synthesized in the burning cone of a cigarette (Hecht et al., 1981; Stedman, 1968). These oxidants in turn generate reactive oxygen species (ROS): superoxide, hydrogen peroxide and hydroxyl radicals that initiate inflammation and tissue damages, and are mainly responsible for emphysema and coronary heart disease (Chow, 1993; Wald and Hackshaw, 1996). Smokers and non-smokers alike may be exposed to dry-distilled vapor droplets containing mostly pyrolyzed and decomposed small molecules in actively inhaled or passive smoke (Hollander and Stober, 1986; Witschi et al., 1997). Epidemiological data showed that total IgE and IL-4 levels in cigarette smokers were elevated, comparable to those in asthmatics (Brand et al., 1993; Ericsson et al., 1993). Rutin, pyrolytically conjugated to degraded proteins in cigarette condensates, augmented IgE production to the conjugated proteins (Francus et al., 1983). Passive smoke was shown to stimulate IgE production in a murine model of airway inflammation (Seymour et al., 1997). The chemical entities in smoke and their mechanisms of potentiating IgE production are not known. The chemical complexity of smoke and its condensates due to pyrolytic transformation poses a daunting task to identify the specific molecule(s) and study the respective underlying immunological mechanisms for IgE production.

On the other hand, smokeless tobacco users are exposed to non-pyrolyzed tobacco products containing naturally occurring polyphenolic antioxidants via oral or intranasal routes of consumption. It appears more straightforward to evaluate whether non-pyrolyzed tobacco polyphenols from tobacco leaves affect IgE production and CD4 T cell development. It is not known whether freely occurring polyphenolic antioxidants exert immune modulation on native protein antigens, concomitantly administered via natural routes of exposure. We identified and purified chlorogenic acid (CGA) as a major form of water-soluble tobacco polyphenols from smokeless tobacco extract (STE) (Chen et al., 2000). We show that CGA as well as small molecules enriched in polyphenols potentiated long-lasting antigen-specific and total IgE production in vivo. Lymphocytes from CGA-treated mice exhibited a polarized Th2 cytokine

phenotype upon antigenic stimulation. We propose that redox modulation by tobacco polyphenols affects Th2 development, and henceforth enhances IgE production in vivo.

MATERIALS AND METHODS

Mice

Eight-week old female BALB/c mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice are put on different drinking regimens with heated-dissolved tobacco polyphenols for different times prior to and during certain periods of antigen immunizations. Alternatively, mice were given polyphenols in a bolus only in conjunction with OVA and KLH antigenic challenges intraperitoneally (i.p.) or intranasally (i.n.). The i.p. challenge consisted of administering 10 µg OVA and 10 µg KLH in 2 mg alum, and the i.n. challenge delivered 10 µg KLH and 10 µg OVA in 25 µl of solution consisting of 1% CGA or rutin. Mice were bled seven to ten days following the last antigenic challenge. In vitro cultures of splenic and mesenteric lymph nodes cells were used for antigen restimulation. Sera were assayed for antigen-specific IgE and IgG as described below.

Reagents and Chemicals

Research loose-leaf chewing tobacco, 1S1 (nicotine at 0.76% dry weight), was obtained from the University of Kentucky Tobacco and Health Research Institute (Lexington, KY). Smokeless tobacco extract (STE) was prepared as follows: Leaves were ground in water at 45% (w/v), supernatant was collected following centrifugation at 3000 rpm, and filtered through 0.22 µm. The protein was determined at 1.4 mg/ml of STE by Micro BCA protein determination with a kit purchased from Pierce Inc. (Rockford, IL). A fraction containing small molecules (SM-STE) was prepared by dialyzing STE against water with a membrane of M.W. 1000 cut-off. The volume of SM-STE was then re-adjusted to that of the original sample before dialysis. Molecular weights of SM-STE were determined by a SCIEX-API 100 mass spectrometer (Perkin Elmer, San Francisco, CA) based on ionic spray and subsequent time-of-flight (TOF) mass determination. Rutin (quercetin-3-rutinoside; 3, 3', 4, 5, 7-pentahydroxyflavone-3-rutinoside; 3-[[6-O-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2-(3, 4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one, M.W. 610.5), and chlorogenic acid [CGA, 3-caffeoylquinic acid, 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate), M.W. 354], were obtained from Sigma (St. Louis, MO). Nicotine was purchased from Sigma. Nicotine was readily dissolved in water. Rutin and CGA were heated as 1% and 3% (w/v) in double distilled water for 10 min and diluted accordingly.

Antibody Assay and ELISAs

Antigen-specific IgE was determined by passive anaphylactic skin test with end-point dilution as described (Chen, 1992). Briefly, each individual serum was serially

diluted twofold in saline with 1% normal rat sera. 100 μ l was then injected into the male Lewis rat. Four hr later, the rat was challenged i.v. with 1 mg KLH in 1% Evan's Blue, and 1 hr later the end point for the blue spots was scored for each individual serum. Means of PCA titers of the group were determined and presented. According to PCA convention, titer difference of 4-fold is considered significant. Moreover, statistical p values were computed among individual differences between groups by Wilcoxon's sum of ranks test. In contrast to ELISA plaque assay (Chen, 1990), serial bleeding and measurements by PCA permit multipoint determination of IgE status of a prolonged period as dictated in this study. Total serum IgE was determined by employing the PharMingen double anti-IgE MAbs for ELISA assay. IgG1 and IgG2a, IgG2b were determined as described (Chen, 1992). Double MAbs sandwich-ELISA kits for determining IL-4 (DuoSetTM, 89-3537-00), IL-12 (InterTest-12XTM, 80-4244-01) and IFN- γ (InterTest- γ TM, 80-3842-04) were purchased from Genzyme Co. (Boston, MA). Statistic p values were computed among groups by Student's t test.

RESULTS

CGA, a Polyphenol Constituent in Tobacco Leaves Potentiates IgE Production

Previously, we identified and purified polyphenolic antioxidants in naturally occurring small molecules in non-pyrolyzed STE by high-pressure liquid chromatography and mass spectrometry. Herein, we determine whether STE and tobacco polyphenol constituents, i.e., CGA and rutin modulate IgE production. Different routes of administration of these materials were evaluated. In the first regimen, mice were pre-fed with polyphenols or STE in drinking water for two weeks. They were then immunized three times at intervals of two weeks with antigens in alum, while continually fed with polyphenols or STE. These mice were then put back on regular drinking water for two weeks, and challenged for the fourth IgE antibody response. As shown in Fig. 1, mice on normal drinking water exhibited a control PCA titer of 40 (panel A), while mice fed with STE, SM-STE, CGA exhibited enhanced antigen-specific IgE production (panel B: PCA titer of 160, $p < 0.01$), while the effect of rutin is marginal (panel B, PCA titer of 80, $p < 0.05$). To evaluate the persistent effect of this treatment, mice were rested for two more months on regular drinking water, followed by two additional challenges. Figure 1 (panel B) also showed that mice previously fed with STE, small molecules of STE (SM-STE), CGA exhibited significantly enhanced IgE responses upon the sixth antigenic challenge (panel B vs. A: PCA titers of 320 versus 80, $p < 0.01$).

In contrast to the continual drinking regimen described above, we examined IgE production in mice following limited exposure to polyphenolic antioxidants, delivered in a bolus only during i.p. immunization. As also shown in Fig. 1 (panel C), SM-STE enriched for rutin and CGA, enhanced IgE production (PCA titers: 160 versus 40; 320 versus 80 for the fourth and sixth antigenic challenge, respectively; $p < 0.01$). Interestingly, injection of crude STE did not enhance IgE production under a regimen of limited exposure (cpd panel A). In contrast, pure tobacco polyphenols, CGA and rutin (panel A) greatly augmented IgE production (PCA titers: 160–320 versus 40, and

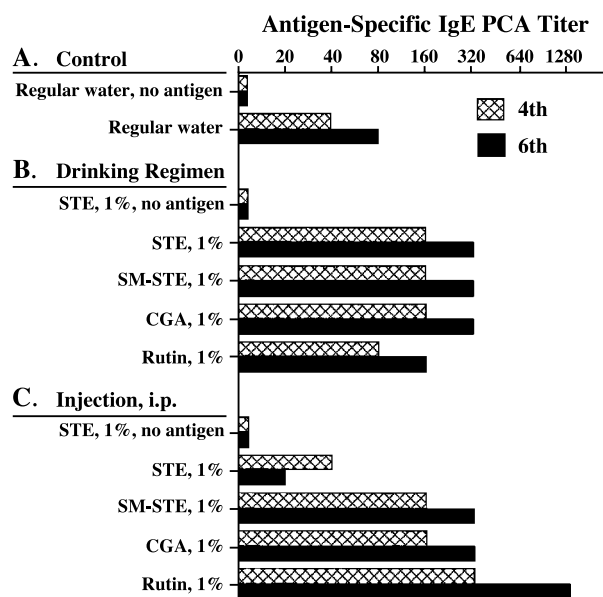


Figure 1. Tobacco polyphenols enhance antigen-specific IgE responses. *Drinking regimen:* BALB/c mice (4 each group) were first put on polyphenol- or tobacco-drinking regimens for two weeks, immunized three times with 10 μ g OVA/KLH each in 2 mg alum i.p. at intervals of two weeks while on the respective drinking regimen. Mice were then put back on regular water, challenged i.p. once with antigens in alum, sera collected (4th immunization), rested for two months, and challenged twice (6th immunization). *i.p. regimen:* Mice are immunized i.p. three times with antigens/alum in 0.5 ml containing 1% polyphenols, extracts or saline. Subsequent challenges were performed with antigen/alum. PCA titer of each individual serum was determined, and the mean PCA titer of each group was then obtained by averaging the four individual PCA titers within the group. Statistical p values were computed between groups by Wilcoxon's sum of ranks test.

320–1280 versus 80 for the fourth and sixth antigenic challenge, respectively; $p < 0.01$). These observations strongly suggested that CGA and rutin are the molecular principles responsible for augmenting IgE responses in SM-STE-fed animals. The magnitude of the fourth and sixth antigen-specific IgE responses is not significantly different. Continual administration of polyphenolic antioxidants is not required for maintaining a heightened IgE response since mice previously treated with polyphenols demonstrate prolonged enhanced IgE responses following the sixth antigenic challenge.

Since CGA is an abundant polyphenolic species present in SM-STE, we determined the efficacies of CGA treatment on IgE production in subsequent experiments. CGA was administered in different concentrations by oral and i.p. routes according to the regimens described above. As shown in Fig. 2A, 2.3- to 3.6-fold more serum IgE than that of control were produced in mice treated with 0.03% to 1% CGA via continual administration by the oral route, or delivered i.p. in a bolus ($p < 0.01$). Th2-dependent IgG1 production was potentiated 1.9- to 3.1-fold higher via oral feeding

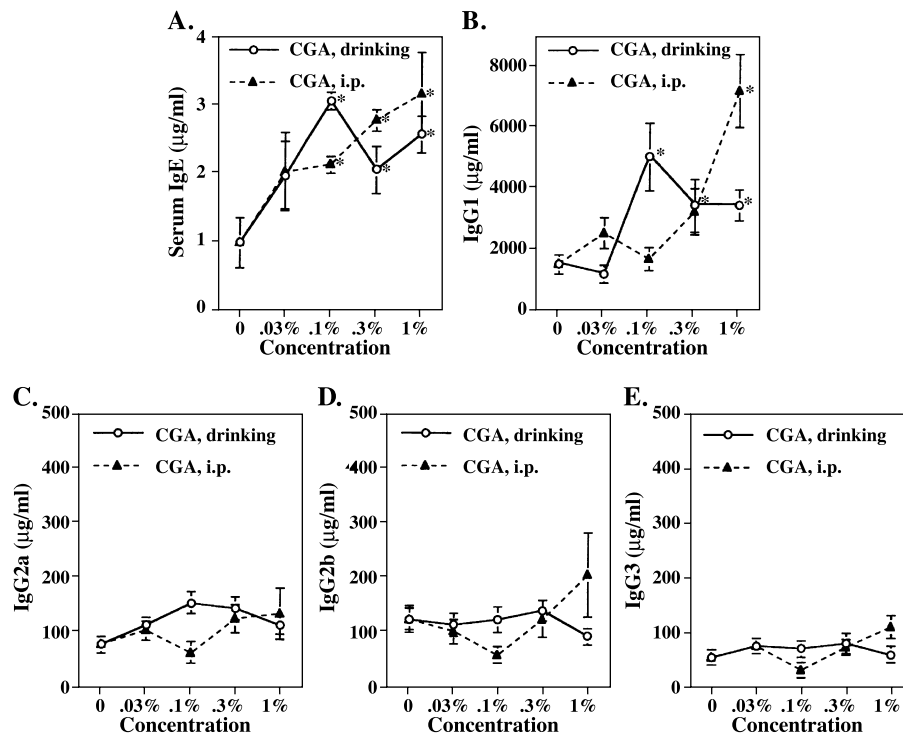


Figure 2. Dosage response curve of CGA in potentiating IgE and subclasses of IgG production. BALB/c female mice in groups of four were fed on normal water, or drinking regimens with different doses of CGA. Mice were then immunized as described in the legend to Fig. 1. Sera were collected after four immunizations. Serum IgE and antigen-specific IgG1, IgG2a, IgG2b and IgG3 were determined. Differences among groups were estimated by Student's t-test. Symbol (*) designated that means $\pm$ s.d. of the CGA-treated groups were significantly different from the non-treated control groups ($p < 0.01$).

with 0.1% to 1% CGA, and 1.9- to 4.2-fold higher via i.p. injection with 0.3% to 1% CGA in a bolus (Fig. 2B, $p < 0.01$). In contrast, IgG2a, IgG2b and IgG3 responses in CGA-treated mice remained not significantly different from the controls on normal drinking water (Fig. 2C–E, $p > 0.05$).

Airway delivery of antigens and tobacco snuff occurs naturally during consumption of smokeless tobacco products; furthermore, nicotine is an important component of tobacco leaves in addition to polyphenols. Therefore, we examined whether intranasal (i.n.) administration of CGA or nicotine together with antigens modulated IgE production. As shown in Fig. 3, CGA administered IN augmented antigen-specific IgE (PCA titer of 160 versus 40, Fig. 3A) as well as 4.6 fold more serum IgE than that of control (Fig. 3B, $p < 0.01$). In contrast, nicotine did not affect antigen-specific IgE production (Fig. 3A, $p > 0.05$), while it exerted only moderate influence (60% more than that of control) on total IgE levels (Fig. 3B, $p < 0.05$).

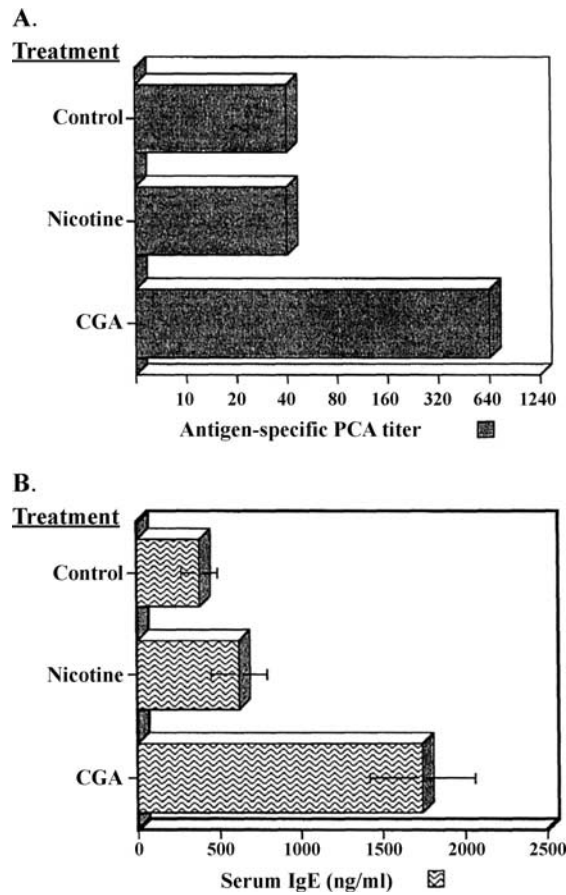


Figure 3. CGA potentiated total IgE production via intranasal immunization. A group of eight BALB/c female mice was primed once with 10 μ g OVA/ KLH each in 2 mg alum i.p. Two weeks later, ten micrograms of antigens each prepared in 50 μ l CGA (3.3%) or nicotine (2 mg/ml) solutions were delivered i.n. three times in a week. Twenty-four hr after the last i.n. challenge, the mice were sacrificed. PCA titers and serum IgE were determined. Means and standard errors from eight mice were determined. The experiment was repeated twice with similar results. Statistical p values were determined.

CGA Polyphenol Preferentially Induces Th2 Dichotomy

We then determine whether enhanced IgE production was correlated to a preferential development to Th2 in mice treated with antigens and CGA together. IL-4 was produced in the range of 28 to 55 pg/ml in antigen-stimulated mesenteric lymph nodes (MLN) cultures from mice, previously immunized with antigens along with CGA or nicotine (Fig. 4A, $p < 0.01$). IL-4 was barely detectable in antigen-challenged control cultures. In contrast, IFN- γ production by antigen-challenged MLN

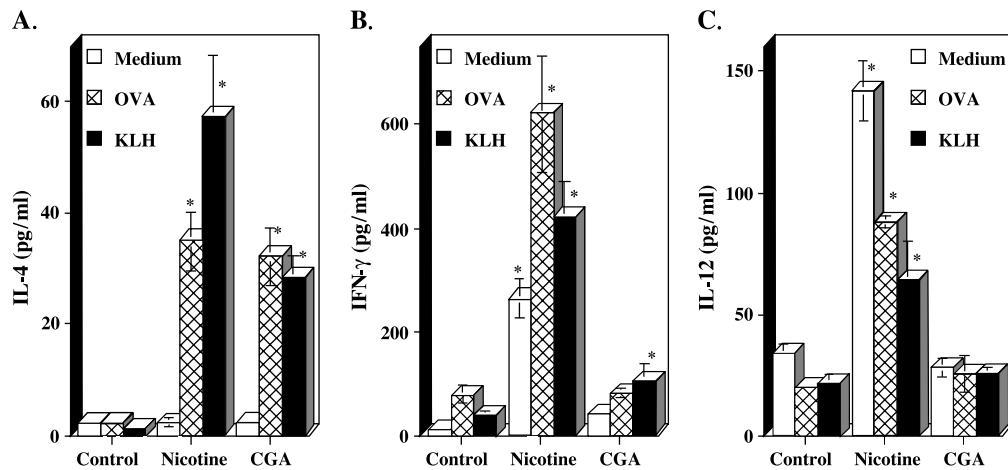


Figure 4. CGA caused an increase in Th2 cytokines, while nicotine generated mixed profile of Th1/Th2 cytokines. Single-cell suspensions of mesenteric lymph node (MLN) were prepared 24 hr following the last i.n. challenge from mice immunized according to the legend to Fig. 3. 1×10^5 MLN cells in 96-well were re-stimulated with OVA or KLH at 10 μ g/ml. Supernatants were harvested day four after stimulation. Levels of IL-4, IL-12 and IFN- γ were determined. This experiment was repeated four times with different antigenic doses and cell sources with similar results. The symbol (*) designated that means $\pm$ s.d. of the CGA or nicotine-treated groups were significantly different from the non-treated control groups ($p < 0.01$).

from nicotine-treated mice was higher than that from CGA-treated mice, i.e., a 6.7-fold increase vs. OVA-challenged control cultures; and an 8.4-fold increase in KLH-challenged cultures (Fig. 4B, $p < 0.01$). Furthermore, production of IL-12 was also preferentially enhanced in cultures prepared from nicotine-treated mice: a 3- to 4.2-fold increase in IL-12 production was observed in cultures of nicotine-treated mice (Fig. 4C, $p < 0.01$), while that of CGA-treated mice remained at the basal levels, comparable to those of antigen-challenged control cultures. Interestingly, primed cells from nicotine-treated mice produced significantly higher levels of IFN- γ and IL-12 even without further antigen restimulation in vitro (Fig. 4B and 4C). Similar cytokine profiles were also observed in culture supernatants of spleen cells (data not shown). Thus, mice primed with antigens and CGA together exhibited polarized Th2 cytokine production (IL-4/IFN- γ ratio = 12.6, while those with antigens and nicotine exhibited a mixed profile (IL-4/IFN- γ ratio = 2.1) of Th1/Th2 cytokines.

DISCUSSION

The salient features of this study are: 1) CGA present in tobacco leaf extracts enhanced antigen-specific and total IgE production via oral feeding, or i.p. and i.n. injection in a bolus; 2) T cells from CGA-treated mice exhibited an increase in the Th2 cytokine profile as compared to that of nicotine-treated mice. We propose that CGA

provided at sustained levels via drinking water or injected concomitantly with antigens, exerts a potent influence on IgE production due to an enhanced Th2 development.

Polyphenols, consisting of flavonoids and non-flavonoids, is known as the largest class of naturally occurring antioxidants, present in food, nutrient supplements and certain consumer products (Hollman and Katan, 1987; Rice-Evans et al., 1996). CGA as a non-flavonoid polyphenol, is one of the most important antioxidants present in coffee, grape and apple skins, and also in tobacco leaves (Chen et al., 2000). It is well-documented that antioxidant activities of polyphenols are mediated by their capacities to scavenge reactive oxygen species (ROS), chelate transition metals, and induce expression of antioxidant genes, i.e., glutathione transferase and quinone oxidoreductase (Dinis et al., 1994; Rice-Evans et al., 1996; Xie et al., 1995). In so doing, antioxidants moderate intracellular redox via enhancement of accessible pools of glutathione (Daniel, 1993). Importantly, signal transduction of IL-1, TNF- α , TGF- β 1, CD28, and HIV replication in lymphocytes depends on the generation of appropriate levels of ROS (Hong et al., 1997; Los et al., 1995). Indeed, ROS was proposed to serve as a second messenger for signal transduction in different cell types (Roederer et al., 1990; Schreck et al. 1991). We and others later also proposed that modulation of ROS levels, henceforth intracellular redox, plays a role in antigen presentation and T cell differentiation (Gong et al., 1999; Peterson et al., 1998; Verhasselt et al., 1999). Intracellular redox levels may also play a role in regulation mast cell activation.

It is interesting to note (Fig. 2) that there exhibits a unimodal enhanced IgE response through all doses of CGA administered via the i.p route, while the optimal response occurred at a lower dose of 0.1% CGA in the oral route. We interpreted that this may be due to cumulatively higher intake of CGA in drinking water, while the i.p injection was provided only once in a bolus for each injection presumably impacting on APC and CD4 + T cell priming. Observations in this study demonstrated that SM-STE and CGA antioxidant favor polarized Th2 development (Fig. 4). CGA may modulate the redox potentiated and subsequent biochemical events of APC and/or CD4 T cells so that Th2 development is changed. It is important to develop in vitro model system whereby effect of polyphenols may be evaluated at different cellular levels (Constant and Bottomly, 1997; O'Garra, 1998; Szabo et al., 1995).

Biochemical mechanisms underlying the effect of polyphenols are not known. These results may be explained at levels of APC and/or CD4 + Helper T cells by modulation of redox-sensitive kinases as well as the pertinent transcription factors by non-pyrolyzed or commercially pure polyphenols. It is known that PKC, phosphatase, PTK, and the downstream p21Ras are redox-sensitive (Nakamura et al., 1997). Several studies suggested that differential activation of these kinase pathways is crucial in determining the Th1 versus Th2 dichotomy (Bonvini et al., 1991; Gajewski et al., 1990; Kawakami and Parker, 1992; Tamura et al., 1995). Differential activation of c-Maf transcription factor plays a critical role in IL-4 production and Th2 dichotomy (Ho et al., 1996). It was found that Nrf-1 and Nrf-2 of the c-Maf gene family were activated by polyphenols and their metabolites, which then enhanced the expression of antioxidant genes via the antioxidant response element (ARE) (Venugopal and Jaiswal, 1996). Interestingly, Nrf-1/Nrf-2 formed activating heterodimers with c-Maf (Kerppola and Curran, 1994; Venugopal and Jaiswal, 1998). Since c-Maf is critically involved in IL-4 expression (Ho et al., 1996), it is possible that polyphenols may favor a differential of IL-4/IFN-gamma production and Th2 development via Maf/Nrf heterodimer.

Unlike cigarette smoking, incidences of cardiovascular and pulmonary inflammatory diseases have not been reported in long-term users of smokeless tobacco. This may be due to the reason that oral or intranasal consumption of smokeless tobacco does not lead to tar deposit and generation of ROS thereof, which cause oxidative damage of the affected organs. Moreover, as discussed above, polyphenols taken up via the two latter routes may indeed scavenge ROS produced by other environmental oxidants, and thus attenuate nonspecific inflammation. Alternatively, antioxidants may indirectly reduce levels of inflammation by antagonizing Th1 development as suggested from this study.

Flavonoid polyphenols and non-flavonoid CGA were previously reported to inhibit histamine release and mast cell cytokine activation by Middleton and colleagues and our laboratory (Chen et al., 2000; Middleton and Drzewiecki, 1984). We hypothesize that polyphenols exert a wide range of biological activities via regulating intracellular redox of different types of leukocytes and tissues. Thus the outcome of allergy protection, i.e., inhibition of mast cell activation versus augmented IgE production may transpire at different leukocyte cell types, dictated by the respective programmed differentiation and is not paradoxical.

It is known that IL-4 and IL-10 secreted by Th2 inhibit inflammation elicited by IFN- γ , IL-1, IL-6, IL-8, TNF- α that were produced by Th1, epithelial and connective tissues (Denizot et al., 1999; Hart et al., 1996; van Roon et al., 1996). This study herein strongly suggests that IgE-mediated allergy may be precipitated by the uptake of tobacco or plant polyphenols. However, chronic IgE-mediated allergies may be better tolerated than emphysema and coronary heart diseases induced by tar oxidants, which claim most casualties in cigarette-smoking population. In conclusion, this study focuses our attention on IgE production, and dichotomous CD4 T cell development in the context of environmental exposure to polyphenol. These observations may be extended to other environmental pollutants; hitherto their importance in the context of lymphocyte differentiation has not been explored. We propose that CGA induces an anti-oxidative defense at the expense of IgE-mediated allergic disorders. IL-4 and IL-10 secreted by Th2 antagonize comparably more severe tissue damages caused by pro-inflammatory cytokines released by Th1 and other cell types, activated by environmental oxidants and pollutants such as cigarette-smoking.

ABBREVIATIONS

CGA	Chlorogenic acid
MLN	Mesenteric lymph node
PAH	Polyaromatic hydrocarbon
SM-STE	Small molecules of smokeless tobacco extract
STE	Smokeless tobacco extract
ROS	Reactive oxygen species
PCA	passive cutaneous anaphylaxis

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REFERENCES

- Bonvini, E., Debell, K. E., Taplits, M. S., Brando, C., Laurenza, A., Seamon, K., Hoffman, T. (1991). A role for guanine-nucleotide-binding proteins in mediating T-cell-receptor coupling to inositol phospholipid hydrolysis in a murine T-helper (type II) lymphocyte clone. *Biochem. J.* 275:689–696.
- Brand, P. L., Kerstjens, H. A., Jansen, H. M., Kauffman, H. F., de Monchy, J. G. (1993). Interpretation of skin tests to house dust mite and relationship to other allergy parameters in patients with asthma and chronic obstructive pulmonary disease. The Dutch CNSLD study group. *J. Allergy Clin. Immunol.* 91:560–570.
- Chen, S. S. (1990). Enumeration of antigen-specific IgE responses at the single-cell level by an ELISA plaque assay. *J. Immunol. Methods* 135:129–138.
- Chen, S. S. (1992). Genesis of host IgE competence: perinatal IgE tolerance induced by IgE processed and presented by IgE Fc receptor (CD23)-bearing B cells. *Eur. J. Immunol.* 22:343–348.
- Chen, S. S., Gong, J., Liu, F. T., Mohammed, U. (2000). Naturally occurring polyphenolic antioxidants modulate IgE-mediated mast cell activation. *Immunology* 100:471–480.
- Constant, S. L., Bottomly, K. (1997). Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. *Annu. Rev. Immunol.* 15:297–322.
- Chow, C. K. (1993). Cigarette smoking and oxidative damage in the lung. *Ann. N. Y. Acad. Sci.* 686:289–298.
- Daniel, V. (1993). Glutathione S-transferases: gene structure and regulation of expression. *Crit. Rev. Biochem. Mol. Biol.* 28:173–207.
- Denizot, Y., Besse, A., Raher, S., Nachat, R., Trimoreau, F., Praloran, V., Godard, A. (1999). Interleukin-4 (IL-4), but not IL-10, regulates the synthesis of IL-6, IL-8 and leukemia inhibitory factor by human bone marrow stromal cells. *Biochim. Biophys. Acta* 1449:83–92.
- Dinis, T. C., Maderia, V. M., Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.* 315:161–169.
- Ericsson, C. H., Svartengren, M., Mossberg, B., Camner, P. (1993). Bronchial reactivity, lung function, and serum immunoglobulin E in smoking-discordant monozygotic twins. *Am. Rev. Respir. Dis.* 147:296–300.
- Gajewski, T. F., Schell, S. R., Fitch, F. W. (1990). Evidence implicating utilization of different T cell receptor-associated signaling pathways by TH1 and TH2 clones. *J. Immunol.* 144:4110–4120.
- Gong, J., Masbad, J., Liu, F. T., Chen, S. S. (1999). Natural occurring polyphenolic antioxidants of tobacco leaves are IgE adjuvant. *FASEB.* 13:255.17.
- Francus, T., Siskind, G. W., Becker, C. G. (1983). Role of antigen structure in the regulation of IgE isotype expression. *Proc. Natl. Acad. Sci. U. S. A.* 80:3430–3434.
- Hart, P. H., Hunt, E. K., Bonder, C. S., Watson, C. J., Finlay-Jones, J. J. (1996). Regulation of surface and soluble TNF receptor expression on human monocytes and synovial fluid macrophages by IL-4 and IL-10. *J. Immunol.* 157:3672–3680.
- Hecht, S. S., Carmella, S., Mori, H., Goffmann, D. (1981). A study of tobacco carcinogenesis XX. Role of catechol as a major cocarcinogen in the weakly acidic fraction of smoke condensate. *J. Natl. Cancer Inst.* 66:163–169.

- Ho, I. C., Hodge, M. R., Rooney, J. W., Glimcher, L. H. (1996). The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. *Cell* 85:973–983.
- Hollander, W., Stober, W. (1986). Aerosols of smoke, respiratory physiology and deposition. *Arch. Toxicol., Suppl.* 9:74–87.
- Hollman, P. C., Katan, M. B. (1987). Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed. Pharmacother.* 51:305–310.
- Hong, Y. H., Peng, H. B., La Fata, V., Liao, J. K. (1997). Hydrogen peroxide-mediated transcriptional induction of macrophage colony-stimulating factor by TGF-beta1. *J. Immunol.* 159:2418–2423.
- Kawakami, K., Parker, D. C. (1992). Differences between T helper cell type I (Th1) and Th2 cell lines in signaling pathways for induction of contact-dependent T cell help. *Eur. J. Immunol.* 22:85–93.
- Kerppola, T. K., Curran, T. A. (1994). Conserved region adjacent to the basic domain is required for recognition of an extended DNA binding site by Maf/Nrl family proteins. *Oncogene* 9:3149–3158.
- Los, M., Schenk, H., Hexel, K., Baeuerle, P. A., Droge, W., Schulze-Osthoff, K. (1995). IL-2 gene expression and NF-kappa B activation through CD28 requires reactive oxygen production by 5-lipoxygenase. *EMBO J.* 14:3731–3740.
- Middleton, E. Jr., Drzewiecki, G. (1984). Flavonoid inhibition of human basophil histamine release stimulated by various agents. *Biochem. Pharmacol.* 33:3333–3338.
- Nakamura, H., Nakamura, K., Yodoi, J. (1997). Redox regulation of cellular activation. *Annu. Rev. Immunol.* 15:351–369.
- O'Garra, A. (1998). Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 8:275–283.
- Peterson, J. D., Herzenberg, L. A., Vasquez, K., Waltenbaugh, C. (1998). Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. *Proc. Natl. Acad. Sci. U. S. A.* 95:3071–3076.
- Rice-Evans, C. A., Miller, N. J., Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* 20:933–956.
- Roederer, M., Staal, F. J., Raju, P. A., Ela, S. W., Herzenberg, L. A. (1990). Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine. *Proc. Natl. Acad. Sci. U. S. A.* 87:4884–4888.
- Schreck, R., Rieber, P., Baeuerle, P. A. (1991). Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J.* 10:2247–2258.
- Seymour, B., Pinkerton, K., Frieberthausen, K., Coffman, R., Gershwin, L. (1997). Second-hand smoke is an adjuvant for T helper-2 responses in a murine model of allergy. *J. Immunol.* 159:6169–6175.
- Stedman, R. L. (1968). The chemical composition of tobacco and tobacco smoke. *Chem. Rev.* 68:153–207.
- Szabo, S. L., Jacobson, N. G., Dighe, A. S., Gubler, U., Murphy, K. M. (1995). Developmental commitment to the Th2 lineage by extinction of IL-12 signaling. *Immunity* 2:665–675.
- Tamura, T., Nakano, H., Nagase, H., Morokata, T., Igarashi, O., Oshimi, A., Miyazaki, S., Nariuchi, H. (1995). Early activation signal transduction pathways of Th1 and

- Th2 cell clones stimulated with anti-CD3. Roles of protein tyrosine kinases in the signal for IL-2 and IL-4 production. *J. Immunol.* 155:4692–4701.
- van Roon, J. A., van Roy, J. L., Gmelig-Meyling, F. H., Lafeber, F. P., Bijlsma, J. W. (1996). Prevention and reversal of cartilage degradation in rheumatoid arthritis by interleukin-10 and interleukin-4. *Arthritis Rheum.* 39:829–835.
- Verhasselt, V., Vanden Berghe, W., Vanderheyde, N., Willems, F., Haegeman, G., Goldman, M. (1999). N-acetyl-L-cysteine inhibits primary human T cell responses at the dendritic cell level: association with NF-kappaB inhibition. *J. Immunol.* 162:2569–2574.
- Venugopal, R., Jaiswal, A. K. (1996). Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. *Proc. Natl. Acad. Sci. U. S. A.* 93:14960–14965.
- Venugopal, R., Jaiswal, A. K. (1998). Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* 17:3145–3156.
- Xie, T., Belinsky, M., Xu, Y., Jaiswal, A. K. (1995). ARE- and TRE-mediated regulation of gene expression. Response to xenobiotics and antioxidants. *J. Biol. Chem.* 270:6894–6900.
- Wald, N. J., Hackshaw, A. K. (1996). Cigarette smoking: an epidemiological overview. *Br. Med. Bull.* 52:3–11.
- Witschi, H., Joad, J. P., Pinkerton, K. E. (1997). The toxicology of environmental tobacco smoke. *Annu. Rev. Pharmacol. Toxicol.* 37:29–52.
- Wynder, E. L., Graham, E. A. (1985). Landmark article May 27, 1950: tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma. A study of six hundred and eighty-four proved cases. By Ernest L. Wynder and Evarts A. Graham. *JAMA* 253:2986–2994.