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Review

Glutathione and infection ☆

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ABSTRACT

Background: The tripeptide γ-glutamylcysteinylglycine or glutathione (GSH) has demonstrated protective abilities against the detrimental effects of oxidative stress within the human body, as well as protection against infection by exogenous microbial organisms.
Scope of review: In this review we describe how GSH works to modulate the behavior of many cells including the cells of the immune system, augmenting the innate and the adaptive immunity as well as conferring protection against microbial, viral and parasitic infections. This article unveils the direct antimicrobial effects of GSH in controlling *Mycobacterium tuberculosis* (*M. tb*) infection within macrophages. In addition, we summarize the effects of GSH in enhancing the functional activity of various immune cells such as natural killer (NK) cells and T cells resulting in inhibition in the growth of *M. tb* inside monocytes and macrophages. Most importantly we correlate the decreased GSH levels previously observed in individuals with pulmonary tuberculosis (TB) with an increase in the levels of pro-inflammatory cytokines which aid in the growth of *M. tb.*
Major conclusions: In conclusion, this review provides detailed information on the protective integral effects of GSH along with its therapeutic effects as they relate to the human immune system and health.
General significance: It is important to note that the increases in the levels of pro-inflammatory cytokines are not only detrimental to the host due to the sequel that follow such as fever and cachexia, but also due to the alteration in the functions of immune cells. The additional protective effects of GSH are evident after sequel that follows the depletion of this antioxidant. This is evident in a condition such as Cystic Fibrosis (CF) where an increased oxidant burden inhibits the clearance of the affecting organism and results in oxidant-induced anti-protease inhibition. GSH has a similar protective effect in protozoans as it does in human cells. Thus GSH is integral to the survival of some of the protozoans because some protozoans utilize the compound trypanothione [$T(SH)_2$] as their main antioxidant. $T(SH)_2$ in turn requires GSH for its production. Hence a decrease in the levels of GSH (by a known inhibitor such as buthionine sulfoximine [BSO] can have adverse effects of the protozoan parasites. This article is part of a Special Issue entitled Cellular functions of glutathione.

1. Introduction

Glutathione (γ-glutamylcysteinylglycine, GSH), an ubiquitous sulfhydryl-containing tripeptide produced by most mammalian cells, is the cells principle mechanism of eliminating reactive oxygen species (ROS) [1–3]. GSH is synthesized *de novo* in a two-step enzymatic process in which glutamine and cysteine are covalently linked by the heterodimeric enzyme γ-glutamylcysteine synthetase or glutamate-cysteine ligase (GCL) to form the product γ-glutamylcysteine (Fig. 1A). This is the rate limiting step in the synthesis of GSH, and cysteine is both the rate limiting reactant and the component that provides GSH with antioxidant activity, as cysteine's sulfhydryl bond is oxidized during the reduction of ROS [1–3]. In the second step of the reaction, γ-glutamylcysteine is bonded to glycine to form a complete GSH molecule (Fig. 1B). Using GSH as a substrate, glutathione peroxidase (GPx) detoxifies hydrogen peroxide, a potent source of ROS within the cell [1–3]. GPx performs the reduction of hydrogen peroxide to water, while linking two GSH molecules together via a disulfide bridge to form oxidized glutathione (GSSG) (Fig. 2A). GSSG is unable to perform antioxidant functions; however, GSH can be reclaimed from GSSG through the use of glutathione reductase (GSR) [1–3]. Utilizing NADPH as a co-factor, GSR performs the reduction of GSSG to GSH, and oxidation of NADPH to NAD^+ (Fig. 2B). Unfortunately, this GSH system can be overwhelmed if ROS are produced in excess. If this occurs the remaining excess free radicals begin to do damage to molecules essential to cellular homeostasis and metabolism, including proteins,

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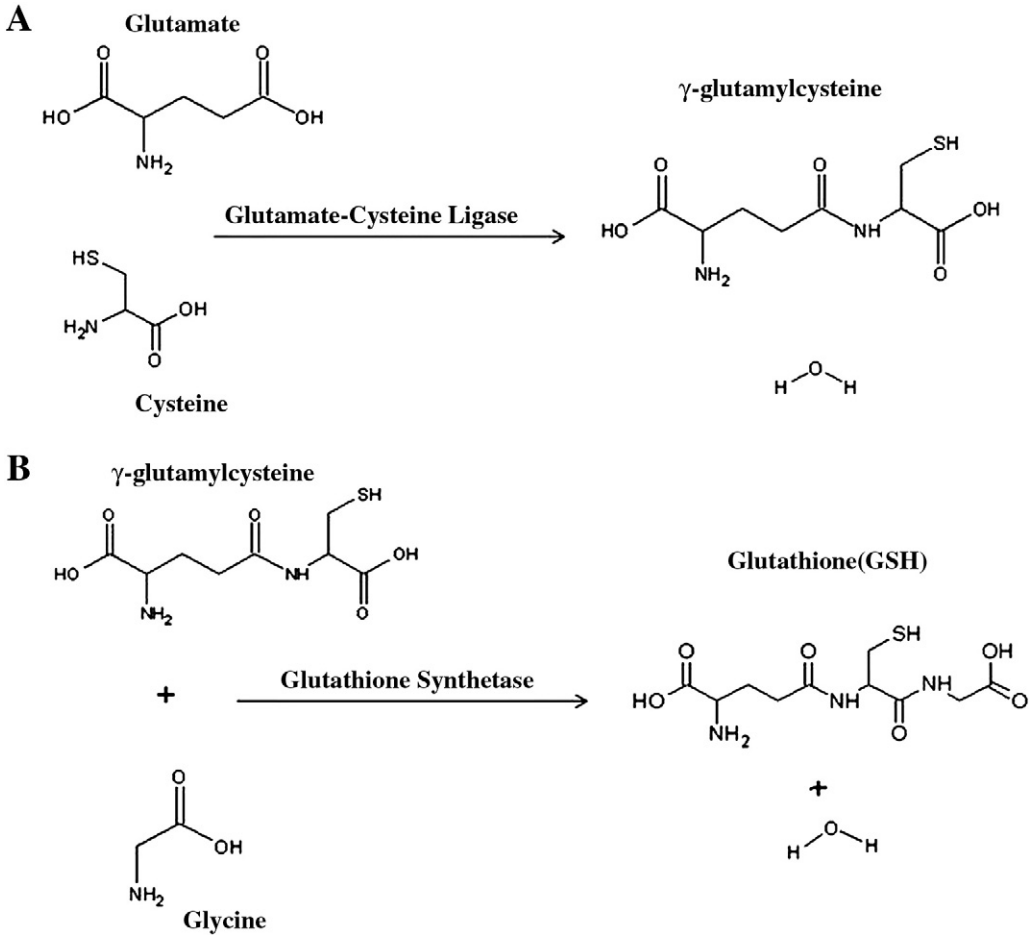

Fig. 1. A. Demonstrates *de novo* synthesis of GSH in twostep enzymatic process in which glutamine and cysteine are covalently linked by the heterodimeric enzyme γ-glutamylcysteine synthetase or glutamate-cysteine ligase (GCL) to form the product γ-glutamylcysteine. B: Demonstrates the second step in the reaction, γ-glutamylcysteine is bonded to glycine to form a complete GSH molecule.

nucleic acids, and lipids. This oxidative damage has real consequences for cellular functions.

2. GSH in prokaryotes

GSH is produced by most eukaryotes and is produced by some but not all prokaryotes. This important antioxidant is synthesized under the control of diverse systems depending on which prokaryote is being considered. In *Escherichia coli* (*E. coli*) oxidative stress induces the synthesis of GSH, through upregulation of two enzymes γ-glutamylcysteine synthetase and GSH synthetase. OxyR a transcription factor can sense and respond to oxidative stress. In its oxidized form it can activate transcription of promoters upstream of genes involved in resistance to oxidative damage and these include genes involved in GSH synthesis. Interestingly GSH is not required for growth of *E. coli* during logarithmic phase but is required for growth during stationary phase [4]. In another species of bacterium, *Salmonella* spp., it is the stringent response, or response to nutrient deprivation that upregulates GSH production [5]. ppGpp is produced in response to conditions of nutrient starvation, which in turn activates a response regulator that upregulates GSH production. Potentially oxidative damage increases during nutrient starvation which GSH combats.

In bacteria GSH is synthesized in locations where it is needed. In *E. coli* GSH is located in the cytoplasm where it serves to reduce the environment in this compartment [6]. The periplasm of bacterial cells such as *E. coli* is much more oxidized. In fact many proteins in this location are linked by disulfide bonds and consequently GSH is low in this bacterial location. It is thus unexpected that GSH from *E. coli* cells accumulates in the growth media and may serve to protect these bacteria from external oxidative stress before it reaches them [6]. Other bacteria such as cyanobacteria which perform aerobic photosynthesis also synthesize GSH. In this case GSH is located in the cell wall as well as the cytoplasm as ROS can localize to the cell envelope [7].

A number of gram positive organisms produce GSH which include *streptococci*, *listeria*, *lactobacilli*, *clostridium*, and *enterococci*. Gram positive bacteria use differing strategies to produce GSH; some use two separate enzymes which are γ-glutamylcysteine synthetase and GSH synthetase. This is similar to what is seen in *E. coli*. Other bacteria such as *Streptococcus agalactiae*, *Listeria monocytogenes*, *Pasturella multocida* and *Streptococcus thermophilus* contain both functions of the above two enzymes in one [8–11]. In addition some gram positives produce GSH transferases that serve to inactivate toxic compounds [12,13].

As discussed above GSH is synthesized by some bacteria in response to oxidative stress. GSH however is consumed during neutralization of oxidative stress in an oxidized form where two GSH molecules are linked by a disulfide bond. In order to recycle the GSH molecule so that it can be used to combat oxidative damage, the enzyme GSR is induced [14]. This enzyme thus reduces GSSG and is regulated by the transcriptional regulator OxyR [6].

There are a number of bacteria that fail to synthesize GSH but nonetheless can import this molecule from an extracellular location. *Francicella tularensis* (*F. tularensis*) is one of these organisms. In this case *F. tularensis* may use GSH as a nutrient for growth [15]. As *F. tularensis* is a fastidious organism it requires cysteine in growth