Published in final edited form as:

Cell Immunol. 2013 March ; 282(1): 38–43. doi:10.1016/j.cellimm.2013.04.005.

Effects of cigarette smoke extract on primary activated T cells

Claudia P. Hernandez^a, Kevin Morrow^a, Cruz Velasco^a, Dorota D. Wyczzechowska^a, Amarjit Naura^a, and Paulo C. Rodriguez^{a,b,c}

^aStanley S. Scott Cancer Center, Louisiana State University-Health Sciences Center, New Orleans, LA, 70112.

^bDepartment of Microbiology Immunology and Parasitology, Louisiana State University-Health Sciences Center, New Orleans, LA, 70112.

Abstract

Tobacco smoking predisposes the development of diseases characterized by chronic inflammation and T cell dysfunction. In this study, we aimed to determine the direct effects of cigarette smoke on primary T cells and to identify the corresponding molecular mediators. Activated T cells cultured in the presence of cigarette smoke extract (CSE) displayed a dose-dependent decrease in cell proliferation, which associated with the induction of cellular apoptosis. T cell apoptosis by CSE was independent of caspases and mediated through reactive oxygen and nitrogen species endogenously contained within CSE. Additional results showed that exposure of T cells to CSE induced phosphorylation of the stress mediator eukaryotic-translation-initiation-factor 2 alpha (eIF2). Inhibition of the phosphorylation of eIF2 in T cells prevented the cellular apoptosis induced by CSE. Altogether, the results show the direct effects of CSE on T cells, which advance in the understanding of how cigarette smoking promotes chronic inflammation and immune dysfunction.

Keywords

T cells; Cigarette smoke; Reactive oxygen species; Peroxynitrites; Phospho-eIF2; apoptosis

1. Introduction

Cigarette smoking is responsible for about 90% of the lung carcinoma cases worldwide [1] and represents a major risk factor for the development of several diseases including chronic obstructive pulmonary disease (COPD), asthma, coronary disease, and cancer [2,3,4,5]. These conditions are characterized by the chronic presence of inflammatory immune responses [6,7], suggesting a potential direct and/or indirect effect of cigarette smoking in immune cells [8]. Accordingly, previous studies have suggested that exposure to cigarette smoke leads to an increased susceptibility to infections such as influenza virus and mycobacterium tuberculosis and significant changes in cellular immune responses [9,10]. However, the direct effects of cigarette smoke on T lymphocytes are still poorly understood.

© 2013 Elsevier Inc. All rights reserved.

^cCorrespondence should be addressed to: Stanley S. Scott Cancer Center, Louisiana State University, Health Sciences Center, 533 Bolivar St Room 452, New Orleans, LA, 70112, US. Phone: (504) 568-3350; Fax: (504) 599-0911; prodri1@lsuhsc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The gaseous and particulate phases of cigarette smoke contain thousands of compounds, many of which are toxic agents. Among them, the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) after tobacco burning play a major role in the cellular alterations induced by cigarette smoke on epithelial cells [11,12]. In fact, previous studies had found an increased oxidative burden and a decreased in anti-oxidant pathways in the systemic compartment of smokers [13,14].

Diverse stress signals including hypoxia, exposure to ultraviolet irradiation, and nutrient starvation, elicit in cells an integrated cellular response that is characterized by the phosphorylation of the eukaryotic translation initiation factor 2 alpha (eIF2) [15]. Phosphorylation of eIF2 on serine residue 51 (phospho-eIF2) inhibits nucleotide exchange on the eIF2 complex, attenuating cellular translation of most mRNAs and reducing protein synthesis [16,15,17]. During transient stress conditions, this process promotes the expression of proteins that function in the cellular adaptation to stress, which occurs through a Cap-independent translation [16,15,17]. However, if the stress conditions are persistent or highly aggressive for the cell, the phosphorylation of eIF2 will lead to the expression of proteins promoting cellular apoptosis.

The use of cigarette smoke extract (CSE) *in vitro* is a standardized method to test the direct effects induced by cigarette smoke on cells [18,19]. Therefore, in this study, we aimed to determine the effect of CSE on primary T cells *in vitro*. Our results suggest that activated T cells cultured in the presence of CSE displayed a dose-dependent decrease in cell proliferation, which associated with the induction of T cell apoptosis. The induction of apoptosis by CSE was independent of caspase activation and mediated through ROS and RNS endogenously contained within the CSE. Additional results showed that the exposure of T cells to CSE induced the phosphorylation of eIF2. Inhibition of the phosphorylation of eIF2 in T cells prevented the cellular apoptosis induced by CSE. Altogether, the results suggest that exposure of activated T cells to CSE induces apoptosis in a phospho-eIF2 - dependent manner. These results advance in the understanding of how cigarette smoking modulates responses in immune cell populations.

2. Materials and Methods

2.1. Cells lines, animals, and reagents

Human primary T cells were isolated from peripheral blood mononuclear cells using T cell enrichment columns (R&D Systems, Minneapolis, MN), as previously reported [20]. T cell purity ranged between 90 and 95%. Samples from healthy donors were obtained from buffy coats purchased from blood banks (around 30 different blood units were used during the study). Then, T cells were activated as previously described [21]. Murine T cells were isolated from spleens and lymph nodes of C57BL/6 mice and gp91^{phox}/Nox-2 null mice (The Jackson Laboratory) by negative selection kits (Dynabeads, Invitrogen, Carlsbad, CA). Then, they were activated using 1 g/ml of plate bound anti-CD3 (Clone 145-2C11) plus anti-CD28 (Clone 37.51) (PharMingen-Becton Dickinson, San Diego, CA) as previously reported [20]. Activated T cells were cultured in RPMI 1640 (Lonza, Hopkinton, MA) supplemented with 5% fetal bovine serum (Hyclone, Logan, UT), 4 mM L-glutamine (Cambrex, Charles City, IA), 25 mM Hepes, and 100 U/ml of penicillin/streptomycin (Invitrogen). T cell line CCRF-CEM (ATCC, Manassas, VA) was transfected with plasmids coding for a wild type eIF2 (eIF2 -S51S), or eIF2 -S51A, a dominant negative non-phosphorylatable form of eIF2 in which serine 51 has been mutated to alanine, as previously described [22]. Transfected CCRF-CEM cells were cultured in RPMI-1640 medium supplemented with 800 g/ml Geneticin (Invitrogen). Carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (Z-VAD-FMK) (50 M) (Promega, Madison, WI), staurosporine (1 M) (Cell Signaling-Millipore, Boston, MA), catalase from bovine liver (600

U/ml), and glutathione reduced ethyl ester (GSH_e) (1 M) (Sigma-Aldrich, St. Louis, MO) were added to T cells cultured in the presence or the absence of CSE.

2.2. T Cell proliferation

Activated T cells (1×10^5) were cultured in medium containing increasing concentrations of CSE (1.25, 2.5 and 5%). After 72 hours, 1 Ci [³H]-thymidine was added to the cultures and incubated for 8 hours. Then, T cell proliferation was tested by measuring the incorporation of [³H]-thymidine. Data are expressed as counts per minute (CPM) (mean $\pm$ SD) of at least triplicate experiments.

2.3. CSE preparation

CSE was prepared as previously described [23] by bubbling smoke from 2 Marlboro 100's cigarettes into 10 ml of serum free-RPMI for approximately 5 minutes and the medium was filtered with a 0.45 μ m filter (Acrodisc® 25 mm Syringe Filter, Pall, Ann Arbor, MI). This solution was considered to be 100% CSE. The nitrite concentration was determined using a Griess Reagent Kit (Invitrogen), and ranged between 12-17 μ M, which is accepted as a standardized concentration of pure CSE.

2.4. Flow cytometry

Expression of annexin V was measured using the annexin V-FITC Apoptosis Detection Kit (BD Biosciences)[24], following manufacturer's recommendations. The results are expressed as the percentage of annexin V⁺ cells. To test the mitochondrial membrane potential, primary T cells cultured with or without CSE were labeled with 3,3'-diethyloxycarbocyanine-iodide (DiOC2(3)), using the MitoProbe™ DiOC2(3) assay kit (Molecular Probes-Invitrogen).

Dichlorodihydrofluorescein diacetate (DCFDA) (Molecular Probes-Invitrogen) was used to determine the production of ROS. One million T cells were labeled for 5 min at 37°C in PBS using 5 μ M of DCFDA, washed twice with warm PBS, cultured in media with or without CSE, and fluorescence determined by flow cytometry.

2.5. Measurement of ATP

Intracellular ATP was measured by the luciferin/luciferase method using the ApoSENSOR™ Cell Viability Assay kit (BioVison, Mountain View, CA), following the vendor's protocol.

2.6. Western Blot

Twenty micrograms of cell lysates, collected as described [20] were ran in 8% Tris-Glycine gels, transferred to PVDF membranes (Invitrogen), and immunoblotted with specific antibodies against phospho-eIF2 α (Epitomics, Burlingame, CA), total eIF2 α (Invitrogen), and -actin (Sigma-Aldrich). Membrane-bound immune complexes were detected using ECL Western blotting detection system (Amersham-Biosciences, Arlington Heights, IL) followed by exposure to BioMax MR films (Kodak, Rochester, NY).

2.7. Statistical Analysis

Comparisons between cells cultured in the presence or the absence of CSE, including proliferation, annexin V expression, and ATP levels were done with suitable ANOVA models accounting for unequal variances with the Satterthwaite correction; followed by means comparisons via simulation of quantiles. Analyses were carried out in SAS 9.3 (SAS Institute, Cary NC). Tests were performed at the 5% significance level with adjustment for multiple comparisons.

3. Results

3.1. T lymphocytes cultured in CSE have a decreased proliferation and high rate of cellular apoptosis

To determine the effect of cigarette smoke on T cells, we have used CSE, a standardized method to test the effects of cigarette smoke on cultured cells [18,19]. Human activated T lymphocytes were cultured in the presence of increasing concentrations of CSE (1.25-5%) and cell proliferation tested after 72 hours. A dose dependent inhibition in cell proliferation was observed in activated T lymphocytes cultured with CSE (Fig. 1A) ($P<0.0001$). The anti-proliferative effect induced by CSE in T cells associated with a high expression of the apoptosis markers annexin V (Fig. 1B-C), depletion of ATP levels (Fig. 1D) ($P<0.0001$), and an impaired mitochondrial membrane potential (Fig. 1E). To determine the role of caspases in the CSE-induced T cell apoptosis, we used the pan caspase inhibitor Z-VAD-FMK. Pre-incubation of T cells with Z-VAD-FMK did not prevent the apoptosis induced by CSE (Fig. 1F) ($P=0.69$). However, it blocked the caspase-dependent cell death induced by staurosporine. These results suggest that CSE impairs T cell proliferation through the induction of cellular apoptosis, which cannot be prevented after caspases inhibition.

3.2. Reactive species within the CSE are responsible for the induction of T cell apoptosis

We tested the possibility that reactive species within CSE were responsible for the alterations induced in the activated T cells. An increased fluorescence of T cells labeled with ROS/RNS-sensitive dye DCFDA was found in the presence of CSE, but not in CSE-free conditions (Fig. 2A). Moreover, the addition of the antioxidant compound GSHe (1 μ M) completely prevented the induction of T cell apoptosis by CSE ($P=0.0002$), whereas H₂O₂ scavenger catalase (600 U) partially blocked CSE-induced T cell apoptosis ($P=0.012$) (Fig. 2B). To determine the role of the ROS produced endogenously in the T cells, we used T cells from gp91^{phox} knockout mice, which plays a major role in the endogenous production of ROS/RNS [25]. A similar rate of apoptosis (Fig. 2C) and an equal production of ROS/RNS (Fig. 2D) was found in CSE-treated T cells from gp91^{phox} knockout mice and control littermates, suggesting a potential role of the exogenous reactive species within the CSE in the induction of T cell apoptosis. Accordingly, pre-treatment of CSE-conditioned medium with catalase induced the same prevention of T cell apoptosis as the catalase used without pre-conditioning (Fig. 2E).

3.3. Role of phospho-eIF2 on the induction of T cell apoptosis by CSE

In response to stress, phosphorylation of eIF2 α initially alleviates cellular injury, but later promotes cellular apoptosis [26,15]. In order to determine if CSE was able to induce phosphorylation of eIF2 α , primary T cells and T cell line CCRF-CEM cells were cultured with or without CSE. Stimulated T cells and CCRF-CEM cells cultured with CSE have an increased expression of phospho-eIF2 α , as compared to control cells cultured without CSE (Fig. 3A-B). In order to determine the role of the phospho-eIF2 α on the induction of apoptosis by CSE, T cell line CCRF-CEM was stably transfected with plasmids coding for the wild type eIF2 α (S51S), or the dominant negative form of eIF2 α (S51A). A significant prevention in the induction of annexin V expression was found in the CSE-treated S51A-CCRF-CEM cells, as compared to treated S51S-transfected cells (Fig. 3C) ($P<0.0001$). This effect correlated with a prevention in the depletion of ATP levels induced by CSE in S51A-expressing cells, but not in control S51S-cells (Fig. 3D) ($P<0.001$). Furthermore, a decrease in the phosphorylation of eIF2 by CSE was found in cells cultured in the presence of GSHe, but not in cells cultured with catalase (Fig. 3E), suggesting the role of reactive oxygen/nitrogen species in the induction of phospho-eIF2 by CSE.

4. Discussion

Cigarette smoking is the leading cause of death in the United States and a major risk for the development of cancer, heart and respiratory diseases. Because of the negative impact of cigarette smoke in health and the persistent damage after cessation, it is important to understand the mechanisms by which cigarette smoke exposure induces cell damage. Our results suggest that ROS/RNS contained within CSE lead to apoptosis in primary T cells through caspase-independent mechanisms that are mediated through the phosphorylation of eIF2. These results advance in the understanding of the mechanisms by which cigarette smoking modulates responses in immune cell populations.

Diverse stress signals including unfolded protein responses (UPR), nutrient starvation, hypoxia, and UV exposure elicit an integrated cellular response that leads to the phosphorylation of eIF2 [16,15]. Previous studies have shown that exposure of cells or rats to cigarette smoke induces the phosphorylation of eIF2 [27,28,29,30,31]. Furthermore, a high induction of UPR stress-related proteins, including phospho-eIF2, was detected in the lung from heavy smokers [12]. Now, what is the effect of the phosphorylation of eIF2 after exposure to cigarette smoke. Initial studies showed a correlation of eIF2 with the induction of cellular apoptosis [27,28,29,30,31]. Accordingly, our *in vitro* results show that inhibition of the phosphorylation of eIF2 using a dominant negative form of eIF2 prevented the T cell apoptosis induced by CSE. Phosphorylation of eIF2 has also been associated with changes in the immune responses and remodeling after combination of cigarette smoke and poly (I:C) [32], suggesting a potential role of this pathway in the alterations of immune responses induced by cigarette smoke. Four different kinases, the double-stranded RNA-dependent protein kinase (PKR), the hemin-regulated inhibitor (HRI), the PKR-like endoplasmic reticulum-related kinase (PERK), and the general control non-repressed 2 kinase (GCN2), phosphorylate eIF2 in response to different stress signals [33,15]. Previous studies have suggested the role of PERK in the phosphorylation of eIF2 induced by CSE [29,27,31]. In contrast, our experiments silencing the expression of eIF2-kinases in CCRF-CEM cells showed neither a retardation in the eIF2 phosphorylation nor a prevention in CSE-induced apoptosis (data not shown), suggesting a potential role of the dephosphorylation of eIF2 as the mechanisms for the high levels of phospho-eIF2 in CSE-treated T cells. Current experiments in our group are testing the role of eIF2 phosphatases in the high levels of phospho-eIF2 induced by CSE.

Reactive species such as hydrogen peroxide (H_2O_2), nitric oxide (NO), superoxide anion (O_2^-), peroxynitrite, and hydroxyl radical (OH^-) are essential for many biological functions, including cell growth and cell differentiation [34]. However, their high accumulation leads to cellular damage and mutagenesis [35,36]. Cigarette smoke contains large quantities of reactive species, some of which can enter the cells, reach the nucleus and cause DNA damage and cell death [34,37,11]. In accordance, an increased in the levels of ROS and RNS has been detected in the systemic compartment of smokers [13]. These reactive species can induce a direct damage to the lung epithelium or alter the phenotype and/or function of immune cells [38,13,39,14]. Our results suggest the relevance of the ROS and RNS in the apoptosis induced by CSE. The potential link between the high levels of reactive species and the phosphorylation of eIF2 lays on the induction of stress in the endoplasmic reticulum (ER) [31,12]. In fact, Tagawa et al [27,31] previously showed that cigarette smoke triggers apoptosis through oxidative stress and PERK-related ER stress-dependent induction of CCAAT/enhancer-binding protein-homologous protein (CHOP). However, the role of this interaction in the chronic inflammation and potentially in the induction of cancer in smokers is still unknown.

The death pathways engaged by cells subjected to CSE have been investigated in different cell types including endothelial and alveolar epithelial cells. These studies have found conflicting results, with the mode of cell death ranging from classical apoptosis to gross necrosis [27,40,41]. Our results showed that CSE induced the expression of annexin V and disrupted mitochondrial membrane stability, both classical markers of cellular apoptosis. However, we found that caspase inhibition in T cells did not prevent the induction of CSE-apoptosis. Because several mechanisms can induce cell death in CSE-treated cells, it is possible that some other mechanisms of cell death can compensate the inhibition of caspases, leading to a similar induction of cell death. The cellular apoptosis triggered after treatment of alveolar cells with CSE has been reported to be mediated by CHOP. ROS/PNT in the CSE activates PERK, leading to the expression of phospho-eIF2 and a subsequent induction of CHOP, which promotes intrinsic apoptosis pathways [28,27,31]. Therefore, the therapeutic use of ROS/PNT scavengers or the generation of inhibitors of CHOP could potentially have an impact in the cell death and the alterations of immune responses induced by cigarette smoke.

In conclusion, our results suggest a new effect of cigarette smoke in normal T cells, in which ROS and RNS within CSE induce the phosphorylation of eIF2, thereby promoting caspase-independent apoptosis. This study advances in the understanding of how cigarette smoking promotes chronic inflammation and immune dysfunction.

Acknowledgments

We thank Dr. Jey Jeyaseelan, PhD (LSU, Baton Rouge) for their input, help, and support during this work

This work was supported in part by NIH-NCRR (COBRE) P20RR021970 to P.C.R. and 1R21CA162133 to PCR

Abbreviations

CSE	Cigarette smoke extract
eIF2	Eukaryotic translation initiation factor 2 alpha
ROS	reactive oxygen species
RNS	reactive nitrogen species
GSHe	glutathione reduced ethyl ester

7. Reference List

- [1]. Alberg AJ, Samet JM. Epidemiology of lung cancer. *Chest*. 2003; 123:21S–49S. [PubMed: 12527563]
- [2]. Anto RJ, Mukhopadhyay A, Shishodia S, Gairola CG, Aggarwal BB. Cigarette smoke condensate activates nuclear transcription factor-kappaB through phosphorylation and degradation of IkappaB(alpha): correlation with induction of cyclooxygenase-2. *Carcinogenesis*. 2002; 23:1511–1518. [PubMed: 12189195]
- [3]. Adcock IM, Caramori G, Barnes PJ. Chronic obstructive pulmonary disease and lung cancer: new molecular insights. *Respiration*. 2011; 81:265–284. [PubMed: 21430413]
- [4]. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. *Lancet*. 2007; 370:765–773. [PubMed: 17765526]
- [5]. Sethi JM, Rochester CL. Smoking and chronic obstructive pulmonary disease. *Clin.Chest Med*. 2000; 21:67–86. viii. [PubMed: 10763090]
- [6]. Gardi C, Valacchi G. Cigarette smoke and ozone effect on murine inflammatory responses. *Ann.N.Y.Acad.Sci*. 2012; 1259:104–111. [PubMed: 22758642]

- [7]. Rom O, Avezov K, Aizenbud D, Reznick AZ. Cigarette smoking and inflammation revisited. *Respir.Physiol Neurobiol.* 2013
- [8]. Gardi C, Valacchi G. Cigarette smoke and ozone effect on murine inflammatory responses. *Ann.N.Y.Acad.Sci.* 2012; 1259:104–111. [PubMed: 22758642]
- [9]. Mehta H, Nazzal K, Sadikot RT. Cigarette smoking and innate immunity. *Inflamm.Res.* 2008; 57:497–503. [PubMed: 19109742]
- [10]. Feng Y, Kong Y, Barnes PF, Huang FF, Klucar P, Wang X, Samten B, Sengupta M, Machona B, Donis R, Tinnereim AR, Shams H. Exposure to cigarette smoke inhibits the pulmonary T-cell response to influenza virus and Mycobacterium tuberculosis. *Infect.Immun.* 2011; 79:229–237. [PubMed: 20974820]
- [11]. Aoshiba K, Tamaoki J, Nagai A. Acute cigarette smoke exposure induces apoptosis of alveolar macrophages. *Am.J.Physiol Lung Cell Mol.Physiol.* 2001; 281:L1392–L1401. [PubMed: 11704535]
- [12]. Kelsen SG, Duan X, Ji R, Perez O, Liu C, Merali S. Cigarette smoke induces an unfolded protein response in the human lung: a proteomic approach. *Am.J.Respir.Cell Mol.Biol.* 2008; 38:541–550. [PubMed: 18079489]
- [13]. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. *Chest.* 2007; 131:1557–1566. [PubMed: 17494805]
- [14]. van, d.; Postma, V.DS.; Timens, W.; ten Hacken, NH. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. *Thorax.* 2004; 59:713–721. [PubMed: 15282395]
- [15]. Holcik M, Sonenberg N. Translational control in stress and apoptosis. *Nat.Rev.Mol.Cell Biol.* 2005; 6:318–327. [PubMed: 15803138]
- [16]. Holcik M, Sonenberg N, Korneluk RG. Internal ribosome initiation of translation and the control of cell death. *Trends Genet.* 2000; 16:469–473. [PubMed: 11050335]
- [17]. Woo CW, Cui D, Arellano J, Dorweiler B, Harding H, Fitzgerald KA, Ron D, Tabas I. Adaptive suppression of the ATF4-CHOP branch of the unfolded protein response by toll-like receptor signalling. *Nat.Cell Biol.* 2009; 11:1473–1480. [PubMed: 19855386]
- [18]. Glader P, Moller S, Lilja J, Wieslander E, Lofdahl CG, von WK. Cigarette smoke extract modulates respiratory defence mechanisms through effects on T-cells and airway epithelial cells. *Respir.Med.* 2006; 100:818–827. [PubMed: 16242311]
- [19]. Yang SR, Chida AS, Bauter MR, Shafiq N, Seweryniak K, Maggirwar SB, Kilty I, Rahman I. Cigarette smoke induces proinflammatory cytokine release by activation of NF-kappaB and posttranslational modifications of histone deacetylase in macrophages. *Am.J.Physiol Lung Cell Mol.Physiol.* 2006; 291:L46–L57. [PubMed: 16473865]
- [20]. Rodriguez PC, Quiceno DG, Ochoa AC. L-arginine availability regulates T-lymphocyte cell-cycle progression. *Blood.* 2007; 109:1568–1573. [PubMed: 17023580]
- [21]. Rodriguez PC, Hernandez CP, Morrow K, Sierra R, Zabaleta J, Wyczehowska DD, Ochoa AC. L-arginine deprivation regulates cyclin D3 mRNA stability in human T cells by controlling HuR expression. *J.Immunol.* 2010; 185:5198–5204. [PubMed: 20889542]
- [22]. Morrow K, Hernandez CP, Raber P, Del VL, Wilk AM, Majumdar S, Wyczehowska D, Reiss K, Rodriguez PC. Anti-leukemic mechanisms of pegylated arginase I in acute lymphoblastic T-cell leukemia. *Leukemia.* 2012
- [23]. Yang SR, Yao H, Rajendrasozhan S, Chung S, Edirisinghe I, Valvo S, Fromm G, McCabe MJ Jr. Sime PJ, Phipps RP, Li JD, Bulger M, Rahman I. RelB is differentially regulated by IkappaB Kinase-alpha in B cells and mouse lung by cigarette smoke. *Am.J.Respir.Cell Mol.Biol.* 2009; 40:147–158. [PubMed: 18688039]
- [24]. Hernandez CP, Morrow K, Lopez-Barcons LA, Zabaleta J, Sierra R, Velasco C, Cole J, Rodriguez PC. Pegylated arginase I: a potential therapeutic approach in T-ALL. *Blood.* 2010; 115:5214–5221. [PubMed: 20407034]
- [25]. Jackson SH, Devadas S, Kwon J, Pinto LA, Williams MS. T cells express a phagocyte-type NADPH oxidase that is activated after T cell receptor stimulation. *Nat.Immunol.* 2004; 5:818–827. [PubMed: 15258578]
- [26]. Raven JF, Koromilas AE. PERK and PKR: old kinases learn new tricks. *Cell Cycle.* 2008; 7:1146–1150. [PubMed: 18418049]

- [27]. Tagawa Y, Hiramatsu N, Kasai A, Hayakawa K, Okamura M, Yao J, Kitamura M. Induction of apoptosis by cigarette smoke via ROS-dependent endoplasmic reticulum stress and CCAAT/enhancer-binding protein-homologous protein (CHOP). *Free Radic.Biol.Med.* 2008; 45:50–59. [PubMed: 18394432]
- [28]. Csordas A, Kreutmayer S, Ploner C, Braun PR, Karlas A, Backovic A, Wick G, Bernhard D. Cigarette smoke extract induces prolonged endoplasmic reticulum stress and autophagic cell death in human umbilical vein endothelial cells. *Cardiovasc.Res.* 2011; 92:141–148. [PubMed: 21676957]
- [29]. Gan G, Hu R, Dai A, Tan S, Ouyang Q, Fu D, Jiang D. The role of endoplasmic reticulum stress in emphysema results from cigarette smoke exposure. *Cell Physiol Biochem.* 2011; 28:725–732. [PubMed: 22178884]
- [30]. Somborac-Bacura A, van der TM, Franciosi L, Slebos DJ, Zanic-Grubisic T, Bischoff R, van Oosterhout AJ. Cigarette smoke induces endoplasmic reticulum stress response and proteasomal dysfunction in human alveolar epithelial cells. *Exp.Physiol.* 2013; 98:316–325. [PubMed: 22848082]
- [31]. Tagawa Y, Hiramatsu N, Kato H, Sakoh T, Nakajima S, Hayakawa K, Saito Y, John H, Takahashi S, Gu L, Yao J, Kitamura M. Induction of CCAAT/enhancer-binding protein-homologous protein by cigarette smoke through the superoxide anion-triggered PERK-eIF2alpha pathway. *Toxicology.* 2011; 287:105–112. [PubMed: 21703327]
- [32]. Kang MJ, Lee CG, Lee JY, Cruz C.S. la, Chen ZJ, Enelow R, Elias JA. Cigarette smoke selectively enhances viral. *J.Clin.Invest.* 2008; 118:2771–2784. [PubMed: 18654661]
- [33]. Blander JM, Amsen D. Immunology. Amino acid addiction. *Science.* 2009; 324:1282–1283. [PubMed: 19498159]
- [34]. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat.Rev.Drug Discov.* 2009; 8:579–591. [PubMed: 19478820]
- [35]. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev.* 2002; 82:47–95. [PubMed: 11773609]
- [36]. Erdelyi K, Bai P, Kovacs I, Szabo E, Mocsar G, Kakuk A, Szabo C, Gergely P, Virag L. Dual role of poly(ADP-ribose) glycohydrolase in the regulation of cell death in oxidatively stressed A549 cells. *FASEB J.* 2009; 23:3553–3563. [PubMed: 19571039]
- [37]. Trachootham D, Lu W, Ogasawara MA, Nilsa RD, Huang P. Redox regulation of cell survival. *Antioxid.Redox.Signal.* 2008; 10:1343–1374. [PubMed: 18522489]
- [38]. Stampfli MR, Anderson GP. How cigarette smoke skews immune responses to promote infection, lung disease and cancer. *Nat.Rev.Immunol.* 2009; 9:377–384. [PubMed: 19330016]
- [39]. Domagala-Kulawik J. Effects of cigarette smoke on the lung and systemic immunity. *J.Physiol Pharmacol.* 2008; 59(Suppl 6):19–34. [PubMed: 19218630]
- [40]. Wickenden JA, Clarke MC, Rossi AG, Rahman I, Faux SP, Donaldson K, MacNee W. Cigarette smoke prevents apoptosis through inhibition of caspase activation and induces necrosis. *Am.J.Respir.Cell Mol.Biol.* 2003; 29:562–570. [PubMed: 12748058]
- [41]. Kim HP, Wang X, Chen ZH, Lee SJ, Huang MH, Wang Y, Ryter SW, Choi AM. Autophagic proteins regulate cigarette smoke-induced apoptosis: protective role of heme oxygenase-1. *Autophagy.* 2008; 4:887–895. [PubMed: 18769149]

Highlights

- ▶ Cigarette smoke extract (CSE) blocks T cell proliferation by inducing apoptosis.
- ▶ Reactive nitrogen and oxygen species from CSE are responsible for T cell apoptosis.
- ▶ Phosphorylation of eIF2 α is the central mediator for T cell apoptosis by CSE.

Figure 1. T lymphocytes cultured in CSE have a decrease proliferation and a high rate of apoptosis

A. T cells (1×10^5) were stimulated with anti-CD3 plus anti-CD28 and cultured in medium containing increasing concentrations of CSE. Proliferation was measured at 72 hours by incorporation of ^3H -thymidine. **B.** Expression of annexin V (24 hours) in stimulated T cells (1×10^6) cultured in the absence or the presence of different concentrations of CSE. **C-E.** Expression of annexin V (**C**), levels of ATP (**D**), and mitochondrial membrane potential (**E**) were measured in activated T cells cultured in medium with or without CSE (5%). **F.** CSE induced T cell apoptosis in a caspase-independent manner. Values are from 3 similar experiments

Figure 2. Reactive species within CSE are responsible for the induction of apoptosis

A. Stimulated T cells (1×10^6) were labeled with the DCFDA and cultured in medium with or without CSE (5%) for 2 hours. Fluorescence was detected by flow cytometry. **B.** Expression of annexin V was tested in stimulated T cells cultured in the presence of CSE plus peroxynitrite inhibitor GSHe or H_2O_2 scavenger catalase. **C.** T cells (1×10^6) from gp91^{phox} knockout or wild type mice cultured in the presence or the absence of CSE (5%) for 24 hours were tested for the expression of annexin V. **D.** Stimulated T cells (1×10^6) from gp91^{phox} knockout mice or wild type controls were labeled with the DCFDA and cultured in medium with or without CSE (5%). Two hours later, the cells were acquired by flow cytometry. **E.** T cells (1×10^6) were cultured in the presence of CSE or medium pre-treated or not with catalase for 2 hours. Annexin V expression was measured 24 hours later.

Figure 3. Role of phospho-eIF2 on the induction of T cell apoptosis by CSE

A. Phosphorylation of eIF2 was determined in stimulated T cells (**A**) or CCRF-CEM cells (**B**) cultured in the presence or the absence of CSE (5%). Densitometry analysis of band intensities (p-eIF2 /teIF2) was performed. **C-D.** CCRF-CEM cells expressing eIF2 -S51S or eIF2 -S51A were cultured in medium containing or not CSE (5%) and the expression of annexin V (**C**) or the levels of ATP (**D**) were determined. **E.** Culture of CCRF-CEM cells in CSE-medium in the presence of GSHe, but not catalase, prevented the induction of phospho-eIF2 after 2 hours of culture.