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Inhibition of Autoantigen Expression by (−)-Epigallocatechin-3-gallate (the Major Constituent of Green Tea) in Normal Human Cells

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ABSTRACT

Autoimmune disorders, characterized by inflammation and apoptosis of target cells leading to tissue destruction, are mediated in part by autoantibodies against normal cellular components (autoantigens) that may be overexpressed. For example, antibodies against the autoantigens SS-A/Ro and SS-B/La are primary markers for systemic lupus erythematosus and Sjögren's syndrome. Recently, studies in animals demonstrated that green tea consumption may reduce the severity of some autoimmune disorders, but the mechanism is unclear. Herein, we sought to determine whether the most abundant green tea polyphenol, (−)-epigallocatechin-3-gallate (EGCG), affects autoantigen expression in human cells. Cultures of pooled normal human primary epidermal keratinocytes and of an immortalized human salivary acinar cell line were incubated with 100 μM EGCG (a physiologically achievable level for topical application or oral administration) for various time periods and then analyzed by cDNA microarray analysis, reverse transcription-polymerase chain reaction, and Western blotting for expression of several major autoantigen candidates. EGCG inhibited the transcription and translation of major autoantigens, including SS-B/La, SS-A/Ro, coilin, DNA topoisomerase I, and α-fodrin. These findings, taken together with green tea's anti-inflammatory and antiapoptotic effects, suggest that green tea polyphenols could serve as an important component in novel approaches to combat autoimmune disorders in humans.

Autoimmune disorders are a significant clinical problem; their prevalence in the United States is estimated at more than 8.5 million (Jacobson et al., 1997). They are often associated with serious morbidity and, in some cases, lead to death. The pathogenesis of most autoimmune disorders, including systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS), remains incompletely understood (Yamamoto, 2003; Venables, 2004). SLE and SS are characterized by the production of autoantibodies against normal cellular components, designated as autoantigens, that may be overexpressed (Gerl et al., 2005). The production of autoantibodies is considered to be a key mechanism of pathogenesis. To date, a large number of autoantigens have been identified in SLE (Sawalha et al., 2003). Sera from lupus patients often have high titers of antinuclear autoantibodies (ANA) that target components of the nucleus (Sawalha and Harley, 2004). These ANA include SS-A/Ro, SS-B/La, centro-

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ABBREVIATIONS: SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; ANA, antinuclear autoantibodies; PARP, poly(ADP)-ribose polymerase; RNP, ribonucleoprotein; RT-PCR, reverse transcription-polymerase chain reaction; GTPP, green tea polyphenol(s); TNF-α, tumor necrosis factor-α; CENP, centomere protein(s); EGCG, generic name: (−)-epigallocatechin-3-gallate, chemical name: (2*R*,3*R*)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-1(2*H*)-benzopyran-3,5,7-triol 3-(3,4,5-trihydroxybenzoate); NHEK, normal human epidermal keratinocytes; NS-SV-AC, immortalized human salivary gland acinar cells; OSC2, oral squamous cell carcinoma-2; PM, perfectly matched; MM, mismatched; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PBS, phosphate-buffered saline; PBST, 0.1% Tween 20 in PBS; IL, interleukin; MES, 4-morpholineethanesulfonic acid.

mere proteins (CENP) A, B, and C, double-stranded DNA, polymyositis-scleroderma, RNA polymerases, poly(ADP)-ribose polymerase (PARP), uridine-rich 1 small nuclear ribonucleoprotein (RNP), Smith antigen, ribosomal-P, histidyl-tRNA synthase (Jo-1), and DNA topoisomerase I (Scl-70) (Reeves, 2004). ANA are also found in approximately 70% of patients with SS, and autoantibodies against SS-A/Ro and SS-B/La are found in approximately 95 and 87% of primary SS patients, respectively (Rehman, 2003). Elevated levels of SS-A/Ro and SS-B/La mRNA were found in the salivary tissues of SS patients with xerostomia (Bolstad et al., 2003). Lupus-associated autoantigens also include golgins present in the Golgi apparatus and coilin proteins (Stinton et al., 2004).

Treatment options for most autoimmune disorders are limited and, in general, seek to suppress the immune response. In contrast, Chinese traditional medicines composed mostly of herbal extracts or green tea contain naturally occurring materials that may provide sources of alternative remedies for autoimmune disorders. Previous studies in animal models suggested that green tea polyphenols (GTPP) are able to reduce inflammation and apoptosis associated with autoimmune activities. In a mouse model for rheumatoid arthritis, 0.2% GTPP in water significantly reduced the protein levels of cyclooxygenase-2, interferon-γ, TNF-α, and IgG in the joints, resulting in >50% reduction of collagen-induced arthritis (Haqqi et al., 1999). In a mouse model for autoimmune encephalomyelitis, oral administration of EGCG reduced clinical severity by reducing brain inflammation and neuronal damage, paralleling diminished proliferation and TNF-α production in encephalitogenic T cells (Aktas et al., 2004). In an SLE mouse model, MRL/MpJ-Fas^{lprcg}/Fas^{lprcg} (MRL-lpr^{cg}) mice fed a 2% green tea powder diet for 3 months had prolonged survival with reduced lymph node hyperplasia and anti-DNA antibody levels, raising the possibility that GTPP could inhibit the expression of autoantigens (Sayama et al., 2003).

Herein, we report the novel finding that GTPP at physiologically achievable levels down-regulates the expression of several major autoantigens in both normal human epidermal keratinocytes (NHEK) and normal human salivary acinar cells. This finding is relevant for studies using GTPP as a treatment component of autoimmune disorders.

Materials and Methods

Chemicals and Antibodies. EGCG (molecular formula: $C_{22}H_{18}O_{11}$, purity, >95%) was purchased from Sigma-Aldrich (St. Louis, MO). Anti-human CENP-C (H-300) rabbit polyclonal antibody, anti-human 52-kDa Ro/SSA (D-12) mouse monoclonal antibody, anti-human PARP (F-2) mouse monoclonal antibody, and anti-human actin (I-19) goat polyclonal antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-human coilin mouse monoclonal antibody was obtained from BD Biosciences Transduction Laboratories (Lexington, KY). The anti-human golgin-67 rabbit polyclonal antibody was a kind gift from Dr. Don Fujita (University of Calgary, Calgary, Canada). The anti-human La/SSB mouse monoclonal antibody was purchased from ImmunoVision (Springdale, AZ).

Cell Lines. NHEK were purchased from Cambrex Bio Science Baltimore, Inc. (Baltimore, MD) and subcultured in keratinocyte growth medium-2 provided by the manufacturer. Subculture of the NHEK was performed by detaching the cells in 0.25% trypsin and transferring into new tissue culture flasks. The immortalized human salivary gland acinar cells (NS-SV-AC) have been described previously (Azuma et al., 1993). These cells were selected after transfection of origin-defective SV40 mutant DNA and maintained in keratinocyte growth medium-2; they were kindly provided by Dr. Masayuki Azuma (Tokushima University School of Dentistry, Tokushima, Japan). The human oral squamous cell carcinoma line OSC2 was described previously (Osaki et al., 1994). The cells were cultured in Dulbecco's modified Eagle's medium/Ham's F-12 50/50 MIX medium (Cellgro, Kansas City, MO) supplemented with 10% (v/v) fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 5 μg/ml hydrocortisone.

Cell Treatment. EGCG was dissolved in cell culture media as a 5 M stock immediately before use. Exponentially growing cells were incubated with 100 μM EGCG for various time periods (0, 0.5, 2, 6, 24, and 48 h) in the growth media described above, and then they were extracted for total RNA or protein before gene array analyses, RT-PCR, or Western blots.

Affymetrix Gene Array Analyses. Total RNA was isolated from the control and EGCG-treated NHEK cells using silica gel membranes and further purified using spin columns (QIAGEN, Valencia, CA). The quantity of the RNA was estimated with an RNA quantification kit (RiboGreen; Invitrogen, Carlsbad, CA), and samples were stored at −70°C.

Samples (10 μg) of total RNA were used to make ds-cDNA using the SuperScript Choice System (Invitrogen) with an oligo(dT) primer containing a T7 RNA polymerase promoter (Genset, San Diego, CA). After second-strand synthesis, the reaction mixture was extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and ds-cDNA was recovered by ethanol precipitation. Biotin-labeled cRNA was then produced from the ds-cDNA by in vitro transcription using the Enzo RNA transcript labeling kit (Enzo Life Sci, Farmingdale, NY). The cRNA was purified using an RNeasy affinity column (QIAGEN) and fragmented randomly to sizes ranging from 35 to 200 bases by incubating with fragmentation buffer (40 mM Tris acetate, pH 8.1, 10 mM KOAc, and 30 mM MgOAc) for 35 min at 94°C. The hybridization solutions contained 100 mM MES, 1 M Na^+, 20 mM EDTA, and 0.01% Tween 20. The final concentration of fragmented cRNA was 0.05 $\mu g/\mu l$ in the hybridization solution. Targets for hybridization were prepared by combining 40 μl of fragmented transcripts with sonicated herring sperm DNA (0.1 mg/ml), bovine serum albumin, and 5 nM control oligonucleotide (Affymetrix, Santa Clara, CA) in a buffer containing 1.0 M NaCl, 10 mM Tris-HCl (pH 7.6), and 0.005% Triton X-100. Target cRNA was hybridized for 16 h at 45°C to a set of human oligonucleotide arrays (HuG133A; Affymetrix), which contained 22,283 genes. Arrays were then washed at 50°C with stringent solution (100 mM MES, 0.1 M (Na^+), and 0.01% Tween 20) and then at 30°C with nonstringent washes [6× saline/sodium phosphate/EDTA (0.18 M NaCl, 0.01 M sodium phosphate, pH 7.7, and 1 mM EDTA) and 0.01% Tween 20]. Arrays were then stained with streptavidin-phycoerythrin (Invitrogen). DNA chips were read with a GeneArray Scanner (Hewlett-Packard, Palo Alto, CA) at a resolution of 3 μm and were analyzed with the GENECHIP (Affymetrix Microarray Suite 5; Affymetrix). Detailed protocols for data analysis of Affymetrix microarrays and extensive documentation of the sensitivity and quantitative aspects of the method have been described previously (Lockhart et al., 1996). In brief, each gene is represented by the use of ~20 perfectly matched (PM) and mismatched (MM) control probes. The MM probes act as specificity controls that allow the direct subtraction of both background and cross-hybridization signals. The number of instances in which the PM hybridization signal is larger than the MM signal is computed, along with the average of the logarithm of the PM:MM ratio (after background subtraction) for each probe set. These values are used to make a matrix-based decision concerning the presence or absence of an RNA molecule.

Subsequent data analysis was performed with GeneSpring analysis software (Agilent Technologies, Palo Alto, CA). Data from the Affymetrix analysis were transformed by setting measurements less than 0.01 to 0.01. Then each chip was normalized to the 50th percentile of the measurements taken from that chip. Finally, the expression of each individual gene in an experimental condition was compared with the control measurement for that gene. Changes in gene expression greater than 2-fold above or below the control (0 time) were considered significant.

Total RNA Extraction and Semiquantitative Reverse-Transcription-Polymerase Chain Reaction. Total RNA was extracted using an RNeasy total RNA isolation system (QIAGEN). RT reactions and PCR reactions were performed according to the manufacturer's protocol (SuperArray Bioscience Corporation, Frederick, MD). For amplification, the following pair of primers was used: GAPDH, sense 5′-TCCCATCACCATCTTCCA-3′ and antisense 5′-CATCACGCCACACGAGTTTCC-3′; SS-A/Ro (469bp), sense 5′-GAACTGCTGCAGGAGGTGATAA-3′ and antisense 5′-GGCACATTCAGAGAAGGAGT-3′; and SS-B/La (95bp), sense 5′-CCAAAATCTGTCATCAAATTGAGTATT-3′ and antisense 5′-CCAGCCTTCATCCAGTTTTATCT-3′. Amplification was started by heating for 1.5 min at 94°C followed by 30 cycles for 52-kDa SSA/Ro, 25 cycles for GAPDH, and 25 cycles for SSB/La, each cycle consisting of 15 s at 94°C, 30 s at 57.3°C, and 1 min at 72°C. A final extension was performed at 72°C for 5 min before gel analysis.

Western Blotting. Cells were washed in ice-cold PBS and lysed for 20 min in radioimmune precipitation buffer containing 1% (v/v) Nonidet P-40, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 10 μg/ml leupeptin, 3 μg/ml aprotinin, and 100 mM phenylmethylsulfonyl fluoride. Samples of lysates containing 5 to 30 μg of protein (it varied in each blot against one specific antibody) were loaded in each lane and electrophoretically separated on a 12% SDS polyacrylamide gel. After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore Corporation, Billerica, MA). The membrane was blocked for 1 h with 5% (w/v) nonfat dry milk powder in PBST and then incubated for 1 h with primary antibody diluted in PBST/milk [CENP-C, PARP, coilin, and Golgin-67 (1:500); 52-kDa Ro/SSA (1:2500); actin (1:2000); and La/SSB (1:50,000)]. The membrane was washed three times with PBST and incubated with peroxidase-conjugated affinity-purified anti-rabbit IgG (Santa Cruz Biotechnology) for 1 h. After extensive washing, the reaction was developed by enhanced chemiluminescent staining using ECL Western blotting detection reagents (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Results

Affymetrix Gene Array Analyses. Gene arrays provide a valuable tool for studying the broad response of cells to agents, and we have used this approach to compare the differential effects of GTPP on gene expression in NHEK and OSC2 cells. The results demonstrated that approximately 2100 genes were either up- or down-regulated by at least 2-fold in response to 100 μM EGCG in both cell types during the 24-h post-treatment (data not shown). Examination of the relative NHEK mRNA levels of genes represented on the gene chip encoding autoantigens previously identified in SLE (Sherer et al., 2004) revealed a 2-fold or greater change in expression of a number of genes during the 24-h post-treatment time course (Table 1).

Several patterns of change were observed. For example, the nuclear autoantigen genes RNA polymerase II E and SS-B/La, a cytoskeletal autoantigen gene (α-fodrin), and a Golgi autoantigen gene showed a general pattern of rapid (0.5–2-h initiation) decrease in mRNA levels and generally persistent 2-fold or greater reduction during the 24-h period. Certain autoantigen genes showed an initial decrease during the first 2 h (e.g., centromere protein C1, coilin), with levels returning to near normal by 24 h. Some autoantigen genes (e.g., nuclear antigen SP100 and scleroderma autoantigen I) showed a rapid initial increase (2–4-fold) followed by a 2-fold or greater decrease. Some autoantigen genes (e.g., one of the three arrayed XRCC5/Ku autoantigen genes) showed a transient initial increase, with levels returning to near normal. In contrast, other autoantigen genes did not show a significant change during the time course of the experiment. For example, 60-kDa SS-A/Ro (SSA2) did not decline significantly (52-kDa SS-A/Ro cDNA was not included in the gene array).

Semiquantitative RT-PCR. The preliminary array analysis described above provided data suggesting that GTPP could alter the expression of some genes encoding autoantigens. To further test this possibility, three cell types, NHEK, NS-SV-AC (an SV40-immortalized cell line derived from human submandibular acinar cells), and OSC2 cells (derived from an oral squamous cell carcinoma), were treated with 100 μM EGCG for different times. A semiquantitative estimate of SS-A/Ro and SS-B/La mRNA levels was then obtained by RT-PCR during the exponential period of amplification. GAPDH was used as a housekeeping gene control. SS-B/La and SS-A/Ro 52 were selected, because elevated mRNA levels (2–3-fold) of SS-A/Ro 52 and SS-B/La are found in salivary tissues of SS patients (Bolstad et al., 2003) and autoantibodies against SS-A/Ro and SS-B/La are found in nearly all (~95 and 87%, respectively) primary SS patients (Hahn, 1998). Consistent with the gene array data, SS-B/La message decreased progressively and substantially in both NHEK and NS-SV-AC cells (Fig. 1). The levels of SS-A/Ro (52 kDa) mRNA also showed a reduction during treatment, although the effect was less pronounced than that seen for SS-B/La and was not prominent until 24 h. In OSC2 cells, the reduction in SS-B/La mRNA was less pronounced and did not occur until 24 h of exposure, whereas SS-A/Ro mRNA showed little change. GAPDH also showed no marked decrease in these cells in response to EGCG, indicating that the reduction in mRNA levels seen for SS-B/La and SS-A/Ro was not due to a generalized effect on the cells.

Western Blotting. To extend the mRNA data, the protein levels of six different autoantigens in NHEK and NS-SV-AC cells were determined by Western analysis after two treatments with 100 μM EGCG at 24 and then 48 h. As shown in Fig. 2, coilin and PARP protein levels were significantly reduced in both cell lines by 24 h and were barely detectable after 48 h. CENP-C showed a similar trend, although the reduction was less pronounced. Neither SS-A/Ro 52 nor SS-B/La was dramatically reduced in either cell line by 24 h but was considerably reduced by 48 h. Golgin-67 was reduced in NS-SV-AC cells by 24 h and barely detectable at 48 h. Golgin-67 was also reduced in NHEK cells, although the reduction was not as marked and was only observed at 48 h. Actin protein levels were unchanged by EGCG during the 48-h treatment period.

Discussion

Studies over the past 20 years have demonstrated that GTPP possess chemoprevention activities and antioxidant, anti-inflammatory, and antiapoptotic properties (Sueoka et al., 2001), suggesting that they could be beneficial to patients with auto-

TABLE 1

cDNA microarray determination of autoantigen expression in NHEK treated with 100 μM EGCG for the indicated hours

Numbers represent -fold up/down at the time points normalized to the time zero levels. Repeats represent different cDNA fragments of one gene. The following autoantigens were not detected: ABCA7, AMPH, ANXA11, ASRGL1, CENPE, CHD3, CHD5, DEAF1, FLJ10613, FLJ12595, GAD1, GAD2, GMEB1, GOLGA6, ICA1, LMOD1, LOC93349, PC, PRTN3, PTPRN, PTPRN2, RCD-8, SC65, SE20–4, SP140, SSA1, STRN, TIGD6, TMPRSS3, and TPX1.

Autoantigens	0	0.5	2	6	24	Descriptions
			h			
Inhibited >2-fold						
AKAP12	1	0.505	0.991	0.4	0.198	A kinase (PRKA) anchor protein (gravin) 12
α-NAC	1	0.632	0.521	0.0443	0.469	*Homo sapiens* α-NAC gene for nascent polypeptide-associated complex component
CENPB	1	0.506	0.773	0.498	0.721	Centromere protein B
CENPC1	1	1.269	0.457	0.869	0.936	Centromere protein C 1
FLJ31657	1	0.833	0.603	0.435	0.959	Hypothetical protein FLJ31657
FLNB	1	0.72	0.92	0.875	0.243	Filamin B, γ (actin-binding protein 278)
FLNB	1	1.379	1.168	0.751	0.249	Filamin B, β (actin-binding protein 278)
GOLGA1	1	0.398	1.132	0.68	0.9	Golgi autoantigen, golgin subfamily a, 1
GOLGA2	1	0.332	0.642	0.769	0.949	Golgi autoantigen, golgin subfamily a, 2
GOLGA3	1	0.533	0.58	0.547	0.419	Golgi autoantigen, golgin subfamily a, 3
GOLGA4	1	0.822	0.748	0.353	0.664	Golgi autoantigen, golgin subfamily a, 4
HSA6591	1	0.456	0.835	0.532	0.562	Nucleolar cysteine-rich protein
HUMAUANTIG	1	0.948	0.779	0.444	0.517	Nucleolar GTPase
LMO4	1	0.272	0.867	0.708	1.011	LIM domain only 4
POLR2A	1	0.13	1.29	1.30	1.02	Polymerase (RNA) II (DNA-directed) polypeptide A, 220 kDa
POLR2A	1	1.03	0.45	0.44	1.17	Polymerase (RNA) II (DNA-directed) polypeptide A, 220 kDa
POLR2D	1	1.14	0.95	1.04	0.29	Polymerase (RNA) II (DNA-directed) polypeptide D
POLR2E	1	0.45	0.70	0.87	0.39	Polypeptide E, 25 kDa
POLR3C	1	0.90	0.73	0.45	0.49	Polymerase (RNA) III (DNA-directed) (62 kDa)
POLR3G	1	1.55	1.00	1.05	0.47	Polymerase (RNA) III (DNA-directed) (32 kDa)
SNRPA	1	1.29	0.93	1.35	0.32	Small nuclear ribonucleoprotein polypeptide A
SP100	1	1.72	1.037	0.59	0.356	Nuclear antigen Sp100
SPTAN1	1	0.41	0.75	0.56	0.42	Spectrin,α, nonerythrocytic 1 (α-fodrin)
SPTAN1	1	0.49	0.97	0.75	0.71	Spectrin,α, nonerythrocytic 1 (α-fodrin)
SNRPB	1	0.96	0.95	0.76	0.52	Small nuclear ribonucleoprotein polypeptides B and B1
SNRPB	1	0.62	0.79	0.67	0.42	Small nuclear ribonucleoprotein polypeptides B and B1
SNRPB2	1	0.49	0.67	0.56	0.82	Small nuclear ribonucleoprotein polypeptide B″
SSA2	1	1.009	0.335	0.739	1.17	602540961F1 NIH_MGC_59 *H. sapiens* cDNA clone IMAGE:4671854 5′, mRNA sequence
SSB	1	0.838	0.878	0.57	0.273	Sjögren's syndrome antigen B (autoantigen La)
SSB	1	0.711	0.739	0.427	0.257	Sjögren's syndrome antigen B (autoantigen La)
STRN3	1	0.425	0.623	0.528	0.759	Striatin, calmodulin binding protein 3
TOP1	1	0.85	1.09	0.84	0.12	Topoisomerase (DNA) I
TOP1	1	0.62	0.62	0.33	0.43	Topoisomerase (DNA) I
UBTF	1	0.498	0.484	0.501	0.546	Upstream binding transcription factor, RNA polymerase I
Induced then inhibited >2-fold						
PMSCL1, 75 kDa	1	3.993	1.09	0.431	0.496	Polymyositis/scleroderma autoantigen 1
PSME3	1	2.554	1.151	1.128	0.421	Proteasome (prosome, macropain) activator subunit 3 (PA28γ; Ki)
SNRPD1	1	2.098	1.285	0.658	0.425	Small nuclear ribonucleoprotein D1 polypeptide, 16 kDa
SP100	1	3.123	1.205	0.636	0.079	Nuclear antigen Sp100
Induced then declined to control levels						
ADPRT	1	3.547	1.201	0.992	0.85	ADP-ribosyltransferase [NAD^+; poly(ADP)-ribose polymerase]
CENPA	1	7.677	1.095	0.672	0.673	Centromere protein A, 17 kDa
MGC5560	1	4.392	1.161	1.646	0.81	Hypothetical protein MGC5560
MGC5560	1	7.07	0.932	2.605	0.552	Hypothetical protein MGC5560
SP110	1	5.725	1.702	1.205	0.978	SP110 nuclear body protein
SP110	1	4.176	1.804	0.997	0.654	SP110 nuclear body protein
SNRP70	1	3.59	1.01	2.28	0.51	Small nuclear ribonucleoprotein 70-kDa polypeptide (RNP antigen)
XRCC5	1	2.338	1.403	0.977	0.771	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand break rejoining; Ku autoantigen, 80 kDa)
Induced >2-fold						
ANXA11	1	1.043	1.26	2.141	1.946	Annexin A11
CTDSPL	1	2.83	1.12	2.42	2.10	Carboxyl-terminal domain, RNA polymerase II, polypeptide A, small phosphatase-like
Changed <2-fold						
CALR	1	0.71	0.943	1.482	1.528	Calreticulin
CBARA1	1	0.506	0.773	0.498	0.721	
CHD4	1	0.766	0.811	0.916	0.84	Chromodomain helicase DNA-binding protein 4
COL17A1	1	1.749	1.102	1.043	0.684	Collagen, type XVII, α1

TABLE 1
Continued

Autoantigens	0	0.5	2	6	24	Descriptions
CTDSP1	1	0.71	1.00	1.25	1.39	Carboxyl-terminal domain, RNA polymerase II, polypeptide A, small phosphatase 1
CTDSP2	1	1.61	1.21	0.92	1.71	Carboxyl-terminal domain, RNA polymerase II, polypeptide A, small phosphatase 2
dJ963E22.1	1	1.842	0.864	0.723	0.88	Contains the 3′ end of a novel gene similar to NY-REN-2 antigen
DLAT	1	1.54	0.924	1.031	0.751	Dihydrolipoamide *S*-acetyltransferase (E2 component of pyruvate dehydrogenase complex)
EPPK1	1	0.793	0.856	1.318	0.889	Epiplakin 1
FBL	1	1.64	1.12	1.09	0.69	Casein kinase II β subunit; complete cds.; fabrillin
G22P1	1	1.15	1.185	1.127	0.792	Thyroid autoantigen, 70 kDa (Ku antigen)
GMEB2	1	0.584	0.739	0.626	0.745	Glucocorticoid modulatory element-binding protein 2
GOLGA5	1	1.566	0.928	0.731	1.494	Golgi autoantigen, golgin subfamily a, 5
GOLGB1	1	0.969	0.704	0.56	0.688	Golgi autoantigen, golgin subfamily b, macrogolgin (with transmembrane signal), 1
HARS	1	1.27	1.11	0.83	0.72	Histidyl-tRNA synthetase
HARSL	1	1.12	1.05	0.88	1.00	Histidyl-tRNA synthetase-like
IMP-2	1	1.615	1.366	0.635	0.665	Insulin growth factor-II mRNA-binding protein 2
LAD1	1	0.799	0.681	0.954	0.553	Human ladinin (LAD) gene, complete cds.
LMO4	1	0.6	1.199	1.133	0.659	LIM domain only 4
NS	1	0.634	0.95	0.519	0.814	Nucleostemin
PDHB	1	1.079	1.14	0.745	0.818	Pyruvate dehydrogenase (lipoamide) β
PDX1	1	1.103	0.765	0.646	0.903	E3-binding protein
PMSCL2	1	0.959	0.938	1.058	0.717	Polymyositis/scleroderma autoantigen 2, 100 kDa
POLR1B	1	0.67	1.03	0.98	1.04	Polymerase (RNA) I polypeptide B, 128 kDa
POLR1D	1	0.90	0.96	1.04	1.31	Hypothetical protein MGC9850
POLR2B	1	1.83	1.02	0.59	0.74	Polymerase (RNA) II (DNA-directed) polypeptide B, 140 kDa
POLR2C	1	1.32	1.60	1.53	1.66	Polymerase (RNA) II (DNA-directed) polypeptide C, 33 kDa
POLR2F	1	0.90	1.00	1.17	0.80	Polymerase (RNA) II (DNA-directed) polypeptide F
POLR2G	1	1.09	1.02	0.74	0.89	Polymerase (RNA) II (DNA-directed) polypeptide G
POLR2H	1	0.88	0.85	1.02	1.15	Polymerase (RNA) II (DNA-directed) polypeptide H
RALY	1	1.556	0.752	1.496	0.612	RNA-binding protein (autoantigenic, human RNP-associated with lethal yellow)
SART1	1	1.982	1.188	1.462	0.98	Squamous cell carcinoma antigen recognized by T cells
SLC25A16	1	1.022	0.886	1.099	1.144	*H. sapiens*, similar to cytochrome *c*-like antigen, clone MGC: 2960 IMAGE:3139311, mRNA, complete cds.
SNRPD1	1	0.755	0.676	0.612	0.517	Small nuclear ribonucleoprotein D1 polypeptide, 16 kDa
SNRPC	1	1.28	1.03	1.28	1.12	Small nuclear ribonucleoprotein polypeptide C
SOX13	1	1.304	0.771	1.24	1.582	SRY-box 13
SP110	1	1.149	1.026	0.7	0.621	SP110 nuclear body protein
SPTBN	1	0.91	0.92	0.95	0.72	Spectrin, β, nonerythrocytic 1
SSA2	1	0.709	0.919	0.687	0.88	Sjögren's syndrome antigen A2 (60 kDa, ribonucleoprotein autoantigen SS-A/Ro)
SSA2	1	0.505	0.957	0.571	1.556	*H. sapiens* cDNA FLJ13982 fis, clone Y79AA1001711, highly similar to human 60-kDa ribonucleoprotein (Ro) mRNA
SSA2	1	0.596	0.98	0.85	0.94	*H. sapiens* cDNA FLJ13982 fis, clone Y79AA1001711, highly similar to human 60-kDa ribonucleoprotein (Ro) mRNA
SSNA1	1	1.354	1.17	1.608	0.948	Sjögren's syndrome nuclear autoantigen 1
SSSCA1	1	1.267	1.694	1.005	1.228	Sjögren's syndrome/scleroderma autoantigen 1
XRCC5	1	1.87	1.141	0.958	0.725	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand break rejoining; Ku autoantigen, 80 kDa)

PRKA, protein kinase A; α-NAC, human nacent peptide-associated complex; SRY, sex-determining region Y.

immune disorders. Green tea consumption has been shown to have protective effects in autoimmune animal models (Yang et al., 1998; Haqqi et al., 1999; Sayama et al., 2003; Roca et al., 2004). There are several mechanisms by which green tea could act. Apoptosis has been implicated in the presentation of autoantigens and tissue destruction in autoimmune disorders, and reduction in this event could ameliorate symptoms. GTPP have been shown to induce differential responses in normal versus tumor cells. Normal cells are protected; e.g., EGCG protects human salivary acinar and duct cells from radiation and chemotherapeutic drug-induced apoptosis (Yamamoto et al., 2004) and induced terminal differentiation in NHEK (Hsu et al., 2003). In contrast, tumor cells are induced to undergo apoptosis (Ahmad et al., 1997, Yamamoto et al., 2003). GTPP potently inhibited TNF-α, a proapoptotic cytokine (Yang et al., 1998), and EGCG inhibited TNF-α-mediated activation of the NF-κB pathway, thereby protecting normal cells from TNF-α-induced apoptosis (Wheeler et al., 2004). Autoimmune disorders have an inflammatory component. Recently, the anti-inflammatory effects of green tea have been explored with promising results (reviewed in Curtis et al., 2004). Studies using transgenic mice suggest that one way in which green tea may block diseases associated with cytokine overexpression is by inhibiting transcription of the proinflammatory cytokines TNF-α and IL-6 (Sueoka et al., 2001). EGCG also showed inhibitory effects on inflammatory pathways mediated by the inflammatory cytokine IL-1 (Wheeler et al., 2004) and effectively inhibited IL-1β-induction of matrix metalloproteinases 1 and 13 (Ahmed et al., 2004). The inhibition of IL-1β signaling may occur through the modulation of the mitogen-activated protein kinase pathway components (Singh et al., 2003). Green tea and EGCG are able to inhibit IL-8 release in TNF-α-stimulated NHEK

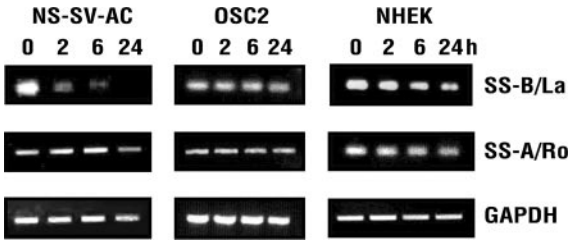

Fig. 1. Expression of SS-B/La and SS-A/Ro (52 kDa) mRNA levels in normal, immortalized, and tumor cells treated with EGCG. The results of semiquantitative RT-PCR from EGCG-treated NHEK, NS-SV-AC, and OSC2 cells at 0, 2, 6, and 24 h are shown for SS-B/La, SS-A/Ro (52 kDa), and GAPDH mRNAs. SS-B/La levels were progressively and significantly reduced in NHEK and NS-SV-AC cells during the time course of exposure. SS-A/Ro (52 kDa) also showed a modest reduction in these cells after 24 h. The reduction in OSC2 cells was much less pronounced. No comparable changes in GAPDH levels were seen. Data are representative of three independent determinations.

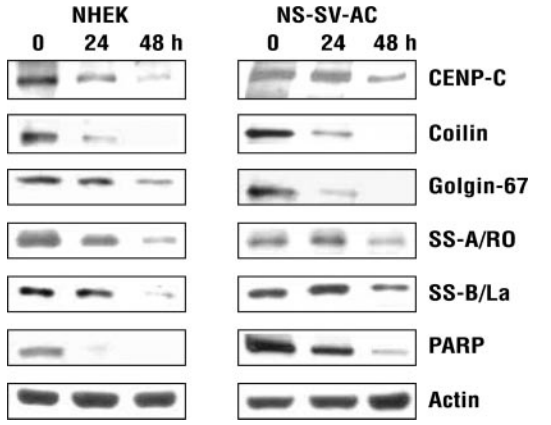

Fig. 2. Western blot results of autoantigen protein levels in EGCG-treated NHEK and NS-SV-AC cells with actin levels as controls. Down-regulation of these autoantigens was observed at 48 h in both cell lines. Data are representative of three independent determinations from two independent treated cell cultures.

(Trompezinski et al., 2003). A recent study showed that EGCG suppressed lipopolysaccharide-induced dendritic cell maturation, therefore reducing the subsequent T cell activation (Ahn et al., 2004).

A significant number of autoantibodies have been described for SLE (Sherer et al., 2004), and there is overlap with the autoantibodies found in the sera of patients with SS. The mechanisms leading to presentation of autoantigens to the immune system are not fully understood. One mechanism may be the translocation of nuclear autoantigens onto the cell membrane during apoptosis, where they are exposed to antigen-presenting cells (Manganelli and Fietta, 2003). During apoptosis, autoantigens redistribute to form apoptotic bodies and blebs, where autoantigens such as SS-A/Ro, SS-B/La, Ku, PARP, fodrin, golgins, and nuclear mitotic apparatus protein (NuMA) are clustered as subcellular structures. An aberrant structure of these autoantigen complexes may contribute to the autoimmune response (Rosen and Casciola-Rosen, 2004). B cells can be stimulated to proliferate and produce autoantibodies by perturbations in the levels of cytokines. Although the exact role of autoantibodies in the pathogenesis of SLE or SS remains unclear, it is thought that they are involved directly in some of the clinical manifestations (Mamula et al., 1994).

Herein, we report the novel finding that EGCG, the most abundant GTPP, down-regulates the expression of different autoantigens at the mRNA and/or protein levels in different epithelial cell types. This represents a third mechanism, in addition to reducing apoptosis and inflammation, by which GTPP could be useful to the prevention of and intervention in autoimmune disorders. The concentration of EGCG used in this study (100 μM) is achievable in the salivary glands (Yang et al., 1999) and topically if the skin barrier is interrupted, as among the patients with SLE and psoriasis.

Results from the Affymetrix gene expression analyses indicated that EGCG modulated the expression of a group of major autoantigens in NHEK, with several genes showing a 2-fold or more reduction in mRNA levels, in some cases after an initial increase. The various different patterns in the kinetics of change among different autoantigens suggest that different regulatory mechanisms could be involved. A reduction in expression of SS-B/La and SS-A/Ro in NHEK and NS-SV-AC cells over a 24-h period was confirmed by RT-PCR (Fig. 1). In OSC2 cells, the effects of EGCG were less pronounced. This difference is consistent with the different response of this cell line toward EGCG (apoptosis instead of differentiation) and suggests that the pathways mediating the inhibitory effect of EGCG on autoantigen expression are altered in tumor cells. It is known that GTPP induces terminal differentiation in NHEK (Hsu et al., 2003), raising the possibility that the decrease in expression seen in these cells is a consequence of this induction. However, because NS-SV-AC cells are immortalized human salivary acinar cells derived from fully differentiated acinar cells, the down-regulation of autoantigens is not necessarily associated directly with cell differentiation.

The SS-A/Ro autoantigen represents at least two independently expressed nucleocytoplasmic ribonucleoproteins, 60-kDa SS-A/Ro and 52-kDa SS-A/Ro, which complex noncovalently with small RNA species Y1 to Y5 (Chan et al., 1990). In patients with SS and SLE, 52-kDa SS-A/Ro is the main epitope recognized by anti-SS-A/Ro autoantibodies (Kubo et al., 2002). Elevated mRNA levels (2–3-fold) of SS-A/Ro 52 and SS-B/La are found in salivary tissues of SS patients (Bolstad et al., 2003), and autoantibodies against SS-A/Ro and SS-B/La are found in nearly all (~95 and 87%, respectively) primary SS patients (Hahn, 1998). During apoptosis, both SS-A/Ro and SS-B/La translocate from the nucleus to the membrane, which may present them as targets for ANA (McArthur et al., 2002). Expression of SS-A/Ro 52 (which was not represented on the Affymetrix array) was shown to be reduced by EGCG at the mRNA and protein level in two different epithelial cell lines using RT-PCR and Western analyses. Likewise, microarray, RT-PCR, and Western analyses demonstrated that EGCG reduced expression of SS-B/La. In contrast, the microarray analysis showed that SS-A/Ro 60 mRNA levels were not significantly altered by EGCG. It is noteworthy that oxidative stress induces cell surface expression of SS-A/Ro 52 but not SS-A/Ro 60 autoantigen on NHEK cells (Saegusa et al., 2002). Because GTPP inhibit the effects of oxidative stress on normal cells (Yamamoto et al., 2004), this may be one mechanism by which EGCG reduces expression of autoantigens. An inhibitory effect of EGCG on protein levels of four other autoantigens was demonstrated by Western analysis in NHEK and NS-SV-AC cells. The kinetics of reduction in protein levels differed somewhat between the

autoantigens. This could reflect regulation via different pathways or differences in mRNA or protein stability or in protein trafficking.

In conclusion, here we report for the first time that the most abundant green tea constituent, EGCG, specifically inhibits expression of certain major autoantigens at the mRNA and protein level in NHEK and human salivary acinar cells, two cell types often damaged by SLE and SS, respectively. Our results suggest that EGCG may be able to modulate the presentation of autoantigens, thereby potentially reducing the targets of autoimmune reactions. Most current approaches to treating SLE and SS focus on modifying the immune response (Goldblatt and Isenberg, 2005). We hypothesize that oral/topical administration of GTPP might delay the onset and reduce the severity of autoimmune disorders by 1) reducing autoimmune responses and decreasing the generation of autoantibodies via inhibition of autoantigen expression, 2) protecting the targeted cells, such as epidermal keratinocytes and glandular secretory cells, from apoptosis, leading to reduced membrane autoantigen presentation, and 3) inhibiting inflammation in affected tissues. Future studies in animal models and clinical trials will be needed to develop novel approaches for the prevention and treatment of autoimmune disorders using active phytochemicals such as EGCG.

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References

Ahmad N, Feyes DK, Nieminen AL, Agarwal R, and Mukhtar H (1997) Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *J Natl Cancer Inst* **89:**1881–1886.

Ahmed S, Wang N, Lalonde M, Goldberg VM, and Haqqi TM (2004) Green tea polyphenol epigallocatechin-3-gallate (EGCG) differentially inhibits interleukin-1 β-induced expression of matrix metalloproteinase-1 and -13 in human chondrocytes. *J Pharmacol Exp Ther* **308:**767–773.

Ahn SC, Kim GY, Kim JH, Baik SW, Han MK, Lee HJ, Moon DO, Lee CM, Kang JH, Kim BH, et al. (2004) Epigallocatechin-3-gallate, constituent of green tea, suppresses the LPS-induced phenotypic and functional maturation of murine dendritic cells through inhibition of mitogen-activated protein kinases and NF-κB. *Biochem Biophys Res Commun* **313:**148–155.

Aktas O, Prozorovski T, Smorodchenko A, Savaskan NE, Lauster R, Kloetzel PM, Infante-Duarte C, Brocke S, and Zipp F (2004) Green tea epigallocatechin-3-gallate mediates T cellular NF-κB inhibition and exerts neuroprotection in autoimmune encephalomyelitis. *J Immunol* **173:**5794–5800.

Azuma M, Tamatani T, Kasai Y, and Sato M (1993) Immortalization of normal human salivary gland cells with duct, myoepithelial, acinar, or squamous phenotype by transfection with SV40 ori- mutant deoxyribonucleic acid. *Lab Investig* **69:**24–42.

Bolstad AI, Eiken HG, Rosenlund B, Alarcon-Riquelme ME, and Jonsson R (2003) Increased salivary gland tissue expression of Fas, Fas ligand, cytotoxic T lymphocyte-associated antigen 4 and programmed cell death 1 in primary Sjogren's syndrome. *Arthritis Rheum* **48:**174–185.

Chan EK, Hamel JC, Peebles CL, Buyon JP, and Tan EM (1990) Molecular characterization and cloning of the 52 kDa SS-A/Ro protein. *Mol Biol Rep* **14:**53–60.

Curtis CL, Harwood JL, Dent CM, and Caterson B (2004) Biological basis for the benefit of nutraceutical supplementation in arthritis. *Drug Discov Today* **9:**165–172.

Gerl V, Hostmann B, Johnen C, Waka A, Gerl M, Schumann F, Klein R, Radbruch A, and Hiepe F (2005) The intracellular 52-kd Ro/SSA autoantigen in keratinocytes is up-regulated by tumor necrosis factor alpha via tumor necrosis factor receptor I. *Arthritis Rheum* **52:**531–538.

Goldblatt F and Isenberg DA (2005) New therapies for systemic lupus erythematosus. *Clin Exp Immunol* **140:**205–212.

Hahn BH (1998) Antibodies to DNA. *N Engl J Med* **338:**1359–1368.

Haqqi TM, Anthony DD, Gupta S, Ahmad N, Lee MS, Kumar GK, and Mukhtar H (1999) Prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green tea. *Proc Natl Acad Sci USA* **96:**4524–4529.

Hsu S, Bollag W, Lewis J, Huang Q, Singh B, Sharawy M, and Schuster GS (2003) Green tea polyphenols induce differentiation and proliferation in epidermal keratinocytes. *J Pharmacol Exp Ther* **306:**29–34.

Jacobson DL, Gange SJ, Rose NR, and Graham NM (1997) Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin Immunol Immunopathol* **84:**223–243.

Kubo M, Ihn H, Asano Y, Yamane K, Yazawa N, and Tamaki K (2002) Prevalence of 52-kd and 60-kd Ro/SS-A autoantibodies in Japanese patients with polymyositis/dermatomyositis. *J Am Acad Dermatol* **47:**148–151.

Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H et al. (1996) Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nature (Lond)* **14:**1675–1680.

Mamula MJ, Fatenejad S, and Craft J (1994) B cells process and present lupus autoantigens that initiate autoimmune T cell responses. *J Immunol* **152:**1453–1461.

Manganelli P and Fietta P (2003) Apoptosis and Sjogren's syndrome. *Semin Arthritis Rheum* **33:**49–65.

McArthur C, Wang Y, Veno P, Zhang J, and Fiorella R (2002) Intracellular trafficking and surface expression of SS-A (Ro), SS-B (La), poly(ADP-ribose) polymerase, and alpha-fodrin autoantigens during apoptosis in human salivary gland cells induced by tumour necrosis factor-alpha. *Arch Oral Biol* **47:**443–448.

Osaki T, Tatemoto Y, Yoneda K, and Yamamoto T (1994) Tumorigenicity of cell lines established from oral squamous cell carcinoma and its metastatic lymph nodes. *Eur J Cancer B Oral Oncol* **30B**:296–301.

Reeves GE (2004) Update on the immunology, diagnosis and management of systemic lupus erythematosus. *Intern Med J* **34:**338–347.

Rehman HU (2003) Sjogren's syndrome. *Yonsei Med J* **44:**947–954.

Roca V, Rosignoli F, Calafat M, and Leiros CP (2004) Lack of nitric oxide-mediated regulation of amylase secretion stimulated by VIP in parotid glands of NOD mice. *Int Immunopharmacol* **4:**1837–1844.

Rosen A and Casciola-Rosen L (2004) Altered autoantigen structure in Sjogren's syndrome: implications for the pathogenesis of autoimmune tissue damage. *Crit Rev Oral Biol Med* **15:**156–164.

Saegusa J, Kawano S, Koshiba M, Hayashi N, Kosaka H, Funasaka Y, and Kumagai S (2002) Oxidative stress mediates cell surface expression of SS-A/Ro antigen on keratinocytes. *Free Radic Biol Med* **32:**1006–1016.

Sawalha AH and Harley JB (2004) Antinuclear autoantibodies in systemic lupus erythematosus. *Curr Opin Rheumatol* **16:**534–540.

Sawalha AH, Potts R, Schmid WR, Scofield RH, and Harley JB (2003) The genetics of primary Sjogren's syndrome. *Curr Rheumatol Rep* **5:**324–332.

Sayama K, Oguni I, Tsubura A, Tanaka S, and Matsuzawa A (2003) Inhibitory effects of autoimmune disease by green tea in MRL-Faslprcg/Faslprcg mice. *In Vivo* **17:**545–552.

Sherer Y, Gorstein A, Fritzler MJ, and Shoenfeld Y (2004) Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. *Semin Arthritis Rheum* **34:**501–537.

Singh R, Ahmed S, Malemud CJ, Goldberg VM, and Haqqi TM (2003) Epigallocatechin-3-gallate selectively inhibits interleukin-1beta-induced activation of mitogen activated protein kinase subgroup c-Jun N-terminal kinase in human osteoarthritis chondrocytes. *J Orthop Res* **21:**102–109.

Stinton LM, Eystathioy T, Selak S, Chan EK, and Fritzler MJ (2004) Autoantibodies to protein transport and messenger RNA processing pathways: endosomes, lysosomes, Golgi complex, proteasomes, assemblyosomes, exosomes and GW bodies. *Clin Immunol* **110:**30–44.

Sueoka N, Suganuma M, Sueoka E, Okabe S, Matsuyama S, Imai K, Nakachi K, and Fujiki H (2001) A new function of green tea: prevention of lifestyle-related diseases. *Ann NY Acad Sci* **928:**274–280.

Trompezinski S, Denis A, Schmitt D, and Viac J (2003) Comparative effects of polyphenols from green tea (EGCG) and soybean (genistein) on VEGF and IL-8 release from normal human keratinocytes stimulated with the proinflammatory cytokine TNFα. *Arch Dermatol Res* **295:**112–116.

Yamamoto K (2003) Pathogenesis of Sjogren's syndrome. *Autoimmun Rev* **2:**13–18.

Yamamoto T, Hsu S, Lewis J, Wataha J, Dickinson D, Singh B, Bollag WB, Lockwood P, Ueta E, Osaki T, et al. (2003) Green tea polyphenol causes differential oxidative environments in tumor versus normal epithelial cells. *J Pharmacol Exp Ther* **307:**230–236.

Yamamoto T, Staples J, Wataha J, Lewis J, Lockwood P, Schoenlein P, Rao S, Osaki T, Dickinson D, Kamatani T, et al. (2004) Protective effects of EGCG on salivary gland cells treated with gamma-radiation or cis-platinum(II)diammine dichloride. *Anticancer Res* **24:**3065–3073.

Yang CS, Lee MJ, and Chen L (1999) Human salivary tea catechin levels and catechin esterase activities: implication in human cancer prevention studies. *Cancer Epidemiol Biomarkers Prev* **8:**83–89.

Yang F, de Villiers WJ, McClain CJ, and Varilek GW (1998) Green tea polyphenols block endotoxin-induced tumor necrosis factor-production and lethality in a murine model. *J Nutr* **128**:2334–2340.

Venables PJ (2004) Sjogren's syndrome. *Best Pract Res Clin Rheumatol* **18:**313–329.

Wheeler DS, Catravas JD, Odoms K, Denenberg A, Malhotra V, and Wong HR (2004) Epigallocatechin-3-gallate, a green tea-derived polyphenol, inhibits IL-1β-dependent proinflammatory signal transduction in cultured respiratory epithelial cells. *J Nutr* **134:**1039–1044.

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