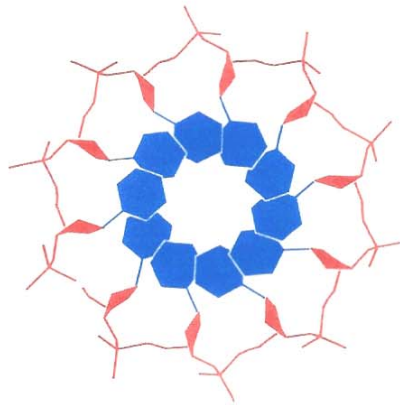


4. Excerpts and Abstracts for Basic Glutathione
References and Summary of Macular Degeneration©

Biochemistry

FOURTH EDITION



Lubert Stryer

STANFORD UNIVERSITY



W. H. Freeman and Company
New York

GLUTATHIONE, A γ -GLUTAMYL PEPTIDE, SERVES AS A SULFHYDRYL BUFFER AND AN ANTIOXIDANT

Glutathione, a tripeptide containing a sulfhydryl group, is a highly distinctive amino acid derivative with several important roles. For example, glutathione protects red cells from oxidative damage (p. 568). The first step in the synthesis of glutathione is the formation of a peptide linkage between the γ -carboxyl group of glutamate and the amino group of cysteine, in a reaction catalyzed by γ -glutamylcysteine synthetase (Figure 28-24). Formation of this peptide bond requires activation of the γ -carboxyl

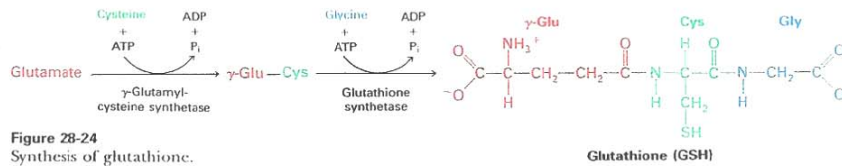


Figure 28-24
 Synthesis of glutathione.

group, which is achieved by ATP. The resulting acyl-phosphate intermediate is then attacked by the amino group of cysteine. This reaction is feedback-inhibited by glutathione. In the second step, which is catalyzed by *glutathione synthetase*, ATP activates the carboxyl group of cysteine to enable it to condense with the amino group of glycine.

Glutathione, present at high levels (~5 mM) in animal cells, serves as a sulfhydryl buffer. It cycles between a reduced thiol form (GSH) and an oxidized form (GSSG) in which two tripeptides are linked by a disulfide bond. GSSG is reduced to GSH by glutathione reductase, a flavoprotein that uses NADPH as the electron source (p. 568). The ratio of GSH to GSSG in most cells is greater than 500.

Glutathione plays a key role in detoxification by reacting with hydrogen peroxide and organic peroxides, the harmful byproducts of aerobic life.



Glutathione peroxidase, the enzyme catalyzing this reaction, is remarkable in having a covalently attached *selenium* (Se) atom. Its active site contains the selenium analog of cysteine, in which Se has replaced S (Figure 28-25). The selenolate (E-Se^-) form of this residue reduces the peroxide substrate to an alcohol and is in turn oxidized to selenenic acid (E-SeOH) (Figure 28-26). Glutathione now comes into action by forming a selenosulfide adduct (E-Se-S-G). A second glutathione then regenerates the active form of the enzyme by attacking the selenosulfide to form oxidized glutathione.

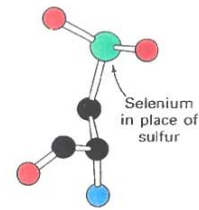
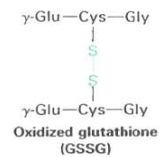


Figure 28-25
 Model of the selenocysteine residue in the active site of glutathione peroxidase. The selenium atom is shown in green. The crystal contains the fully oxidized seleninate form of this modified amino acid. [Drawn from 1gp1.pdb. O. Epp, R. Ladenstein, and A. Wendel. *Eur. J. Biochem.* 133(1983):51.]

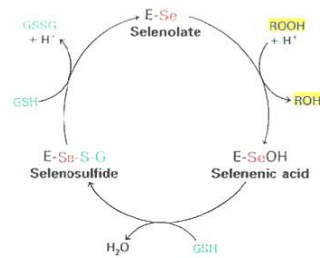


Figure 28-26
 Proposed catalytic mechanism of glutathione peroxidase. [Based on O. Epp, R. Ladenstein, and A. Wendel. *Eur. J. Biochem.* 133(1983):51.]

**GLUTATHIONE REDUCTASE TRANSFERS ELECTRONS FROM
NADPH TO OXIDIZED GLUTATHIONE THROUGH FAD**

The regeneration of reduced glutathione is catalyzed by *glutathione reductase*, a dimer of 50-kd subunits. The electrons from NADPH are not directly transferred to the disulfide bond in oxidized glutathione. Rather, they are transferred from NADPH to a tightly bound flavin adenine dinucleotide (FAD), then to a disulfide bridge between two cysteine residues in the subunit, and finally to oxidized glutathione. Each subunit consists of three structural domains: an FAD-binding domain, an NADP⁺-binding domain, and an interface domain (Figure 22-5). The FAD domain and NADP⁺ domain resemble each other and are similar to nucleotide-binding domains in other dehydrogenases. FAD and NADP⁺ are bound in an extended form, with their isoalloxazine and nicotinamide rings next to each other. It is interesting to note that the binding site for oxidized glutathione is formed by the FAD domain of one subunit and the interface domain of the other subunit.

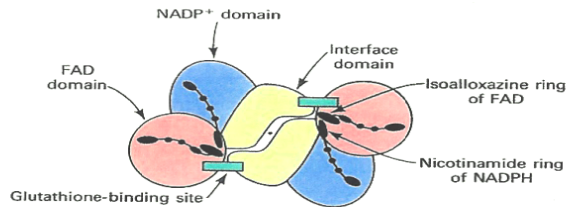


Figure 22-5
Schematic diagram of the domain structure of glutathione reductase. Each subunit in this dimeric enzyme consists of an NADP⁺ domain, an FAD domain, and an interface domain. Glutathione is bound to the FAD domain of one subunit and the interface domain of another. [After G.E. Schultz, R.H. Schirmer, W. Sachsenheimer, and E.F. Pai. *Nature* 273(1978):123.]

NADPH

↓

FAD

↓

Cys₄₆-S

↓

Cys₄₁-S

↓

G-S

↓

G-S

Oxidant-sensitive transcription factor and cyclooxygenase-2 by Helicobacter pylori stimulation in human gastric cancer cells.

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Helicobacter pylori (*H. pylori*) infection might activate nuclear factor-kappaB (NF-kappaB), an oxidant-sensitive transcription regulator of inducible expression of inflammatory genes such as cyclooxygenase-2 (COX-2). We studied the role of NF-kappaB on expression of COX-2 in *H. pylori*-stimulated gastric cancer cell lines by using antioxidants, glutathione (GSH), and N-acetylcysteine (NAC) as well as an NF-kappaB inhibitor, pyrrolidine dithiocarbamate (PDTC). Gastric adenocarcinoma cell lines derived from Caucasian (AGS) cells and Korean (SNU-484) cells were used to study the role of NF-kappaB on COX-2 expression by *H. pylori*. They were treated with GSH, NAC, or PDTC in the presence of *H. pylori*. mRNA expression and protein level for COX-2 were determined by Northern blot and RT-PCR analysis as well as Western blot analysis. NF-kappaB activation was examined by electrophoretic mobility shift assay. As a result, *H. pylori* induced a time-dependent expression of mRNA and protein for COX-2 via activation of NF-kappaB, which was inhibited by GSH, NAC, and PDTC in the cells. In conclusion, oxidant-sensitive transcription factor NF-kappaB may play a novel role in expression of COX-2 by *H. pylori* stimulation in gastric cancer cells.

PMID: 12086398 [PubMed - indexed for MEDLINE]

In vivo antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation.

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We hypothesized that endotoxin injection in rats would stimulate in vivo nuclear factor-kappa B (NF-kappa B) activation in lung tissue and that antioxidant treatment before endotoxin injection would attenuate endotoxin-induced NF-kappa B activation, chemokine gene expression, and neutrophilic lung inflammation. We studied NF-kappa B activation in rat lung tissue following a single i.p. injection of endotoxin (6 mg/kg). After in vivo endotoxin treatment, lung NF-kappa B activation peaked at 2 h and temporally correlated with the expression of cytokine-induced neutrophil chemoattractant mRNA in lung tissue. Treatment with the antioxidant N-acetylcysteine (NAC) 1 h before endotoxin resulted in decreased lung NF-kappa B activation in a dose-dependent manner (from 200-1000 mg/kg) and diminished cytokine-induced neutrophil chemoattractant mRNA expression in lung tissue. Treatment with NAC significantly suppressed endotoxin-induced neutrophilic alveolitis. The average total lung lavage neutrophil count was 5.5×10^6 with endotoxin treatment vs 0.9×10^6 with NAC treatment before endotoxin. The NF-kappa B pathway represents an attractive therapeutic target for strategies to control neutrophilic inflammation and lung injury.

PMID: 8759749 [PubMed - indexed for MEDLINE]

Identification of NF-kappaB-dependent gene networks in respiratory syncytial virus-infected cells.

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Respiratory syncytial virus (RSV) is a mucosa-restricted virus that is a leading cause of epidemic respiratory tract infections in children. In epithelial cells, RSV replication activates nuclear translocation of the inducible transcription factor nuclear factor kappaB (NF-kappaB) through proteolysis of its cytoplasmic inhibitor, IkappaB. In spite of a putative role in mediating virus-inducible gene expression, the spectrum of NF-kappaB-dependent genes induced by RSV infection has not yet been determined. To address this, we developed a tightly regulated cell system expressing a nondegradable, epitope-tagged IkappaBalpha isoform (Flag-IkappaBalpha Mut) whose expression could be controlled by exogenous addition of nontoxic concentrations of doxycycline. Flag-IkappaBalpha Mut expression potently inhibited IkappaBalpha proteolysis, NF-kappaB binding, and NF-kappaB-dependent gene transcription in cells stimulated with the prototypical NF-kappaB-activating cytokine tumor necrosis factor alpha (TNF-alpha) and in response to RSV infection. High-density oligonucleotide microarrays were then used to profile constitutive and RSV-induced gene expression in the absence or presence of Flag-IkappaBalpha Mut. Comparison of these profiles revealed 380 genes whose expression was significantly changed by the dominant-negative NF-kappaB. Of these, 236 genes were constitutive (not RSV regulated), and surprisingly, only 144 genes were RSV regulated, representing numerically approximately 10% of the total population of RSV-inducible genes at this time point. Hierarchical clustering of the 144 RSV- and Flag-IkappaBalpha Mut-regulated genes identified two discrete gene clusters. The first group had high constitutive expression, and its expression levels fell in response to RSV infection. In this group, constitutive mRNA expression was increased by Flag-IkappaBalpha Mut expression, and the RSV-induced decrease in expression was partly inhibited. In the second group, constitutive expression was very low (or undetectable) and, after RSV infection, expression levels strongly increased. In this group, NF-kappaB was required for RSV-inducible expression because Flag-IkappaBalpha Mut expression blocked their induction by RSV. This latter cluster includes chemokines, transcriptional regulators, intracellular proteins regulating translation and proteolysis, and secreted proteins (complement components and growth factor regulators). These data suggest that NF-kappaB action induces global cellular responses after viral infection.

PMID: 12050393 [PubMed - indexed for MEDLINE]

Oxidative stress and TNF-alpha induce histone acetylation and NF-kappaB/AP-1 activation in alveolar epithelial cells: potential mechanism in gene transcription in lung inflammation.

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Oxidants and inflammatory mediators such as tumour necrosis factor-alpha (TNF-alpha) activate nuclear factor kappa B (NF-kappaB) and activator protein-1 (AP-1) transcription factors, and enhance the expression of both pro-inflammatory and protective antioxidant genes. Remodelling of chromatin within the nucleus, controlled by the degree of acetylation/deacetylation of histone residues on the histone core around which DNA is coiled, is important in allowing access for transcription factor DNA binding and hence gene transcription. Unwinding of DNA is important in allowing access for transcription factor DNA binding and hence gene transcription. Nuclear histone acetylation is a reversible process, and is regulated by a group of acetyltransferases (HATs) which promote acetylation, and deacetylases (HDACs) which promote deacetylation. The aim of this study was to determine whether oxidative stress and the pro-inflammatory mediator, TNF-alpha, altered histone acetylation/deacetylation and the activation of NF-kappaB and AP-1, leading to the release of the pro-inflammatory cytokine IL-8 in human alveolar epithelial cells (A549). Hydrogen peroxide (H₂O₂) (100 microM) and TNF-alpha (10 ng/ml) imposed oxidative stress in A549 cells as shown by depletion of the antioxidant reduced glutathione (GSH) concomitant with increased levels of oxidised glutathione (GSSG). Treatment of A549 cells with H₂O₂, TNF-alpha and the HDAC inhibitor, trichostatin A, TSA (100 ng/ml) significantly increased acetylation of histone proteins shown by immunostaining of cells and increased HAT activity, compared to the untreated cells. H₂O₂, and TNF-a, and TSA all increased NF-kappaB and AP-1 DNA binding to their consensus sites assessed by the electrophoretic mobility shift assay. TSA treatment potentiated the increased AP-1 and NF-KB binding, produced by H₂O₂ or TNF-alpha treatments in A549 cells. Both H₂O₂ and TNF-alpha significantly increased IL-8 release, which was further enhanced by pre-treatment of A549 cells with TSA compared to the individual treatments. This study shows that the oxidant H₂O₂ and the pro-inflammatory mediator, TNF-a induce histone acetylation which is associated with decreased GSH levels and increased AP-1 and NF-kappaB activation leading to enhanced proinflammatory IL-8 release in alveolar epithelial cells. This indicates a mechanism for the pro-inflammatory effects of oxidative stress.

PMID: 12162440 [PubMed - indexed for MEDLINE]

Thiol regulation of endotoxin-induced release of tumour necrosis factor alpha from isolated rat Kupffer cells.

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Proinflammatory cytokines released by hepatic macrophages (Kupffer cells) have a central role in the pathogenesis of liver injury and the cardiovascular abnormalities of sepsis. Because cytokine release is controlled primarily at the level of gene expression, intracellular signalling mechanisms that control the transcription of cytokine genes are critical links to organ injury. Oxidant stress up-regulates and antioxidants down-regulate the pleiotropic transcription factor NF-kappa B, a DNA-binding protein that induces the expression of cytokines and vascular adhesion molecules. Thiol-bearing molecules are also important inhibitors of NF-kappa B activation, but whether this inhibition represents an antioxidant effect is unknown. This study was undertaken to determine whether important endogenous and pharmacological thiols modulate the activation of NF-kappa B and the release of tumour necrosis factor alpha (TNF-alpha) from Kupffer cells and to ascertain whether these effects are mediated through glutathione. Exposure of rat Kupffer cells to a physiologically relevant concentration of lipopolysaccharide (10 ng/ml) activated NF-kappa B within 1 h and induced the release of TNF-alpha over 5 h. Cellular glutathione content remained unchanged after lipopolysaccharide exposure, but both glutathione monoethyl ester and N-acetyl-L-cysteine increased cellular glutathione levels, blocked NF-kappa B activation and inhibited the release of TNF-alpha. Inhibition of glutathione synthesis prevented the NAC-induced increase in Kupffer cell glutathione, yet it did not prevent the inhibition of TNF-alpha release by NAC. Thus the inhibition of NF-kappa B activation by pharmacological thiols such as NAC might reflect a more general role of the intracellular thiol redox status in NF-kappa B regulation rather than the antioxidant properties of these agents.

PMID: 9003392 [PubMed - indexed for MEDLINE]

J Biol Chem 2000 Feb 4;275(5):3693-8

Molecular mechanism of decreased glutathione content in human immunodeficiency virus type 1 Tat-transgenic mice.

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Human immunodeficiency virus (HIV) progressively depletes GSH content in humans. Although the accumulated evidence suggests a role of decreased GSH in the pathogenesis of HIV, significant controversy remains concerning the mechanism of GSH depletion, especially in regard to envisioning appropriate therapeutic strategies to help compensate for such decreased antioxidant capacity. Tat, a transactivator encoded by HIV, is sufficient to cause GSH depletion in vitro and is implicated in AIDS-associated Kaposi's sarcoma and B cell lymphoma. In this study, we report a decrease in GSH biosynthesis with Tat, using HIV-1 Tat transgenic (Tat⁺) mice. A significant decline in the total intracellular GSH content in liver and erythrocytes of Tat⁺ mice was accompanied by decreased gamma-glutamylcysteine synthetase regulatory subunit mRNA and protein content, which resulted in an increased sensitivity of gamma-glutamylcysteine synthetase to feedback inhibition by GSH. Further study revealed a significant reduction in the activity of GSH synthetase in liver of Tat⁺ mice, which was linearly associated with their GSH content. Therefore, Tat appears to decrease GSH in vivo, at least partially, through modulation of GSH biosynthetic enzymes.

PMID: 10652368 [PubMed - indexed for MEDLINE]

Cysteine metabolism and whole blood glutathione synthesis in septic pediatric patients.

Lyons J, Rauh-Pfeiffer A, Ming-Yu Y, Lu XM, Zurakowski D, Curley M, Collier S, Duggan C, Nurko S, Thompson J, Ajami A, Borgonha S, Young VR, Castillo L.

Department of Anesthesia, Children's Hospital, Boston, MA, USA.

OBJECTIVE: To investigate whole body in vivo cysteine kinetics and its relationship to whole blood glutathione (GSH) synthesis rates in septic, critically ill pediatric patients and controls. **DESIGN:** Prospective cohort study. **SETTING:** Multidisciplinary intensive care unit and pediatric inpatient units at a children's hospital. **PATIENTS:** Ten septic pediatric patients and ten controls (children admitted to the hospital for elective surgery). **INTERVENTIONS:** Septic patients (age, 31 months to 17 yrs) and controls (age, 24 months to 21 yrs) received a 6-hr primed, constant, intravenous tracer infusion of l-[1-¹³C]cysteine. Blood samples were obtained to determine isotopic enrichment of plasma cysteine and whole blood [1-¹³C]cysteinyl-glutathione by gas-chromatography mass spectrometric techniques. The plasma flux and oxidation rate of cysteine and the fractional and absolute synthesis rates of GSH were determined. Septic patients received variable protein and energy intake, as per routine clinical management, and controls were studied in the early postabsorptive state. **MEASUREMENTS AND MAIN RESULTS:** Plasma cysteine fluxes were increased in the septic patients when compared with the controls (68.2 +/- 17.5 [sd] vs. 48.7 +/- 8.8 micromol x kg(-1) x hr(-1); p <.01), and the fraction of plasma cysteine flux associated with oxidative disposal was similar among the groups. The absolute rates of GSH synthesis in whole blood were decreased (p <.01) in the septic patients (368 +/- 156 vs. 909 +/- 272 micromol x L(-1) x day(-1)). The concentration of whole blood GSH also was decreased in the septic group (665.4 +/- 194 vs. 1059 +/- 334 microM; p <.01) **CONCLUSIONS:** Whole blood glutathione synthesis rates are decreased, by about 60%, in critically ill septic children receiving limited nutritional support. Plasma cysteine fluxes and concentration of cysteine were increased in the septic patients, suggesting a hypermetabolic state with increased protein breakdown. The mechanisms whereby GSH synthesis rates are decreased in these patients are probably multifactorial, presumably involving an inflammatory response in the presence of limited nutritional support. The role of nutritional modulation and the use of cysteine prodrugs in maintaining GSH concentration and synthesis remain to be established.

PMID: 11373484 [PubMed - indexed for MEDLINE]

Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses.

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Oxidative stress is a component of diseases and degenerative processes associated with aging. However, no means are available to assess causative oxidative events separately from decline in function of protective antioxidant systems. Previous studies show that ongoing oxidative processes maintain plasma cysteine/cystine redox at a value that is more oxidized than the antioxidant glutathione/glutathione disulfide (GSH/GSSG) system, suggesting that redox analysis of these plasma thiols could allow separate evaluation of an increase in oxidative events from a decline in antioxidant function. The present study uses measurement of cysteine/cystine and GSH/GSSG redox in plasma of 122 healthy individuals aged 19-85 years to determine whether thiol-disulfide redox changes occur with age. The results show a linear oxidation of cysteine/cystine redox state with age at a rate of 0.16 mV/year over the entire age span. In contrast, GSH/GSSG redox was not oxidized prior to 45 years and subsequently was oxidized at a nearly linear rate of 0.7 mV/year. These data suggest that there is a continuous, linear increase in oxidative events throughout adult life but that the capacity of the GSH antioxidant system is maintained until 45 years and then declines rapidly. The data further suggest that redox states of cysteine/cystine and GSH/GSSG provide an approach to clinically distinguish between increased causative oxidative events and decreased GSH antioxidant function. In principle, such analyses can be used to assess efficacy of intervention strategies against oxidative stress prior to or early after onset of clinical symptoms in aging and age-related disease.

PMID: 12398937 [PubMed - in process]

Kidney Int. 2002 Feb;61(2):599-608.

Glucose-induced oxidative stress in mesangial cells.

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BACKGROUND: Hyperglycemia is a well-recognized pathogenic factor of long-term complications in diabetes mellitus. Hyperglycemia not only generates reactive oxygen species but also attenuates antioxidant mechanisms creating a state of oxidative stress. **METHODS:** Porcine mesangial cells were cultured in high glucose (HG) for ten days to investigate the effects on the antioxidant defenses of the cell. **RESULTS:** Mesangial cells cultured in HG conditions had significantly reduced levels of glutathione (GSH) compared with those grown in normal glucose (NG). The reduced GSH levels were accompanied by decreased gene expression of both subunits of gamma-glutamylcysteine synthetase (gamma-GCS), the rate-limiting enzyme in de novo synthesis of GSH. Elevated levels of intracellular malondialdehyde (MDA) were found in cells exposed to HG conditions. HG also caused elevated mRNA levels of the antioxidant enzymes CuZn superoxide dismutase (SOD) and MnSOD. These changes were accompanied by increased mRNA levels of extracellular matrix proteins (ECM), fibronectin (FN) and collagen IV (CIV). Addition of antioxidants to high glucose caused a significant reversal of FN and CIV gene expression; alpha-lipoic acid also up-regulated gamma-GCS gene expression and restored intracellular GSH and MDA levels. **CONCLUSIONS:** The results demonstrate the existence of glucose-induced oxidative stress in mesangial cells as evidenced by elevated MDA and decreased GSH levels. The decreased levels of GSH are as a result of decreased mRNA expression of gamma-GCS within the cell. Antioxidants caused a significant reversal of FN and CIV gene expression, suggesting an etiological link between oxidative stress and increased ECM protein synthesis.

PMID: 11849402 [PubMed - indexed for MEDLINE]

Negative association between erythrocyte reduced glutathione concentration and diabetic complications.

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1. Multiple logistic regression analysis of biochemical and clinical variables in diabetic patients was performed to identify those associated with the presence of diabetic complications (retinopathy, neuropathy and nephropathy). 2. The presence of diabetic complications correlated positively with duration of diabetes and patients age and negatively with the concentration of reduced glutathione in erythrocytes. Individually, retinopathy, neuropathy and nephropathy correlated with duration of diabetes, but retinopathy also correlated positively with haemoglobin A1C in diabetic patients. In insulin-dependent patients, the concentration of methylglyoxal was also in the logistic model for retinopathy and diabetic complications, but the logistic regression coefficient was not significant. 3. Multiple linear regression analysis indicated that erythrocyte reduced glutathione concentration correlated negatively with D-lactate concentration and positively with duration of diabetes in insulin-dependent patients and correlated negatively with glucose concentration in non-insulin-dependent diabetic patients. 4. In non-diabetic subjects, erythrocyte glyoxalase I activity correlated positively with methylglyoxal concentration. There was no similar correlation in diabetic patients. In insulin-dependent patients, methylglyoxal concentration correlated positively with duration of diabetes. 5. Glyoxal and methylglyoxal are detoxified by the glyoxalase system with reduced glutathione as co-factor. The concentration of reduced glutathione may be decreased by oxidative stress and by decreased in situ glutathione reductase activity in diabetes mellitus. A reduced concentration of reduced glutathione may predispose diabetic patients to oxidative damage and to alpha-oxoaldehydemediated glycation by decreasing the in situ glyoxalase I activity. Recent studies of vascular endothelial cells in vitro have suggested that alpha-oxoaldehydes detoxified by glyoxalase I are the major precursors of advanced glycation end products implicated in the development of diabetic complications. The role of these factors in the development of diabetic complications and the prospective prevention of diabetic complications by supplementation of reduced glutathione and/or alpha-oxoaldehyde-scavenging agents now deserve investigation.

PMID: 8942396 [PubMed - indexed for MEDLINE]

The effect of diesel exhaust particles (DEP) and carbon black (CB) on thiol changes in pulmonary ovalbumin allergic sensitized Brown Norway rats.

Al-Humadi NH, Siegel PD, Lewis DM, Barger MW, Ma JY, Weissman DN, Ma JK.

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Brown Norway rats were exposed by intratracheal instillation of saline, carbon black (CB), or diesel exhaust particles (DEP) (5 mg/kg) on day 1, followed by exposure to ovalbumin (OVA, 90 mg/m³) or saline for 30 minutes on days 1, 8, 15, and 29. Animals were sacrificed on day 30. The DEP, CB, or OVA exposure alone did not result in abnormal levels of inflammatory cells, lactate dehydrogenase (LDH), or total protein in the lavage fluid. In combined OVA-DEP or OVA-CB exposure, however, these markers were significantly increased. The adjuvant effect of CB and DEP on OVA sensitization was evidenced by the marked increases in serum OVA-specific IgG (5.6-fold) and IgE (3.5-4 fold) levels, and the increase in interleukin-4 (IL-4) mRNA levels in lung tissue. The OVA exposure markedly reduced glutathione (GSH) levels in both cell types. In combined DEP-OVA exposure, the level of GSH in lymphocytes was further decreased, indicating a possible interactive effect between DEP and OVA exposures. These results show that both DEP and CB augmented OVA-induced allergic sensitization, and that particle composition of DEP may not be a critical factor for the adjuvant effect. OVA exposure causes significant depletion of intracellular GSH in lymphocytes, which may play a key role in OVA-mediated immune responses.

PMID: 12097228 [PubMed - indexed for MEDLINE]

Proc Natl Acad Sci U S A. 1998 Mar 17;95(6):3071-6.

Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns.

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Current thinking attributes the balance between T helper 1 (Th1) and Th2 cytokine response patterns in immune responses to the nature of the antigen, the genetic composition of the host, and the cytokines involved in the early interaction between T cells and antigen-presenting cells. Here we introduce glutathione, a tripeptide that regulates intracellular redox and other aspects of cell physiology, as a key regulatory element in this process. By using three different methods to deplete glutathione from T cell receptor transgenic and conventional mice and studying in vivo and/or in vitro responses to three distinct antigens, we show that glutathione levels in antigen-presenting cells determine whether Th1 or Th2 response patterns predominate. These findings present new insights into immune response alterations in HIV and other diseases. Further, they potentially offer an explanation for the well known differences in immune responses in "Th1" and "Th2" mouse strains.

PMID: 9501217 [PubMed - indexed for MEDLINE]

Cell and molecular biology of chemical allergy.

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OBJECTIVE: The objective of this review is to provide current approaches to gain increased understanding of the molecular basis of chemical allergenicity. Chemical allergy refers to an allergic reaction to a low molecular weight agent (ie, <1 kD). The symptoms and pathology of chemical asthma resemble those of allergy to larger sized agents, such as pollens, weeds, and danders. The differences relate to mechanisms of disease. To stimulate an immune response, low molecular weight chemicals function as haptens and bind to carrier macromolecules. This article focuses on the chemical reactions and physicochemical characteristics of chemical allergens. **DATA SOURCES:** Data were obtained from published clinical reports and from the Documentation of Threshold Limit Values (1998) published by the American Congress of Governmental Industrial Hygienists. **RESULTS:** In vitro studies indicate the stoichiometric reaction of some chemical allergens with glutathione and the subsequent transfer of the allergen from glutathione to other nucleophiles. Computer-generated structure-activity relationship models have been developed for chemicals that induce respiratory allergy. The models, based on physicochemical properties of the agents, have high sensitivity and specificity. **CONCLUSIONS:** The structure-activity relationship model suggests that chemical binding is the essential feature of chemical allergens. Their in vivo reactions with thiols may result in glutathione deficiency with consequent alteration in cellular reduction-oxidation (redox) status, release of cytokines, and promotion of the T helper cell 2 phenotype. Prevention of permanent disease is dependent on periodic medical surveillance of affected workers. When detected early, the disease can frequently be reversed.

Publication Types:

- Review
- Review, Tutorial

PMID: 11770680 [PubMed - indexed for MEDLINE]

Plasma membrane and mitochondrial transport of hepatic reduced glutathione.

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The tripeptide glutathione (GSH) is a key nonprotein thiol that plays multiple critical functional and regulatory roles in cells. Hepatic transport of GSH is a key process in the interorgan homeostasis of GSH. Hepatocellular GSH is available to other extrahepatic organs by its release into blood and bile through the sinusoidal and canalicular GSH carriers, respectively. Their characterization at the molecular level has been recently accomplished using the functional expression cloning strategy utilizing *Xenopus laevis* oocytes microinjected with the corresponding cRNA from the sinusoidal (RsGshT) and canalicular (RcGshT) clones previously isolated and identified from cDNA libraries constructed from hepatic size-fraction mRNAs expressing separately the sinusoidal and canalicular GSH transporters. These clones of 2.8 and 4.0 kb encode for proteins of 39.9 and 95.8 kD for RsGshT and RcGshT, respectively, with 3 to 5 and 6 to 10 putative membrane-spanning domains. Their tissue distribution reveals that RsGshT is exclusively found in liver, contrasting with the distribution of RcGshT, which is found in nearly all tissues examined. Cellular GSH is also found in the mitochondrial matrix at a concentration similar to that in cytosol. However, mitochondria do not synthesize their own GSH, which originates from the operation of a transport carrier localized within the inner mitochondrial membrane. Its role is critical in maintaining a functionally competent organelle and in cell viability. Expression studies in *Xenopus* oocytes have allowed the identification of the hepatic mitochondrial GSH carrier (RmGshT), which displays distinct functional features from both RsGshT and RcGshT, such as ATP stimulation and inhibitor specificity, suggesting that RmGshT is encoded by a gene distinct from that of the plasma membrane GSH carriers.

Publication Types:

- Review
- Review, Tutorial

PMID: 8781020 [PubMed - indexed for MEDLINE]

Role of two recently cloned rat liver GSH transporters in the ubiquitous transport of GSH in mammalian cells.

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Recently our laboratory has cloned both the rat canalicular and sinusoidal GSH transporters (RcGshT and RsGshT, respectively; Yi, J., S. Lu, J. Fernandez-Checa, and N. Kaplowitz. 1994. *J. Clin. Invest.* 93:1841-1845; and 1995. *Proc. Natl. Acad. Sci. USA.* 92:1495-1499). The current work characterized GSH transport and the expression of these two GSH transporters in various mammalian cell lines. The average cell GSH levels (nmol/10(6) cells) were 25, 22, 32, 13, and 13 in HepG2, HeLa, CaCo-2, MDCK, and Cos-1 cells, respectively. GSH efflux was temperature dependent and averaged 0.018, 0.018, 0.012, 0.007, and 0.019 nmol/10(6) cells/min from HepG2, HeLa, CaCo-2, MDCK, and Cos-1 cells, respectively. Dithiothreitol (DTT), which stimulates rat sinusoidal GSH efflux, stimulated GSH efflux only in HepG2 and HeLa cells which was partially reversed by subsequent cystine treatment. GSH uptake (1 mM plus 35S-GSH) was temperature dependent, linear up to 45 min, and Na⁺-independent with average rates of 1.12, 0.91, 0.45, and 0.45 nmol/10(6) cells/30 min for HepG2, HeLa, CaCo-2, MDCK, and Cos-1 cells, respectively. BSP-GSH (2mM), which cis-inhibits sinusoidal GSH uptake in rat liver and HepG2 cells, inhibited GSH uptake only in HeLa cells. mRNA and polypeptide of RcGshT are expressed in all cells whereas those of RsGshT are expressed only in HepG2 and HeLa cells. In conclusion, bidirectional GSH transport, mediated by the "canalicular" GSH transporter, is ubiquitous in mammalian cells. Sinusoidal GSH transporter expression is more restricted, being present in HepG2 and HeLa cells. DTT and BSP-GSH affect GSH transport only in cells expressing the sinusoidal transporter confirming their selective action on this transporter.

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High-affinity transport of glutathione is part of a multicomponent system essential for mitochondrial function.

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Glutathione, an essential cellular antioxidant required for mitochondrial function, is not synthesized by mitochondria but is imported from the cytosol. Rat liver mitochondria have a multicomponent system that underlies the remarkable ability of mitochondria to take up and retain glutathione. At external glutathione levels of less than 1 mM, glutathione is transported into the mitochondrial matrix by a high-affinity component (K_m , approximately 60 μ M; V_{max} , approximately 0.5 nmol/min per mg of protein), which is saturated at levels of 1-2 mM and stimulated by ATP. Another component has lower affinity (K_m , approximately 5.4 mM; V_{max} , approximately 5.9 nmol/min per mg of protein) and is stimulated by ATP and ADP. Both components are inhibited by carbonylcyanide p-(trifluoromethoxy)phenylhydrazone (FCCP), glutamate, and ophthalmic acid. Increase of extramitochondrial glutathione promotes uptake and exchange; the intermembranous space seems to function as a recovery zone that promotes efficient recycling of matrix glutathione. The findings are in accord with *in vivo* data showing that (i) rapid exchange occurs between mitochondrial and cytosolic glutathione, (ii) lowering of cytosolic glutathione levels (produced by administration of buthionine sulfoximine) decreases export of glutathione from mitochondria to cytosol, and (iii) administration of glutathione esters increases glutathione levels in mitochondria more than those in the cytosol.

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GSH transport in mitochondria: defense against TNF-induced oxidative stress and alcohol-induced defect.

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Mitochondria generate reactive oxygen species (ROS) as byproducts of molecular oxygen consumption in the electron transport chain. Most cellular oxygen is consumed in the cytochrome-c oxidase complex of the respiratory chain, which does not generate reactive species. The ubiquinone pool of complex III of respiration is the major site within the respiratory chain that generates superoxide anion as a result of a single electron transfer to molecular oxygen. Superoxide anion and hydrogen peroxide, derived from the former by superoxide dismutase, are precursor of hydroxyl radical through the participation of transition metals. Glutathione (GSH) in mitochondria is the only defense available to metabolize hydrogen peroxide. A small fraction of the total cellular GSH pool is sequestered in mitochondria by the action of a carrier that transports GSH from the cytosol to the mitochondrial matrix. Mitochondria are not only one of the main cellular sources of ROS, they also are a key target of ROS. Mitochondria are subcellular targets of cytokines, especially tumor necrosis factor (TNF); depletion of GSH in this organelle renders the cell more susceptible to oxidative stress originating in mitochondria. Ceramide generated during TNF signaling leads to increased production of ROS in mitochondria. Chronic ethanol-fed hepatocytes are selectively depleted of GSH in mitochondria due to a defective operation of the carrier responsible for transport of GSH from the cytosol into the mitochondrial matrix. Under these conditions, limitation of the mitochondrial GSH pool represents a critical contributory factor that sensitizes alcoholic hepatocytes to the prooxidant effects of cytokines and prooxidants generated by oxidative metabolism of ethanol. S-adenosyl-L-methionine prevents development of the ethanol-induced defect. The mitochondrial GSH carrier has been functionally expressed in *Xenopus laevis* oocytes microinjected with mRNA from rat liver. This critical carrier displays functional characteristics distinct from other plasma membrane GSH carriers, such as its ATP dependency, inhibitor specificity, and the size class of mRNA that encode the corresponding carrier, suggesting that the mitochondrial carrier of GSH is a gene product distinct from the plasma membrane transporters.

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Pigment-Epithelium–Derived Factor — A Key Coordinator of Retinal Neuronal and Vascular Functions

Precise regulation of vascular development is critical in the eye, where a few microns' deviation can cause blood vessels to move into normally avascular areas such as the cornea, vitreous, and fovea, thereby diminishing vision. Ample vascular supply is essential in the retina, which is one of the most metabolically active tissues in the body. The retina, which consists of cell layers that are less than 1 mm thick, is nourished by two vascular beds: the choriocapillaris in the posterior region and the retinal vasculature in the region beneath the vitreous. Separating these two vascular beds are Bruch's membrane and retinal pigment epithelial cells, which are critical for vision and the regeneration of photoreceptors ([Figure 1](#)). Interestingly, the avascular zones of the retina, such as the macular region, which includes the fovea, are much thinner than the rest of the retina and can receive nourishment by diffusion from the surrounding vasculature and choriocapillaris. Tissues like the cornea and vitreous do not require a vascular supply, since they contain few cells within the stroma and are not very metabolically active. For all these reasons, it is likely that a host of angiogenic and antiangiogenic factors in the retina coordinate vascular flow and regeneration with the corresponding metabolic requirements of the retina.

Multiple growth factors in the eye, including insulin-like growth factors, epidermal growth factors, and fibroblast growth factors, have been postulated to have a role in ocular diseases.¹ However, these growth factors are present in the normal eye and are not regulated by oxygenation, suggesting that they have only a minor role in coordinating vascularization and metabolic needs in the retina. The production of growth factors such as vascular endothelial growth factor is regulated by tissue oxygenation. In patients with diseases such as proliferative diabetic retinopathy and retinopathy of prematurity, in which tissue hypoxia promotes neovascularization, the levels of vascular endothelial growth factor in the ocular tissues are elevated,² and they decline when treatment with laser photocoagulation induces remission of these diseases. These results strongly suggest that growth factors that are regulated by the metabolic needs of the retina participate in ocular diseases in which hypoxia has an important role.

Numerous inhibitors of angiogenesis have been reported to counteract the effects of growth factors. Extracts of cornea and vitreous harbor substances that inhibit the proliferation and angiogenesis of endothelial cells.

However, most of the known antiangiogenic factors are not regulated by tissue oxygenation, nor are they specifically located in the avascular regions of the eye. Recently, Dawson et al. isolated a factor from retinoblastoma cells that has strong antiangiogenic activity³ and that is identical to pigment-epithelium-derived factor, a glycoprotein that can also induce the differentiation of neuronal cells.⁴

Dawson et al. also showed that neutralizing antibodies against pigment-epithelium-derived factor block the antiangiogenic effects of extracts of cornea and vitreous, suggesting that pigment-epithelium-derived factor is the main antiangiogenic factor in these tissues. Even more exciting is the finding that the production of pigment-epithelium-derived factor, unlike that of other antiangiogenic factors, is increased by hyperoxia and decreased by hypoxia. These properties are critical in retinal tissues, especially in the layers where avascularity and the levels of metabolic fuels are maintained in precise balance. For these reasons, pigment-epithelium-derived factor has the attributes of an important angiostatic factor in the retina.

In diseases such as retinopathy of prematurity and proliferative diabetic retinopathy, the main causes of visual loss are the development of new blood vessels at the junction of vascularized tissue and the avascular retina and vascular growth into the vitreous, resulting in hemorrhage and retinal detachment. The excessive neovascularization in these diseases has been attributed to the increased production of vascular endothelial growth factor caused by tissue hypoxia.² The severity of the abnormal vascularization in these retinopathies has been correlated with the degree of elevation of vascular endothelial growth factor. The levels of this factor fall after a remission has been achieved by laser therapy. The new results regarding pigment-epithelium-derived factor suggest that the induction of angiogenesis in the eye requires not only an elevation of vascular endothelial growth factor but also a decrease in pigment-epithelium-derived factor. To test this idea, we need to determine whether the levels of pigment-epithelium-derived factor are decreased in the ocular fluids of patients with active retinopathy of prematurity, diabetic proliferative retinopathy, and other eye diseases marked by neovascularization due to tissue hypoxia ([Figure 1](#)). We should also confirm that the levels of pigment-epithelium-derived factor in the ocular fluid of these patients increase during remissions.

A decrease in pigment-epithelium-derived factor may also be involved in retinal diseases characterized by neovascularization, but not by hypoxia, such as age-related macular degeneration.

The main abnormality in this disorder is the growth of choroidal blood vessels into the degenerating layer of retinal pigment epithelial cells, especially in the macular region ([Figure 1](#)). The degeneration and eventual loss of these cells and the scarring in the macular region resulting from neovascularization lead to decreased vision.

The factors that promote the growth of new vessels into the macular region are unclear,⁵ but the degeneration of retinal pigment epithelial cells may result in a decrease in the production of pigment-epithelium-derived factor. This, in turn, would permit the choriocapillaris to respond to endogenous tissue growth factors and invade the layer of retinal pigment epithelial cells, including the macula. In addition to allowing angiogenesis to proceed, the loss of pigment-epithelium-derived factor could also contribute to the degeneration of neuronal retina and retinal pigment epithelial cells, since this factor has potent trophic effects on neurons and neuroglia. In this way, changes in the level of pigment-epithelium-derived factor may have a role in diabetic neuropathy, which involves abnormalities in both the neuronal and vascular components of the peripheral nerves.

Therapeutically, agonists of pigment-epithelium-derived factor could be used to enhance neuronal differentiation in diabetic neuropathy and to inhibit neovascularization in diabetic and other ocular diseases, even if the responsible growth factors have not been clearly identified. It is also possible that the antiangiogenic effects of pigment-epithelium-derived factor itself will be therapeutically helpful in tissues in which increases in vascularization are pathogenic. The regulation and actions of pigment-epithelium-derived factor and its receptors in normal and abnormal states offer important new areas of study in ophthalmology.

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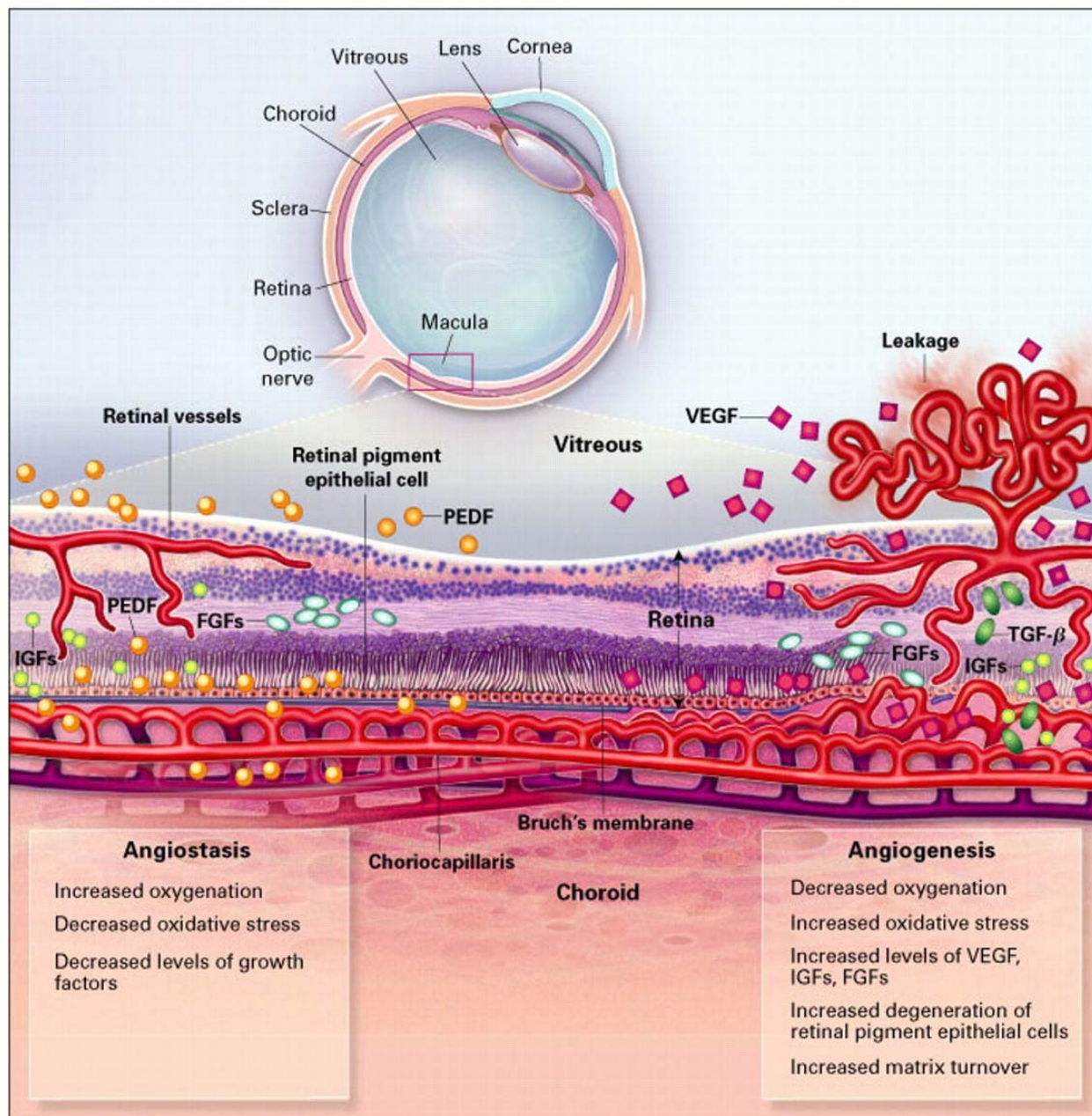


Figure 1. Functions of Pigment-Epithelium–Derived Factor (PEDF), Vascular Endothelial Growth Factor (VEGF), and Other Vascular Trophic Factors in Various Ocular Tissues during Angiostasis and Angiogenesis. The effects on vascularization coordinated by pigment-epithelium–derived factor are indicated in orange, and the effects coordinated by vascular endothelial growth factor are indicated in magenta. The conditions listed under angiostasis and TGF- β transforming growth factor β .

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