Mitochondrial Membrane Potential: A Novel Biomarker of Oxidative Environmental Stress

Muriel Vayssier-Taussat,1 Sarah E. Kreps,1 Christophe Adrie,1 Josette Dall’Ava,1 David Christiani,2 and Barbara S. Polla1

1Laboratory of Respiratory Physiology, UFR Cochin-Port Royal, Université Paris V René Descartes, Paris, France; 2Harvard Medical School, Charlestown, Massachusetts, USA

Epidemiologic analyses, traditionally based on long-term cohort or case–control studies, provide retrospective causal associations between exposure to a particular environmental stressor and an exposure-related disease end point. Recent research initiatives have propelled a shift toward exploring molecular epidemiology and molecular biological markers (biomarkers) as a means of providing more immediate, quantitative risk assessment of potentially deleterious environmental exposures. We compared, in normal human monocytes isolated from the blood of healthy donors, variations in Hsp70 expression and mitochondrial membrane potential (Δψm) in response to exposure to either tobacco smoke or γ-irradiation, two models for environmentally mediated oxidant exposure. On the basis of its mechanistic specificity for oxidants and little baseline variation in cells from distinct individuals, we propose that Δψm represents a selective in vitro and in vivo biomarker for oxidant exposure. Δψm may be used to gauge risks associated with oxidant-mediated air pollution and radiation. Key words: biomarker, environmental exposure, gamma-radiation, heat-shock proteins, Hsp70, mitochondrial membrane depolarization, oxidant, tobacco smoke. Environ Health Perspect 110:301–305 (2002). [Online 14 February 2002] http://ehpnet1.nih.gov/docs/2002/110p301-305vayssier-taussat/abstract.html

Risk assessment is used to estimate the magnitude, likelihood, and uncertainty of environmentally induced toxic effects. Epidemiologic analysis, traditionally based on long-term cohort or case–control studies, provides retrospective causal associations between exposure to a particular environmental stressor and an exposure-related disease end point. Recent research initiatives have propelled a shift toward exploring molecular epidemiology and molecular biological markers (biomarkers) as a means of providing more immediate, quantitative risk assessment of potentially deleterious environmental exposures (1–4).

Three categories of biomarkers have been identified: biomarkers of effect, which are cellular responses that reflect sublethal exposure-related damage to a system; biomarkers of exposure, which are reversible upstream markers that respond before cellular damage occurs; and biomarkers of susceptibility, which refer to individual variations in the genes coding for stressor-induced cellular response (5). Proteins whose genes are influenced and induced by environmental stimuli or ecologic variations are called ecoproteins, in contrast to the constitutive, structural, “eco-free” proteins. Ecoproteins, which are generally highly inducible and conserved in nature, represent protective mechanisms against environmental stress and amplify the system’s ecophysiologic adaptation to environmental conditions.

Among ecoproteins, stress proteins have been abundantly studied as a biomarker of effect for pollutants (6,7). Heat-shock proteins (HSPs) represent the most abundant and widely studied group of stress proteins. HSPs are induced as an adaptive response on exposure to a variety of cellular injuries including oxidative damage. In particular, the cytosolic, inducible, 72 kDa HSP (Hsp70) is induced by oxidants both in vitro and in vivo (8–10), and its expression has been used as an indicator of response to environmental stress and an interesting candidate as biomarkers of effect (11–14). Optimal biomarkers would be biomarkers of exposure, which could provide the earliest, most upstream warning signs of environmental stress exposure. Mitochondria are highly sensitive to oxidants and to toxic exposure–mediated cell death (15–17). We have previously shown that mitochondria were a target for reactive oxygen species–mediated effects of in vitro tobacco smoke (TS) exposure in human monocytes (18). We thus hypothesized that mitochondrial alterations, as determined by mitochondrial membrane potential (Δψm), could represent a primary target for oxidant toxicity and could be used as a specific biomarker for oxidant-mediated exposure.

In this study, we first compared the variability of baseline Δψm to baseline Hsp70 expression in human monocytes isolated from the blood of healthy donors. We then analyzed the effects of two oxidant-mediated environmental stressors, TS and γ-radiation, on Δψm. Our data suggest that the inherent variability of baseline levels of Hsp70 detected in human cells renders Hsp70 expression difficult to use as a biomarker for in vitro exposures, though it remains adequate for in vitro studies. In contrast, Δψm might be an adequate biomarker of oxidant-mediated environmental stress, both in vitro and in vivo.

Materials and Methods

Reagents. We purchased paraformaldehyde and saponin from Sigma (St Louis, MO, USA), and culture medium (RPMI 1640 and Dulbecco’s modified Eagle medium), fetal calf serum (FCS), phosphate-buffered saline (PBS), t-glutamine, bovine serum albumin (BSA, fraction V), and HEPEs buffer from ICN Biochemicals (Costa Mesa, CA, USA). We purchased 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarboxyanine iodide (JC-1) from Molecular Probes (Eugene, OR, USA). The monoclonal antibodies directed against the inducible form of Hsp70 [mouse immunoglobulin (Ig)G1, SPA-810] were from Stressgen (Victoria, Canada). The F(ab′)2 fragment of rabbit anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC), used as secondary antibody, was from Dako (Glostrup, Denmark).

Cells and culture conditions. We obtained anonymous donated blood through the Assistance Publique Hôpitaux de Paris (APHP), in the form of buffy coats. We isolated monocytes by Ficoll gradient centrifugation and purified them by adherence as described previously (18). We maintained monocytes (2.5 × 106/mL) in RPMI-1640 medium containing 10% fetal calf serum, 2 mmol/L glutamine, and 25 mmol/L HEPEs.

In vitro exposure to TS. A peristaltic pump-smoke machine (Heinr. Borgwaldt RM1/G, Hamburg, Germany) generated TS-bubbled PBS from mainstream smoke of standard cigarettes (reference 2R1, University of Kentucky, Lexington, KY, USA) through a puffing mechanism mimicking the human smoking pattern (one puff = 2 sec, 15–17 puff/min).

We are grateful to O. Bensaud, Ecole Normale Supérieure, Paris, for critical review and stimulating discussions. M.V.-T. was supported by Electricité de France; S.E.K., by the U.S. Air Force; D.C., by NIH grant CA 74 586; and B.S.P., by INSERM.

Received 9 February 2001; accepted 10 September 2001.

Address correspondence to B.S. Polla, Forever Laser Institut, 46 rue du Rhône, 1204 Geneva, Switzerland. Telephone: 41-79-200-90-36; Fax: 41-22-810-32-82. E-mail: barbara.polla@vtx.ch
35 mL/min (18,19). The smoke of one cigarette corresponds to 10 puffs bubbled in 5 mL of PBS, the final dilutions being expressed as puff/mL of culture medium. We incubated TS-exposed monocytes for 3 hr before analysis.

**Exposure to in vitro γ-radiation.** We performed in vitro γ-radiation at room temperature, in air, using a γ-ray source (137Cs, irradiator IBL637) at a fixed dose rate of 2 Gy/min. We exposed cells to 5 Gy and allowed them to recover for 6, 24, 48, or 72 hr. At indicated time points, we collected radiated and control cells and determined Δψm disruption.

**Detection of Hsp70 levels in human monocytes.** We determined baseline levels of Hsp70 in control human monocytes by flow cytometry analysis (20). Briefly, we fixed cells for Hsp70 analysis for 10 min in paraformaldehyde 3% and then washed and incubated them with 50 µL saponin 0.6%, allowing permeabilization of the cell membrane. We detected intracellular Hsp70 with the anti-human antibody against the cytotoxic inducible Hsp70 at a dilution of 1/100 in PBS/BSA for 10 min before flow cytometry analysis. We performed flow cytometry on 5,000 cells/sample using an EPICS Elite 4,01 software; we express the results as percentage of cells expressing Hsp70 and by mean fluorescence intensity.

**Table 1.** Mean percentage of cells with disrupted Δψm.

| Experiment | Control | 0.03 | 0.06 | 0.12 | 0.18 | 0.24 | 0.3 | r Value |
|------------|---------|------|------|------|------|------|-----|-------|
| 1          | 11.6    | 18.1 | 13.7 | 55.0 | 20.4 | 97.8 | 98.9 | 0.888 |
| 2          | 10.5    | 7.5  | 50.7 | 96.5 | 95.7 | 83.1 | 99.4 | 0.856 |
| 3          | 5.9     | 6.1  | 7.3  | 21.6 | 48.4 | 66.9 | 73.8 | 0.979 |
| 4          | 17.7    | 13.4 | 17.1 | 23.4 | 70.7 | 75.8 | 84.0 | 0.943 |
| 5          | 8.2     | 23.2 | 17.1 | 20.0 | 27.1 | 31.1 | 75.3 | 0.835 |
| 6          | 7.6     | 9.3  | 5.9  | 5.6  | 6.6  | 22.0 | 38.0 | 0.799 |
| 7          | 13.0    | 12.0 | 10.6 | 22.6 | 21.7 | 22.9 | 37.9 | 0.926 |
| 8          | 12.2    | 13.5 | 13.9 | 17.3 | 21.9 | 22.0 | 38.1 | 0.914 |
| 9          | 23.0    | 11.3 | 25.7 | 35.9 | 58.1 | 87.2 | 80.5 | 0.952 |
| 10         | 7.3     | 8.3  | 13.3 | 14.5 | 36.5 | 40.8 | 47.4 | 0.970 |
| **Mean**   | 12.5    | 12.3 | 17.3 | 31.2 | 40.7 | 55.9 | 67.3 |       |
| **SEM**    | 1.7     | 1.8  | 4.0  | 8.4  | 8.8  | 9.9  | 7.9  |       |

Human monocytes from 10 anonymous blood donors were exposed for 3 hr to in vitro increasing concentrations of TS solution, Δψm decreased with increasing concentrations of TS (18). Here we report on our extension of that study to a larger population. Using the same protocol, we studied over 100 donors and achieved the same results: a decrease in Δψm with increasing concentrations of TS. Correlation coefficients ranging from 0.799 to 0.970 indicated a strong linear association between TS concentrations and Δψm. Table 1 shows 10 representative experiments.

**Variability in baseline Δψm: relation to in vivo exposure to TS.** Because we tested the donors anonymously, determining whether the small yet perceptible baseline variation of Δψm resulted from such differences as age, sex, smoking status, or other environmental exposure was not feasible. We therefore performed another set of experiments using monocytes from volunteers with known smoking status. Baseline levels of Δψm were determined in monocytes from eight young and otherwise healthy smokers and seven nonsmokers. Monocytes from smokers (representing in vivo TS exposure) had significantly higher Δψm disruption than those from their nonsmoking counterparts. The mean percentage of cells with Δψm disruption in the smoking population (n = 8) was 13.3 ± 1.3 compared with 7.4 ± 0.9 (n = 7) for the nonsmokers (p < 0.05; Figure 2).

**Effects of in vitro γ-radiation exposure on Δψm in human monocytes.** Δψm susceptibility to oxidants and the scarcity of reliable any optimal biomarker to be used in vivo have to be stable from one individual to another, in human monocytes the percentage of cells with baseline Δψm disruption ranged from 1.4% to 23.0%. The inherent variability observed for Hsp70, measured as percentage of cells expressing Hsp70, varied from 0.7% to 90%, and mean fluorescence intensity varied from 1 to 3.6 (Figure 1).
and reproducible methods for testing radiation exposures motivated our study of the effects of another oxidant-mediated environmental exposure, ionizing radiation. We exposed human monocytes to a single dose of \(\gamma\)-radiation (5 Gy) and studied \(\gamma\)-radiation-induced modifications in \(\Delta\psi_m\) at 6, 24, 48, and 72 hr after radiation exposure (Figure 3, Table 2). Although \(\Delta\psi_m\) did not vary significantly in unexposed cells at each recovered time, significant \(\gamma\)-radiation-induced \(\Delta\psi_m\) disruption was detectable 24 hr after radiation \((p < 0.05)\), whereas cell death evaluated as a control for radiation toxicity was detected after 48 hr \((30\% \pm 7.8\%\) of cell death; \(n = 10\)) and peaked at 72 hr (data not shown).

**Discussion**

In this study, we report low variability of \(\Delta\psi_m\) compared with established biomarkers such as Hsp70, and a high sensitivity of \(\Delta\psi_m\) disruption to oxidant exposure. Both findings favor the use of \(\Delta\psi_m\) as a sensitive and specific probe for oxidative stress.

To maximize its utility, a biomarker must have the ability to respond to a large number of chemical exposures. We thus chose to study exposure to oxidants because of their significance in environmental exposure. They are indeed involved in the toxicity of many products and in the pathogenesis of many diseases \((24–26)\). We chose TS exposure as a model for oxidative stress, first, because it has well-known effects on the health of smokers \((27)\) and, second, because oxidants play a central role in TS-mediated toxicity and carcinogenesis \((18,28–30)\). TS could also represent a prototype for studying the cellular response to other chemicals whose toxicity is enhanced by oxidants.

Collapse of \(\Delta\psi_m\) can occur by several mechanisms independent of oxidant exposure, such as various exposure to toxins or physical damage. However, TS is a complex pollutant \(\text{(it contains} > 3,600 \text{different compounds)}\). Previous studies indicate that TS-mediated \(\Delta\psi_m\) disruption relates to its oxidants content \((18,31,32)\). Furthermore, TS induces other markers of oxidative stress such as heme oxygenase \((22,31,33)\), promotes lipid peroxidation, and decreases glutathione levels, thus providing direct evidence of the oxidative stress induced by TS \((32,34,35)\). The high sensitivity of \(\Delta\psi_m\) to TS exposure and the role of oxidants in TS-mediated toxicity led us to study the use of \(\Delta\psi_m\) as a biomarker for oxidants.

By comparing the baseline levels of \(\Delta\psi_m\) to a well-known biomarker, Hsp70, we showed that the variability of Hsp70 expression as measured by flow cytometry in human monocytes was up to 10 times higher than that of \(\Delta\psi_m\) disruption. The variability in the percentage of cells expressing Hsp70 was approximately 1:130, which is the lower range of what has been described by others using the same technique or other, less sensitive methods \((20,36)\). To be an adequately useful indicator for in vivo exposure, a biomarker has to demonstrate as little variability as possible from one donor to another. Thus, the inherent variability of baseline levels of Hsp70 detected in human cells renders Hsp70 expression difficult to use as a biomarker for in vivo exposures, though it remains a most adequate biomarker for in vitro and ecosystem studies. In contrast, the variability of \(\Delta\psi_m\) was approximately 1:16 among 100 subjects, thus making this parameter potentially more suitable for studying the effects of in vivo exposures.

We have already shown that TS induces Hsp70 expression as well as mitochondrial alterations in several mammalian cells, including normal human monocytes \((18,31)\). \(\Delta\psi_m\) disruption, as an early pre-requisite step toward programmed cell death \((16,17)\), has been detected after 3 hr of exposure to TS in human monocytes, whereas cell death has not been detected before 16 hr of exposure \((18,19,31)\), thereby meeting the criterion of sensitivity with respect to the cell death end point. Moreover, we performed detection of \(\Delta\psi_m\) disruption using the lipophilic cation JC-1, chosen as a specific and sensitive probe for cytometric analysis of \(\Delta\psi_m\) disruption. Indeed, it has been previously shown that JC-1 is a reliable probe for analyzing \(\Delta\psi_m\) changes with flow cytometry, whereas it is not sensitive to the depolarization of plasma membrane \((37)\).

We tested the role of oxidant-specific mechanisms in mediating the effects of TS by pre-exposing cells to the antioxidant N-acetyl-L-cysteine \((\text{NAC})\) for 1 hr before TS exposure. NAC pretreatment abolished TS-mediated \(\Delta\psi_m\) disruption \((18)\). Moreover, hydrogen peroxide \((\text{H}_2\text{O}_2)\), used at concentrations estimated similar to those found in TS, also induced \(\Delta\psi_m\) disruption in a concentration-dependent manner (data not shown). In contrast, nonoxidative compounds of TS such as the carcinogen benzo\[a\]pyrene had no effect on \(\Delta\psi_m\) even when used at toxic concentrations \((> 50 \mu\text{M})\) \((31)\). Based on these data, we suggest that \(\Delta\psi_m\) disruption may be an early, oxidant-specific biomarker.

Interestingly, for those donors with a high baseline percentage of cells with \(\Delta\psi_m\) disruption \((\text{Figure 2}; \text{donors} 2, 4, \text{and} 9)\), initial exposure to TS \((0.03 \text{ pM/mL})\) had no effect on \(\Delta\psi_m\), whereas those with low baseline percentage of cells with \(\Delta\psi_m\) disruption \((\text{donors} 1, 3, 5, 6, 7, 8)\) were sensitive to this low concentration of TS exposure. According to \(\Delta\psi_m\) sensitivity to in vivo TS exposure, we anticipated that the observed differences in baseline \(\Delta\psi_m\) and in the in vivo sensitivity to TS might be the result of voluntary in vivo exposure to TS: cells with relatively high levels of baseline \(\Delta\psi_m\) disruption would be from smokers and cells with relatively low levels of \(\Delta\psi_m\) disruption would be from nonsmokers. By testing donors with known smoking status, we corroborated the hypothesis that in vivo smoking status may influence \(\Delta\psi_m\) because smokers had a significantly higher baseline percentage of cells with \(\Delta\psi_m\) disruption than nonsmokers. The possibility that the observed differences in \(\Delta\psi_m\) of smokers and nonsmokers...
nonsmokers could be related to impaired oxidative metabolism caused by carbon monoxide should be verified. Moreover, additional experiments would test Δψm in larger cohorts of subjects controlled for other environmental susceptibility factors.

Data on environmental exposures to γ-radiation are generally based on estimates of radiation-induced cancer risk derived from studies of atomic bomb survivors, irradiated victims of Chernobyl, or patients irradiated for therapeutic purposes. Data obtained with these high doses are then extrapolated for low-level exposures. The carcinogenic effect of chronic, low-level radiation exposure can be assessed from epidemiologic studies of cancer among workers in the nuclear industry. However, these studies provide only retrospective and uncertain (because of extrapolation) information rather than sensitive and rapidly detectable biomarkers of ionizing radiation. Recent research has focused on the analysis of genomic translations or other chromosome aberrations as biomarkers of radiation exposure (38–40). However, current biomarkers for radiation are still imprecise and insufficient (41). Because ionizing radiation, particularly γ, generates abundant amounts of oxidants as a result of water radiolysis (42), we tested Δψm disruption as a biomarker for in vitro γ-radiation exposure. According to our study, Δψm appears to be a sensitive and early indicator of in vitro radiation exposure, substantiating our other results showing Δψm to be an effective early biomarker of oxidant-mediated exposures. Further studies will test the effects of in vivo γ-radiation exposure.

Future epidemiologic applications of Δψm as a biomarker include gauging health risks associated with in vitro exposures to oxidant-mediated stressors such as radiation and air pollution. Δψm could also be used for in vitro studies as a screening procedure to detect any oxidative toxicity of new industrial compounds.

REFERENCES AND NOTES

1. Mayer FL. Biochemical, physiological and histological markers of anthropogenic stress. In: Biomarkers (Huggett RJ, Kimerle RA, Mehrle PM Jr, Bergman HL, eds). Chelsea, MI:Lewis Publishers, 1992:85–95.

2. Albertini RJ, Nicklas JA, O’Neil JP. Future research directions for evaluating human genetic and cancer risk from environmental exposures. Environ Health Perspect 104(suppl 3):503–510 (1996).

3. Christiani DC. Utilization of biomarker data for clinical and environmental intervention. Environ Health Perspect 104(suppl 4):941–955 (1997).

4. Hammink K, Kumar R, Bykov VJ, Louhelainen J, Vodicka P. Future research directions in the use of biomarkers. Environ Health Perspect 104(suppl 3):459–464 (1997).

5. Ward JB Jr, Henderson RE. Identification of needs in biomarker research. Environ Health Perspect 104:895–900 (1996).

6. Kreps SE, Banzet N, Christiani DC, Polla BS. Molecular biomarkers of early responses to environmental stressors: implications for risk assessment and public health. Rev Environ Health 12:261–290 (1997).

7. Sanders BM, Martin LS. Stress proteins as biomarkers of contaminant exposure in archived environmental samples. Sci Total Environ 129:459–470 (1993).

8. Ryan JA, Hightower LE. Stress proteins as molecular biomarkers for environmental toxicology. In: Stress-Inducible Cellular Responses (Feige U, Marimorto R, Yahara I, Polla BS, eds). Basel: Birkhäuser Verlag, 1996:411–424.

9. Jornot L, Mirault ME, Junod AF. Differential expression of Hsp70 as biomarkers in ecotoxicological studies. Sci Total Environ 93:201–215 (1991).

10. Sanders BM, Martin LS. Stress proteins as biomarkers of contaminant exposure in archived environmental samples. Sci Total Environ 129:459–470 (1993).

11. Crepa SE, Banzet N, Christiani DC, Polla BS. Molecular biomarkers of early responses to environmental stressors: implications for risk assessment and public health. Rev Environ Health 12:261–290 (1997).

12. Sanders BM, Martin LS. Stress proteins as biomarkers of contaminant exposure in archived environmental samples. Sci Total Environ 129:459–470 (1993).

13. Christiani DC. Utilization of biomarker data for clinical and environmental intervention. Environ Health Perspect 104(suppl 4):941–955 (1997).

14. Dunlap DY, Matsumura F. Development of broad spectrum antibodies to heat shock protein 70 as biomarkers for detection of multiple stress by pollutants and environmental factors. Ecotox Environ Saf 27:238–244 (1997).

15. Saraste M. Oxidative phosphorylation at the end of the die. Science 283:1488–1493 (1999).

16. Green DR, Reed JC. Mitochondria and apoptosis. Science 281:1309–1312 (1999).

17. Kroemer G, Petrilli P, Zamzami N, Vayssier-Le, Mignonette B. The biochemistry of programmed cell death. FASEB J 9:1277–1287 (1995).

18. Banzet N, Francais D, Polla BS. Tobacco smoke induces mitochondrial depolarization along with cell death: effects of antioxidants. Redox Rep 4:229–236 (1999).

19. Vayssier M, Banzet N, Francais D, Bellmann K, Polla BS. Tobacco smoke induces both apoptosis and necrosis in mammalian cells: differential effects of Hsp70. Am J Physiol 275:L771–L779 (1998).

20. Bachelet M, Mariethoz E, Banzet N, Souil E, Pinot F, Polla C, Durand P, Bouhaert I, Polla BS. Flow cytometry is a rapid and reliable method for evaluating heat shock protein 70 expression in human monocytes. Cell Stress & Chaper 3:168–176 (1998).

21. Cossarizza A, Bazzocchi-Lucenti M, Kalachnikova G, Franceschi CA. New method for the cytofluorimetric analysis of mitochondrial membrane potential using the J-aggregate forming lipophilic cation 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzimidazol-carboxylic acid iodide (JC-1). Biochem Biophys Res Commun 197:40–45 (1993).

22. Pinot F, El Yagoubi A, Christie P, Dinh-Xuan AT, Polla BS. Induction of stress proteins by tobacco smoke in human monocytes: modulation by antioxidants. Cell Stress & Chaperon 2:156–161 (1997).

23. Polla BS. The heat shock response in human phagocytes. Immunit Lett 30:159–163 (1991).

24. Eiserich JP, Patel RP, O’Donnell VB. Pathophysiology of nitric oxide and related species: free radical reactions and modification of biomolecules. Mol Asp Med 19:221–257 (1998).

25. Hogg N. Free radicals in disease. Semin Reprod Endocrinol 16:241–248 (1998).

26. Li X, Sun AY. Paracado induced activation of transcriptional factor AP-1 and apoptosis in PC12 cells. J Neuroal Transm 106:21–26 (1999).

27. Hanrahan JP, Sherman CB, Brezsnitz EA, Mannino DM. Cigarette smoking and health. Am J Respir Crit Care Med 153:861–865 (1996).

28. Church DF, Priyor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 64:111–126 (1985).

29. Priyor WA. Biological effects of cigarette smoke, wood smoke, and the smoke from plastics: the use of electron spin resonance. Free Radic Biol Med 13:699–776 (1992).

30. Churg A, Cherukupalli K. Cigarette smoke causes rapid lipid peroxidation of rat tracheal epithelium. Int J Exp Pathol 74:127–132 (1993).

31. Vayssier-Taussat M, Camill T, Aron R, Meplian C, Hainaut P, Polla BS, Weksler B. Effects of tobacco smoke and benzo[a]pyrene on human endothelial cells and monocytes stress response. Am J Physiol 280:H1926–H1930 (2001).

32. Bachelet M, Pinot F, Richard MJ, Francais D, Polla BS, Vayssier-Taussat M, Polla BS. Toxicity of cadmium in tobacco smoke: protection by antioxidants and chelating resins. Free Radic Res (in press).

33. Favalet F, Polla BS. Tobacco smoke-inducible human hem oxygenase-1 gene expression: role of distinct transcription factors and reactive oxygen intermediates. Biochem J 363:475–482 (2001).

34. Ayaso M, Hisada T, Suzukiwa M, Yoshida H, Nishikawa M, Ito T, Nakajima K, Hisakgi Y, Yonemura A, Ishikawa T, et al. Plasma levels of redox status of ascorbic acid and levels of lipid peroxidation products in active and passive smokers. Environ Health Perspect 108:105–108 (2000).

Figure 3. Effects of γ-radiation on Δψm disruption of human circulating monocytes: Δψm disruption of irradiated human monocytes (5 Gy) as a function of recovery time (6, 24, 48, and 72 hr). Data are from one representative experiment out of seven, which are listed in Table 2.
35. Gupta MP, Khanduja KL, Sharma RR. Effect of cigarette smoke inhalation on antioxidant enzymes and lipid peroxidation in the rat. Toxicol Lett 41:107–114 (1988).
36. Durand P, Bachelet M, Brunet F, Richard MJ, Dhainaut JF, Dali Ava J, Polla BS. Inducibility of the 70 kD heat shock protein in peripheral blood monocytes is decreased in human acute respiratory distress syndrome and recovers over time. Am J Respir Crit Care Med 161:286–292 (2000).
37. Salvioli S, Ardizzoni A, Franceschi C, Cossarizza A. JC-1, but not DiOC6(3) or rhodamine 123, is a reliable fluorescent probe to assay delta psi changes in intact cells: implications for studies on mitochondrial functionality during apoptosis. FEBS Lett 411:77–82 (1997).
38. Goodhead DT. Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. Int J Radiat Biol 65:7–17 (1994).
39. Goodhead DT. The initial physical damage produced by ionizing radiations. Int J Radiat Biol 56:623–634 (1989).
40. Wilson VL, Taffe BG, Shields PG, Povey AC, Harris CC. Detection and quantification of 8-hydroxydeoxyguanosine adducts in peripheral blood of people exposed to ionizing radiation. Environ Health Perspect 99:261–263 (1993).
41. Cadet J, Douki T, Ravanat J-L. Artifacts associated with measurement of oxidized DNA bases. Environ Health Perspect 105:1034–1039 (1997).
42. Dubner D, Gisone P, Jaitovich I, Perez M. Free radicals production and estimation of oxidative stress related to gamma irradiation. Biol Trace Elem Res 47:265–270 (1995).

Excellence in basic research at the National Institute of Environmental Health Sciences

NIEHS scientists and grantees are performing basic studies of our susceptibility to environment-related disease: demonstrating that a carcinogen in cigarette smoke (benzo(a)pyrene) alters part of a gene to cause lung cancer . . . showing the effects of fetal exposure to PCBs . . . developing a strain of mouse that lacks functional estrogen receptors and that helps evaluate how some pesticides and other estrogen-like compounds might affect development and reproduction . . . discovering the genes for breast, ovarian, and prostate cancers . . . identifying women’s optimal days of fertility . . . seeking to reverse the damage from lead exposure . . . finding alternatives to traditional animal tests . . . pinpointing the functions of specific genes by eliminating them from specially bred mouse lines . . . discovering a way, using ordinary yeast cells, to isolate and clone genes and other fragments of genetic material more quickly . . . showing the effects of urban air on lung function . . .