The Maintenance of Antioxidant Defenses during Inflammation

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Introduction

Invasion of the body by pathogens, or trauma and chronic neoplastic and inflammatory disease, brings about activation of the immune system. A range of molecules with high biological potency are produced by the system, with the objective of defeating the invasion, destroying damaged and aberrant cells, and restoring tissue function to normal. Cytokines, free radicals and other oxidant products are among these potent molecules. The cytokines comprise the interleukins 1–12 (IL1–12), tumour necrosis factors (TNF) and interferons [1]. Free radicals comprise the superoxide, hydroxyl and nitric oxide radicals. Other oxidant products which are related to free radical biology are hypochlorous acid and hydrogen peroxide [2, 3].

The very nature of these substances potentially exposes the host to damage. However, natural systems exist to afford a high degree of protection of the host against the deleterious effects of cytokines, and free radicals and other oxidant molecules. An increasing body of evidence suggests that enhanced production of all three types of molecule occurs under physiological as well as pathological circumstances. The utilization of oxygen results in production of various reactive oxygen species which have the potential to cause tissue damage. Thus, organisms which utilize oxygen have developed sophisticated anti-oxidant defenses. Strenuous exercise increases free radical and cytokine production [4, 5]. Ovulation is accompanied by increased synthesis of TFN [6]. Mild psychological stress may also enhance IL-1 production [7].

Cytokines, free radicals and other oxidant molecules which are produced as a consequence of activation of the immune system, arise largely from phagocytic cells. These molecules have the ability to enhance each others production. TNF and IL-1 elicit reactive oxygen and nitrogen oxide species from macrophages [8, 9]. Conversely, enhanced TNF production has been observed in experimental situations in which free radical generation is enhanced [10]. Antioxidant defenses are increased by the action of a number of cytokines including IL-1, TNF and IL-6. Thus within mammals, there are attempts to balance oxidant and antioxidant systems even under pathological circumstances. The achievement of a balance may not however occur with total success, as will be seen later in this chapter.

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The Nature of Free Radical and other Oxidant Production by Immune Cells

Before considering the oxidant products which are synthesized by the immune system, it is first necessary to consider the consequences of the synthesis of the superoxide radical, which is a major product of the oxidant burst, that occurs when phagocytic cells are activated [2]. Figure 1 gives an overview of possible products from the superoxide radical.

The superoxide radical is the least active of the free radicals, with the exception of the α-tocopherol radical. Superoxide is removed by the action of superoxide dismutases (SOD). Hydrogen peroxide is the product of the reaction and acts as substrate for a number of competing reaction, some of which result in biologically inactive products, and others in substances of greater potency than the original superoxide. Glutathione peroxidase and catalase convert hydrogen peroxide to water. Myeloperoxidase catalyses the reaction of the peroxide, with chloride, to produce a hypochlorite radical. Hydrogen peroxide may also react nonenzymically with superoxide to produce hydroxyl radicals in the presence of trace amounts of iron and other transition metals. In the presence of the same metals, the hydroxyl radical will react with lipids to produce lipid peroxides which rapidly decompose, producing lipid free radicals. The appearance of these substances leads to a chain reaction of lipid peroxidation. The end-products from this sequence of events are cytotoxic aldehydes, such as malonaldehyde, and hydrocarbons such as ethane and pentane. The chain of lipid peroxidation is arrested or broken if α-tocopherol reacts with a peroxyl radical to produce its own radical, which has virtually no reactivity with lipids. Superoxide production is not limited to immune cells. Fibroblasts and endothelial cells are also capable of production [11, 12]. The physiological role of such production in unclear, however fibroblast proliferation is stimulated, suggesting a role in healing and tissue repair [11]. Nitric oxide (NO) is also among the oxidant substances produced by activated immune cells. It is produced as the result of the action of argin-

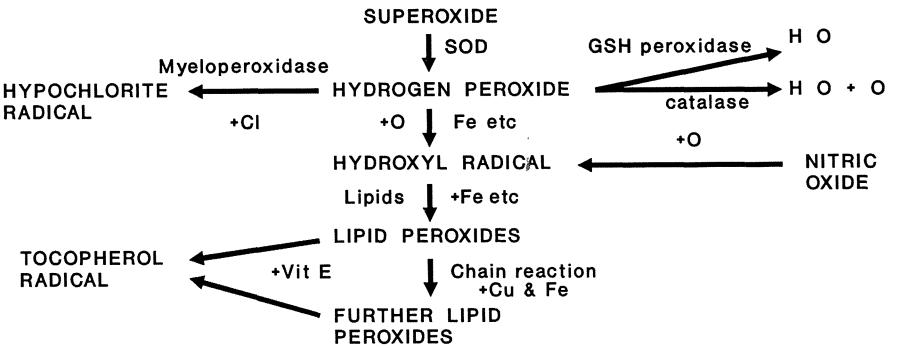

Fig. 1. Metabolism and consequences of superoxide radical formation

ine deimidase on intracellular arginine. NO, like superoxide, can also be produced from other cells such as endothelial cells. The physiological functions of NO in each context is however different. For endothelial cells, NO influences vascular tone by causing vasodilation. For macrophages, NO acts as a killing agent for invading cells. The manner and amount of NO production from each cell source are different. Stimulation of endothelial cells results in rapid, transient production of relatively small amounts of NO, the production does not involve a protein synthetic step. Stimulation of macrophages results in massive, prolonged production after a lag period of several hours. The enhanced production involves a protein synthetic step [3]. Due to the ability of NO to cause vasodilation, production in response to pathogens carries with it the risk of hypotension [13]. Thus, it can be seen from this simplified view of superoxide and NO metabolism that the process carries benefits of killing of pathogens, wound healing, and tissue repair, but also a twofold risk of producing potentially toxic substances and of initiating processes which can lead to cell damage, should antioxidant defenses fail to meet the challenge. The importance of the superoxide and NO radical in the morbidity of severe trauma and sepsis is illustrated by the improved condition which occurs on administering recombinant SOD or a metabolic inhibitor of NO production, n-methyl L-arginine (NMDA) [14, 15].

The cells of the immune system which are mainly responsible for the production of free radicals and other oxidant molecules, are eosinophils and neutrophils. The most potent inducers of free radical and other oxidant molecule production (oxidant or respiratory burst) from neutrophils are phagocytozable microorganisms. A number of physiological secretagogues such as immune complexes, arachidonic acid, leukotrienes and platelet activating factor (PAF) elicit transient release. The amount produced is well below that caused by contact with bacteria. Furthermore, some of the secretagogues are active at concentrations which are unlikely to be present during inflammation.

A wide range of cytokines, including IL-1, IFNγ, and TNFα and β stimulate transient production of reactive oxygen radicals. Cytokines exert their effects at concentrations that are three to five orders of magnitude lower than those required for agents such as leukotrienes or PAF. The ability of polymorphonuclear cells (PMN) to produce oxidant bursts is considerably enhanced when the cells have adhered to surfaces, or have been sensitized by agents such as endotoxin. Adherence of cells to endothelial cells resulted in prolonged production of oxygen radicals in the presence of TNFα that is similar in amounts to that produced by stimulation with bacteria [16]. Production of oxygen radicals by eosinophils in response to TNF is dependent upon adhesion to surfaces [17]. Thus while such sensitization might increase resistance to infection, it might also predispose the patient to oxidant damage. Indeed increased evidence of oxidant damage is present in patients undergoing major inflammatory reactions such as in ARDS [18].

The Actions of Free Radicals and other Oxidant Molecules

There are many ways in which free radicals can exert damaging effects upon bacterial, and malignant and normal cells of the host. Superoxide radicals will, by conversion to hydrogen peroxide, damage cell membrane proteins. The peroxide can rapidly diffuse into cells, inhibit ATP synthesis and produce breaks in the DNA molecule. The latter is more likely to occur in the presence of transition metals which encourage the formation of hydroxyl radicals. Hydrogen peroxide, in the presence of chloride ions and myeloperoxidase, is transformed to hypochlorous acid which will damage the cell membrane by attacking the sulphydryl groups of constituent proteins [19, 20].

A number of studies carried out *in vivo* and *in vitro* suggest that TNF may exert its lethal effects by induction of free radicals. Hydroxyl radical scavengers, anaerobic conditions, and inhibitors of arachidonic acid metabolism, prevent TNF from exerting lethal effects on tumor cell lines *in vitro*. *In vivo,* the lethality of TNF will be increased in animals which have antioxidant defenses impaired by reduction of tissue glutathione concentrations by diethylmaleate. The converse is true, since a reduction in lethality was observed in animals whose defenses were enhanced by feeding N-acetyl cysteine (NAC) [21].

The Nature of Antioxidant Defenses

Defenses are comprised of lipid and water soluble substances and enzymes, while the components of the defenses are derived ultimately from the diet, many are derived directly from the diet. Vitamins E and C, carotenes, and the copper and zinc moities of ceruloplasmin (CP) and metallothionien (MT), fall into this category. Glutathione (GSH) arises to a large extent from dietary supplies of its three constituent amino acids: glycine, cysteine, and glutamate, although *de novo* synthesis of the three amino acids may make a significant contribution. The importance of the dietary contribution is underlined by the major fall in tissue GSH content that occurs when food intake decreases (Fig. 3). The enzyme systems include SOD, glutathione peroxidase and catalase (Fig. 1). The acute phase protein CP also has SOD-like activity [22].

The defenses are strategically placed throughout the body to afford maximum protection from free radicals. Ceruloplasmin, MT, GSH and nutrient antioxidants are present in the circulation. The latter three are also present within the cell. GSH, MT and vitamin C reside within the aqueous parts of the cell. Vitamin E (α-tocopherol) resides within the cell membrane where it acts as the main antioxidant at that location. The vitamin is also present in tissue fluids and within the cell, attached to low density lipoproteins and lipids. The enzyme systems are situated throughout the organelles of the cell.

A number of constituents of antioxidant defenses, which depend upon nutrient intake for their adequacy, interrelate. Tocopherol may be regenerated

from its radical by the action of GSH [23] and ascorbic acid [24]. Despite the ubiquitous nature of antioxidant defenses, it is possible for free radical generation to deplete defenses at a local level. This phenomenon is illustrated in rheumatoid arthritis where α-tocopherol concentrations in serum are normal while the antioxidant concentration in synovial fluid is severely depleted [25].

As well as direct defenses which destroy free radicals, indirect systems exist which prevent free radicals from propogating. Hydrogen peroxide which is produced during the metabolism of superoxide is transformed into hydroxyl radicals if trace amounts of transition metals, such as iron and copper, are present to catalyse the action. Thus safe storage of these ions is crucial at all time. Two thirds of the 4 g of iron present in the human body is stored as hemoglobin, one tenth as myoglobin, a smaller portion is bound to transferrin (the iron transport protein), and the remainder is stored bound to intracellular proteins such as ferritin and hemosiderin. The substantial ability of ferritin to bind iron and the fact that transferrin is only 30% saturated with iron, result in virtually no free iron within the cell [26]. Ceruloplasmin also inhibits transition metal ion catalyzed production of radicals by sequestration of metal ions. The biological importance of adequate sequestration of transition metal ions, in conditions in which the immune system is activated, is illustrated in patients who have suffered iron overload as a consequence of their treatment. Such patients exhibited liver damage and joint inflammation [27, 28]. Studies in mice suggest that iron overload increases hepatic synthesis of glutathione, thereby increasing the demand for its constituent amino acids [29].

The importance of adequate transition metal ion binding is also underlined by the potential of iron to convert ascorbic acid from an antioxidant to a prooxidant role. The transformation occurs due to the ability of ascorbic acid to convert unsequestered iron from ferric to ferrous ions. Since oxidation of ascorbic acid can result in hydrogen peroxide, all the necessary components for production of hydroxyl radicals will be in place [25, 26].

Influence of Cytokines on Antioxidant Defenses

As outlined earlier, it is necessary for antioxidant defenses to be enhanced in situations in which the immune system is activated, to protect host tissues from the damaging effects of free radicals and other oxidant substances produced by the system. Cytokines, in particular IL-1, TNF, and IL-6, marshall a number of components of these defenses. Cytokines bring about large increases in glutathione synthesis, thereby enhancing and maintaining the tissue content. The stimulatory effect on synthesis is necessary since glutathione synthesis is greatly dependent upon dietary protein intake. In particular, on the intake if sulphur amino acids. IL-1 and TNF released during inflammation may bring about a substantial fall in food intake, thus the effects of the reduction in substrate intake are circumvented by the stimulatory ef-

fects of the cytokines on GSH synthesis. The enzymes involved in the metabolism of the superoxide radical and hydrogen peroxide are increased by the actions of a number of cytokines. For example, IL-1 and TNF induce production of manganese SOD (MnSOD) in pulmonary endothelial cells in rats [30, 31].

In addition to increasing the active antioxidant defenses, cytokines also induce changes which minimize free radical proliferation by inducing the synthesis of the substances which limit the exposure of tissues to iron and copper ions. Cytokines bring about substantial changes in the synthesis of hemoglobin, transferrin, ferritin, CP and MT.

That of the first three, hemoglobin, transferrin and ferritin is increased, thereby exerting close control on iron sequestration. The sequestration may however not be completely assured. At a pH of 6, iron dissociates from ferritin. The pH within close proximity to an activated phagocyte may fall below this value permitting iron to enhance free radical production [25]. The acute phase protein haptoglobin also acts to reduce the possibility of iron to becoming available, in catalytic amounts, by binding to haem, released from the destruction of red blood cells which is a consequence of tissue injury and infection. Circulating CP concentrations are enhanced by a number of inflammatory agents such as endotoxin, IL-1 and TNF [32, 33].

An increasing body of evidence is emerging that cytokines and free radicals enhance each others production. Chaudhri and Clark [10] demonstrated that the ability of endotoxin to stimulate TNF production was enhanced by alloxan, a prooxidant drug, and that the effect could be prevented by administration of butylated hydroxyanisole, an antioxidant, or desferrioxymine an iron chelating agent. Other antioxidant substances may be able to suppress cytokine production in situations in which a prooxidant environment has been created. Ku et al. [34] showed that probucol, a hypocholesterolemic drug with antioxidant properties, was able to suppress IL-1 production from murine peritoneal macrophages, that were incubated with endotoxin. The production of TNF was however unaffected. N-acetyl-cysteine which is a substrate for glutathione synthesis, and an antioxidant in its own right, was however able to inhibit production of TNF by splenocytes from mice injected with endotoxin. IL-6 production by the same tissue was unaffected [35].

Subsequently it was shown that TNF, IL-1 and IL-2 production could be stimulated by hydrogen peroxide *in vitro* [10, 36]. The mechanism underlying the enhansive effects of free radicals upon cytokine production may be the ability of oxidants to stimulate production of the transcription factor NFkB. The factor is involved in production of cytokines from stimulated immune cells [37]. Thus, antioxidant substances may prevent the stimulatory effect of oxidants and oxidant situations on cytokine production at the level of the transcription factor.

Interactions between Nutrition, Cytokines and Antioxidant Defenses

The Increased Demand for Nutrients

The metabolic events which occur during inflammation carry with them a nutritional cost. The nutritional implications of inflammation are set out in Table 1. These nutritional implications include increased demands for protein, specific amino acids, and trace elements. A number of these nutrients are involved in the elaboration and maintainance of antioxidant defenses. In addition, the ability of certain nutrients such as fatty acids to alter the intensity of the inflammatory process may alter both the magnitude of synthesis of antioxidants and oxidant production [38].

A number of studies have shown that a low protein diet reduces the GSH content of liver and lungs [33, 34, 36]. The question arises as to whether the reduction is due to protein deficiency *per se*, or to a deficiency in one of the constituent amino acids of the antioxidant i.e. cysteine, glycine or glutamate. Studies carried out by our group on rats suggest that the adequacy of dietary sulphur amino acid intake can influence tissue GSH content. Furthermore, Hong et al. [39] have shown that glutamine supplementation helps to restore

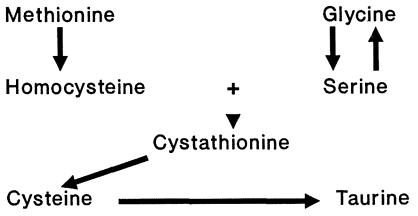

Fig. 2. Metabolic relationship of glycine, serine, cysteine and taurine

Table 1. Nutritional implications of inflammation

Process	Nutrient implication
Fever	Energy requirements
Antioxidant defenses	
– Glutathione	Cysteine, glycine, glutamine
– Antioxidant enzymes	Cu, Mn, Zn, Se
– Nutrient derived	Vitamins A, C, E
Acute phase proteins	Protein, specific amino acids, Cu, Zn, Fe
Connective tissue remodelling	Protein, Glycine, Vitamin C
Hematopoesis	Protein, Vitamin B12, folate, Fe
Inflammatory intensity	n6 to n3 polyunsaturated fatty acid ratio
Immune cell increase and activation	Protein, glutamine, glucose, vitamins, trace elements

liver GSH and plasma content, following acetoaminophen poisoning, in rats consuming a diet with an adequate sulphur amino acid content. Thus, there may be a nutritional hierarchy in the relative importance of nutrient provision, to maintain GSH synthesis. There are several pieces of circumstantial evidence which suggest that the requirement for sulphur amino acids, methionine and cysteine, and amino acids that are metabolically related to them (glycine and serine) are increased during inflammation. Askanazi et al. [40] observed decreases in plasma concentrations of serine, glycine and taurine of 11, 35 and 45%, respectively, in patients with severe trauma and sepsis. As shown in Fig. 2 glycine is a precursor of serine and serine forms the carbon skeleton of cysteine. Several tissue components such as glutathione and metallothionein, which show enhanced syntheses during inflammation are particularly rich in the amino acids related to cysteine (Table 2). Following fractures and burns, urinary sulphur excretion is enhanced to a lesser extent than that of nitrogen [41, 42]. This may indicate preferential retention of sulphur amino acids. The decrease in plasma taurine concentration during sepsis and trauma may likewise indicate diversion of cysteine, from the synthesis of this major end-product, into molecules with a high cysteine content. Indeed in rats given TNF, decreases in urinary sulphate excretion occur in concert with increases in liver and lung glutathione content [43]. We examined the influence of glycine, methionine, cysteine, serine and taurine supplementation on the response of protein depleted rats to TNF injections. Protein depletion was achieved by feeding a diet containing 8% casein instead of 20%. A number of responses to TNF were blunted in animals receiving the 8% diet (Table 3). In animals fed the low protein diet with contained alanine, as an isonitrogenous control for the amino acids interrelated to cysteine, liver glutathione, protein and zinc content did not increase in response to TNF, as it did in animals receiving the 20% casein diet. Furthermore, lung GSH content fell by 42% in the former group in response to the cytokine. No significant change in lung glutathione occurred in animals consuming the high protein diet. Addition of cysteine, and methionine to the low protein diet permitted an increase in GSH content of both liver and lung in response to TNF. While

Table 2. Amino acid composition of proteins and peptides which show increased rates of synthesis during inflammation

(% of residues)	Glycine	Serine	Methionine + Cysteine	Total
C3 complement	6	6	4	16
Haptoglobin	7	5	4	16
Ceruloplasmin	7	6	3	16
C-reactive protein	8	10	3	21
Serum amyloid A	12	9	4	25
Phospholipase A2	5	9	11	25
Collagen	34	3	1	38
Metalothionein	11	13	32	56
Glutathione	33	0	33	66

Table 3. Influence of supplementary glycine, serine, methionine, cysteine and taurine on the response of protein depleted rats to TNFα[a]

Injection diet	Liver [Zn]		Liver [Protein]		Liver [GSH]		Lung [GSH]		Plasma [CP]	
	S	T	S	T	S	T	S	T	S	T
20% Casein	100	124	100	137	100	121	100	95	100	230
8% Cas+ala	91	85	99	102	21	28	71	42	180	353
8% Cas+gly	38	56	106	110	13	28	78	78	133	303
8% Cas+ser	71	85	116	110	33	29	115	74	183	285
8% Cas+met	88	86	72	99	39	105	38	106	192	252
8% Cas+cys	65	92	94	134	79	104	75	110	100	220
8% Cas+tau	85	85	110	106	29	25	96	72	187	365

[a] Values shown are for rats 24 h after an injection of 100 μg/kg TNFα (T) and are expressed as a percentage of the value observed for saline (S) injected rats receiving the 20% casein diet. Amino acid additions to the diets are isonitrogenous and equivalent to 6 g alanine/kg diet

addition of the provider of the carbon skeleton of cysteine (serine), or a major end-product of cysteine (taurine) did not assist an increase in liver GSH content, both reduced the extent of the fall in lung GSH in response to TNF. The data suggest that the sulphur moiety of cysteine is of prime importance in maintaining and enhancing tissue glutathione, in situations in which cytokines such as TNF may be operating.

TNF may play a role in the extensive weight loss observed in cancer and AIDS [44]. It is thus interesting to note that, in asymptomatic HIV-infected individuals, substantial reductions occur in GSH concentrations in plasma, immune cells, and lung endothelial lining fluid [49]. The decreases in tissue GSH in AIDS may therefore indicate the requirement for sulphur amino acids is not being satisfied. There may indeed be an enhanced requirement for cysteine for GSH synthesis in AIDS since alveolar macrophages from such patients are activated and exhibit exaggerated production of oxidants, and urinary malonaldehyde production is enhanced [46, 47]. Furthermore, administration of GSH to the lungs of patients by aerosol resulted in greatly enhanced concentrations of oxidized glutathione in the endothelial lining fluid, suggesting enhanced utilization of this antioxidant [48].

Compensatory Adaptations in Defenses

Our studies suggest that when the nature of the diet limits supplies of substrate for maintaining antioxidant defenses, adaptations occur to maintain defense from the resources available. The data for ceruloplasmin concentrations shown in Table 3 indicates that a compensatory increase occurs in this component of antioxidant defenses, when the ability to synthesize gluta-

thione is impaired by the low protein diet. Compared with the response of well fed animals, an enhanced effect occurred in animals consuming the low protein diet supplemented with alanine. Addition of serine, cysteine and methionine to these diets resulted in a lesser degree of enhancement. Thus when sulphur amino acid intake limits the ability to synthesize GSH, which contains 33% cysteine; ceruloplasmin, which contains 3% of sulphur amino acids, is synthesized in an attempt to maintain antioxidant defenses.

Compensatory increases in components of the antioxidant defenses may also take place in other experimental contexts where defenses are modulated by nutritional means. We studied effects of vitamin E sufficiency on liver and lung GSH concentrations when food intake was decreased. Rats were fed diets deficient in vitamin E, or containing an adequate (50 mg/kg diet) or supplemental (250 mg/kg) level α-tocopherol. The diets were fed in an *ad libitum* amount for 3 weeks prior to 24 h in which feeding continued at 100 or 50 or 25% of the *ad libitum* rate. The data in Fig. 3 show that an enhanced concentrations of hepatic GSH occurs in the vitamin E deficient animals at all levels of food intake, the drive to synthesize GSH overrides the inhibitory effect of reduced sulphur amino acid intake which would occur at the reduced levels of food intake. Lung GSH does not exhibit the same characteristics.

The Effectiveness of Defenses in Nutritional Deficiency

The extent to which such adaptations in antioxidant defenses protect the animals from antioxidant damage is not clear. However, studies carried out by Swords et al. [49] suggest that, at best, the compensatory increase in liver

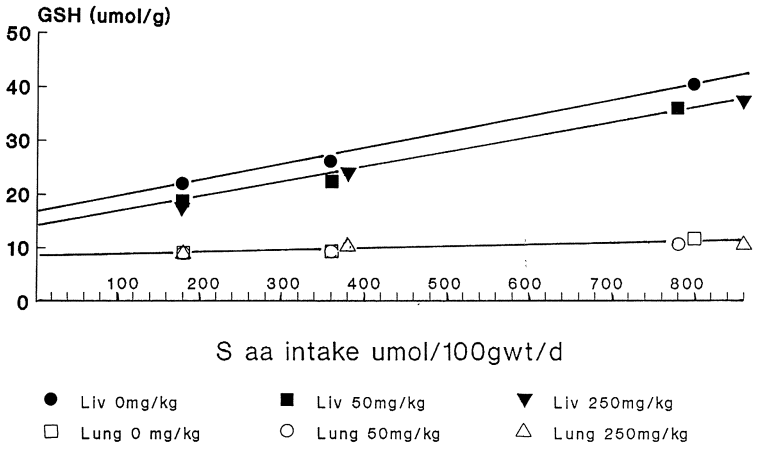

Fig. 3. Influence of sulphur amino acid and vitamin E intake on gluthatione content of liver and lung in rats

GSH would limit, rather than prevent, the increase in oxidant damage which occurs in vitamin E deficiency. As selenium is an important component of glutathione peroxidase, Swords et al. examined the effects of selenium and vitamin E deficiency on lipid peroxidation following endotoxin injections in rats. The investigators also examined the relative effectiveness of vitamin E and selenium supplementation in suppressing the induced lipid peroxidation. Ethane production rose from 0.5 nmol/kgBW/h, in animals receiving adequate vitamin E and selenium, to 5 nmol/kg/h in deficient animals. Treatment with endotoxin increased production to 1 and 15 nmol/kg/h in the well fed and deficient animals. Supplementation of the latter group with selenium and vitamin E reduced production to 4.2 and 1.8 nmol/kg, respectively. Thus vitamin E was more protective than selenium against lipid peroxidation induced by inflammation.

Deficiencies in dietary copper and zinc may also limit antioxidant defenses in a number of ways. Both metals are cofactors in copper-zinc SOD which acts as the first line of defense against the superoxidase free radical (Fig. 1), as well as being components of ceruloplasmin and metalothionein, respectively. In rats, copper deficiency reduced the activity of the enzyme to 11 and 74% of normal activity in liver and lung, respectively. Manganese SOD activity was unchanged in both tissues, while catalase was reduced by 15% in liver by this dietary treatment [50]. Ceruloplasmin activity fell to 18% of normal values. Zinc deficiency had no effect upon the antioxidant enzymes, but reduced liver and lung MT content to 6 and 73% of normal values. Deficiencies in copper and zinc effect CP and MT synthesis, in a different manner, in response to an inflammatory stimulus. In copper deficient rats, synthesis of CP protein in response to IL-1 is unimpaired, while the oxidase activity of the molecule is severely reduced [32]. In zinc deficient rats, the synthesis of MT protein is inhibited [50]. Thus copper deficiency acts posttranscriptionally, while that of zinc acts at the level of transcription. In the study, reduction of CP and MT had little influence on the concentrations of plasma α-tocopherol and ascorbate, however zinc deficiency led to a reduction of 40% and 10% in GSH in liver and lung, respectively. It would appear from this finding that zinc deficiency had the greatest impact on antioxidant defense despite having no effect on SOD activity [50]. Clearly the situation is complex.

It is not clear from the study how the deficiencies in Cu and Zn would influence the effectiveness of the defenses during an inflammatory stress. Exposure to high oxygen concentrations as occurs in care of the premature infant, can cause inflammation of lungs that may be mediated by free radicals and cytokines. Animal models have been used to study this problem. A greater degree of lung damage occurred in the zinc deficient rats when exposed to high oxygen tensions for 7 days, than in rats that were zinc repleted. While increases in lung SOD, glutathione peroxidase and catalase occurred in the latter animals, deficient animals showed none of these changes. In the same animal model, copper deficiency did not prevent the increase in lung CuZn SOD which occurred in response to oxygen exposure, despite major decreases in lung copper content [52]. The data reported in the studies illus-

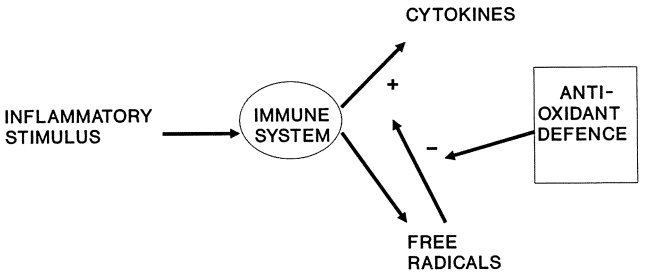

Fig. 4. Influence of antioxidant defenses on the interaction of cytokines and free radicals during inflammation

trate the relative sensitivities of the antioxidant defenses in liver and lung to the dietary intake of precursors. Since free radicals produced as a consequence of cytokine action are able to enhance cytokine production, the question arises as to whether nutrients can indirectly modulate cytokine production by enhancing antioxidant defenses as shown in Fig. 4.

We examined the effects of altering the effectiveness of antioxidant defenses, on the responsiveness to inflammatory agents, by studying the effects of vitamin E sufficiency on the response to endotoxin in rats (Table 4) [53]. Rats were fed diets which contained 0,5 or 250 mg/kg of α-tocopherol for 3 weeks prior to injection. A greater degree of anorexia and increase in α-1-acid glycoprotein occurred in the deficient animals suggesting an enhanced production of cytokines. However infiltration of PMN into lung was similar in all groups after injection with endotoxin. In vitamin E deficient control animals, which had experienced only the minor day-to-day exposure to inflammatory agents in the environment of the animal colony, significantly greater amounts of PMN appeared in lung tissue, than in animals receiving 50 mg/kg α-tocopherol. Animals receiving the supernormal amount of α-

Table 4. Influence of vitamin E status on the response to endotoxin in rats

Diet Vit E (mg/kg)	0		50		250	
Injection	S	E	S	E	S	E
Food intake (g/24 h post injury)	23	3	27	13	24	17
Liver wt (% body wt)	4.0	5.1	3.7	5.1	4.1	5.3
Plasma a-1-acid glycoprotein (units/ml)	22	256	14	157	18	163
Plasma albumin (mg/ml)	34.5	30.9	34.4	28.5	33.4	31.5
Lung PMN (% cells)	9.2	10.6	7.9	10.9	7.0	9.2
Plasma Vit E (mg/ml)	0.32		0.76		0.97	
Red cell Vit E (mg/ml)	0.41		0.73		1.12	

S and E are saline and endotoxin injections, respectively, 800 μg/kg of endotoxin given i.p. Saline injected animals pair fed to intakes of E animals. Effects measured 24 h post-injection. (From [53] with permission)

tocopherol had significantly lower concentrations of PMN in lung than animals given a normal amount of α-tocopherol. Evidence of enhanced responsiveness to endotoxin is also apparent in the study of Omer and Millward [54] in which rats had antioxidant defenses depleted by feeding diets deficient in vitamin E and protein. A greater increase in hepatic protein synthesis and fall in plasma zinc occurred in these, than in animals receiving a diet adequate in these two nutrients.

Conclusion and Clinical Implications

In patients responding to infections, trauma and chronic inflammatory challenges, cytokines orchestrate events to bring about repair and restoration of health. In so doing, cytokines lead to the generation of oxidant molecules which are potentially toxic to the patient. However cytokines also induce defenses against these substances. It is apparent from the studies described above that nutrition may exert substantial effects on antioxidant defenses and cytokine biology (Fig. 5).

The potential for preventing free radicals from leading to overproduction of cytokines by means of the strategy of maintaining and enhancing antioxidant defenses exists. A numer of clinical and laboratory studies, in which the intake of sulphydryl compounds such as cysteine, glutathione and N-acetyl-cysteine have been enhanced, illustrates this potential. N-acetyl-cysteine (NAC), GSH and cysteine all inhibit NFkB expression in stimulated T-cell lines [55, 56]. N-acetyl-cysteine produced beneficial effects in ARDS patients and in a sheep model of ARDS [57, 58]. It also improved inflammation in healthy smokers, as assessed by examination of bronchoalveolar lavage fluid for eosinophil basic protein, lactoferrin, α-chymotrypsin, and chemotaxis of neutrophils [59]. As NAC has antioxidant properties, it is unclear whether NAC is acting directly, or indirectly, by undergoing deacetylation and delivering cysteine directly to tissues. Certainly the importance of cysteine in optimum immune function is highlighted by the fact that macrophages act as a

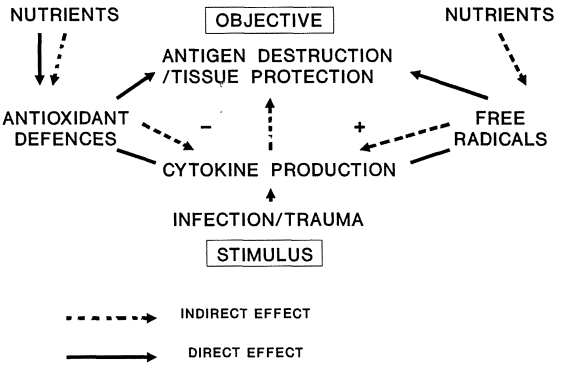

Fig. 5. Effects of nutrients on cytokine and free radical interactions during infection and trauma

cysteine pump to deliver the sulphur amino acid to lymphocytes at variable but controlled rates. Macrophages take up cysteine and cystine from tissue fluid and release cysteine under the action of inflammatory stimuli such as endotoxin and TNF. Close proximity of activated macrophages and lymphocytes facilitates the transfer. In addition to supressing NFkB expression, cysteine enhances a number of lymphocyte functions such as cytotoxic T-cell activity. Uptake of cystine into macrophages is competitively inhibited by glutamate. It is therefore interesting to note that raised plasma glutamates have been observed in AIDS and advanced malignancy, since immunosuppression is apparent in both conditions [60].

The drug diethylthiocarbamate, which has antioxidant capabilities due to glutathione peroxidase like activity, reduces the rate of progression of new opportunistic infections in AIDS. A study in which Zidovudine produced a slowing in the development of AIDS led to a rise in plasma GSH [48]. Vitamin E sufficiency may also play a key part in the antioxidant defenses in AIDS since many of the abnormalities seen in components of the immune system, as a result of HIV infection, are stimulated, or restored, by a high intake of the vitamin e.g. 400 iu/day in man. Feeding studies of normal subjects have shown that α-tocopherol increased delayed type hypersensitivity, and lymphocyte proliferation and IL-2 production in response to mitogens [61].

The inhibitory actions of the sulphydryl compounds on NFkB expression, mentioned earlier on, takes on an additional importance in AIDS since the transcription factor enhances HIV mRNA expression. Thus free radicals cytokines produced during the progression of AIDS will enhance replication of the virus via this route. Since *in vitro* studies have shown that the stimulatory effect of TNF in HIV replication in monocytes can be inhibited by GSH and NAC, the sufficiency of antioxidant defenses take on an additional importance, apart from protecting the patient from the potentially damaging outcome of activation of the immune system by encounters with bacteria, viruses and other inflammatory agents and situations.

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