Mini-review

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Stress proteins as agents of immunological change: some lessons from metallothionein

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INTRODUCTION

Under stressful conditions, the immune system can function in ways that are dramatically different from those found under normal circumstances. These stress-induced changes can correlate with the development of autoimmune disease (Bigazzi 1988), immunodeficiency (Lawrence 1981), or neoplasia (Kirkvliet and Baecher-Steppan 1982). Cells under stressful conditions will respond by producing a variety of stress response proteins, in large part to counter the deleterious effects of exposure to stressors, but a growing body of evidence suggests that these stress response proteins can also contribute to changes in immune function. That increases in stress protein synthesis can alter the immune response is not surprising, since a number of these proteins have roles essential to the development of normal immune function. For example, some of the heat shock proteins can contribute to antigen processing (Pierce et al 1991). Members of the heat shock family of proteins are encoded within the gene complex that regulates antigen presentation (Sargent et al 1989). Other stress proteins facilitate appropriate folding and assembly of intracellular immunoglobulin heavy chains (Munro and Pelham 1986). One of the contributions that ubiquitin makes is to direct cells of the immune response into appropriate circulation patterns within the body (Parakh and Kannan 1993). Under some circumstances, the production of stress proteins has been associated with the development of autoimmunity specific for the stress protein itself (for review see Kaufmann 1994), but it is also possible that stress proteins interact with other proteins or peptides to present them as targets for immune attack (Udono and Srivastava 1993).

Metallothionein (MT) is an interesting example of a stress response protein that has the potential for profound effects on the development of immune function, and lessons learned from the study of this protein may illuminate avenues of investigation that might be similarly pursued in the context of other stress proteins. Some of the potential interactions of MT are illustrated in Figure 1. One might expect that a thorough understanding of the roles that these stress proteins play in the immunomodulation caused by stressors would not only enable new therapeutic approaches to the treatment of immune disease, but would also offer the potential for management of immune responses under normal circumstances. Since MT has several unusual characteristics that distinguish it from other stress response proteins, we will first describe some details regarding the basic structure and function of this protein before going on to consider potential roles for MT as an intermediary in the development of stress-induced changes in immune response.

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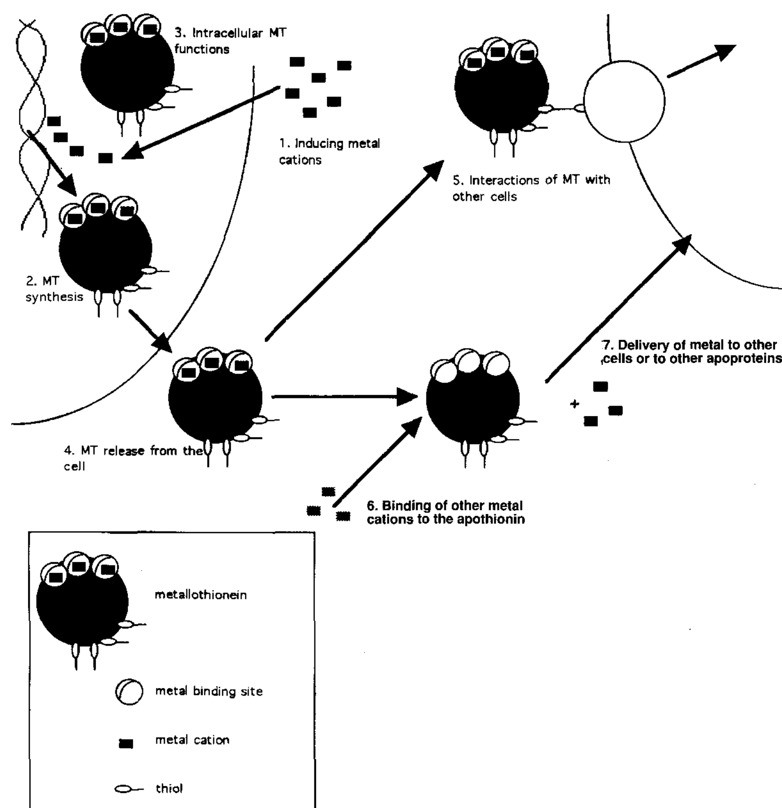
PROTEIN STRUCTURE AND METAL COMPOSITION

MT is a low molecular weight molecule (approximately 7 kDa) originally recognized as a metal-binding protein in equine kidney (Margoshes and Vallee 1957). MTs are characterized by an unusually rich cysteine content (approximately 30 mol %) and these stress proteins have



Fi⌣. 1 Potential interactions of MT with the immune response.

since been demonstrated to be present in a variety of species and synthesized by nearly every tissue. Mammalian MTs are comprised of four major isoforms (MT-I, -II, -III, and -IV), each of which is encoded by an individual gene. The two isoforms that are ubiquitously expressed, MT-I and MT-II, differ by a single amino acid charge at neutral pH (Huang et al 1977; Soumillion et al 1992). MT-III is a brain-specific isoform containing two insertions that distinguish it from MT-I and -II (a threonine after position 4 and six amino acids in the carboxy terminus) (Palmiter et al 1992). MT-IV also contains an insertion (glutamate after position 4) and is expressed only in differentiating squamous epithelia (Quaife et al 1994). There are two immunodominant epitopes present in MT, residues 1–5 (MDPNC) and 20–25 (KCKECK) (Garvey 1984).

Mammalian MTs consist of two domains: the β domain (amino acids 1-30) which has 9 cysteines, and the α domain (amino acids 31–61) which has 11 cysteines (Furey et al 1986; Hamer 1986). These cysteines are arranged in highly conserved Cys-Cys or Cys-X-Cys clusters that are distributed evenly throughout the amino acid sequence. Group Ib and IIb transition metals are organized within the protein backbone into a tetrahedral arrangement in which each metal cation is coordinated to four cysteines (Furey et al 1986; Robbins et al 1991). The general order of metal binding affinity to the protein is (Ag > Hg > Cu > Cd > Zn > Co = Ni) (Klaassen and Lehman-McKeeman 1989).

The specific metal content of MT varies depending upon the tissue and history of environmental exposure of the organism (Hamer 1986; Kagi and Schaffer 1988). In normal fetal and adult mammalian liver, the predominant metals associated with MT are copper and zinc (Webb 1987). These metals are thought to serve as a reservoir for metalloproteins essential to the growth and development of the organism. Despite the high affinity with which MT binds to metal cations, MT does have the capacity to exchange metals with other metalloproteins. In vitro, Zn-thionein can donate zinc to metal-requiring apoenzymes (Li et al 1980; Udom and Brady 1980; Churchich et al 1989) as well as extract zinc from metal-bound Sp1 (Zeng et al 1991).

GENE ORGANIZATION

The MT genes are arranged into a tripartite structure in which three exons are separated by two introns in precisely conserved positions. Traditional TATAA boxes, GC-rich regions and poly (A) adenylation sites are well

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defined (Glanville et al 1981; Shworak et al 1993) and both inducible and constitutive regulatory regions have been identified (for review see Andrews 1990).

Murine MT genes are tandemly arranged on chromosome 8 and expression of MT-I and MT-II is coordinately regulated, although MT-I is consistently induced approximately 1.4-fold more than MT-II (Palmiter 1987). The human MTs, which are located on chromosome 16, display much greater diversity than murine MTs. As in the mouse, MT-I and -II vary at position 11, but in man, MT-I itself displays microheterogeneity (Hunziker and Kagi 1985). While only one isoform of MT-II (hMT-IIA) has been identified, MT-I is recognized to have at least 8 distinct subforms (hMT-IA, -IB, -IE, -IF, -IG, -IH, -IX, and MT-0) (Karin et al 1984; Soumillion et al 1992; Pauwels et al 1994; Stennard et al 1994). In both mouse and human, MT-III and -IV are distinguished from MT-I and -II by characteristic amino acid insertions and highly restricted gene expression. There are also several MT pseudogenes which do not transcribe functional proteins (Karin and Richards 1982; Schmidt et al 1985; Stennard et al 1994).

INDUCING AGENTS

The predominant site of MT synthesis is the liver, although most non-hepatic cells, including lymphocytes and lymphoid tissues, are capable of producing MT under the appropriate stimulus (Harley et al 1989; Huerta et al 1989; Pauwels 1994). The only tissue specifically demonstrated not to produce MT is the testes of certain strains of rodents (Durnam and Palmiter 1981; Shiraishi et al 1995). Expression of MT is influenced by an array of stressors including heavy metal cations, inflammatory agents, free radicals and organic compounds.

The most potent inducers of MT are heavy metal cations. Cadmium and zinc can induce up to a 100-fold increase in hepatic MT (Klaassen and Lehman-McKeeman 1989). In hepatocytes, cadmium has been demonstrated to induce specific isoforms differentially (hMT-IE > IIA > IG > IF > IA > IB) (Taplitz et al 1986; Shworak et al 1993). Other heavy metals, including Au, Bi, Cu, Co, Hg, Fe, In, Mn, Ni and Pt, have been demonstrated to increase MT transcription, again to a different extent depending upon the physiologic tissue (Durnam and Palmiter 1981; Palmiter 1987; Klaassen and Lehman-McKeeman 1989). Differential induction may reflect differences in the strength of cis-acting metal regulatory elements (Carter et al 1984; Searle 1987; Radtke et al 1993), variations in the MRE-specific DNA-binding proteins (Labbe et al 1993; Radtke et al 1993; Heuchel et al 1994; Otsuka et al 1994), as well as accessibility of the tissue to the metal cation.

Physical stresses such as starvation, immobilization and exposure to extreme temperature also elevate MT synthesis (5- to 10-fold increase) (Hidalgo et al 1986; Giralt et al 1993; Sasagawa et al 1993). In these circumstances, there is evidence of a role for endocrine control in MT production, although the precise influence is unclear. Receptor-bound glucocorticoids which bind to cis-acting glucocorticoid regulatory elements (GRE) (Plisov et al 1994a, 1994b) have been demonstrated to both positively and negatively regulate MT synthesis (Hempe et al 1991; Min et al 1992). In part, it may be that different physiological pools of MT are individually regulated. For example, while both serum and hepatic MT levels are increased by acute immobilization, adrenalectomy prior to immobilization reduced serum MT but promoted an increase in hepatic MT (Hidalgo et al 1988).

Inflammatory agents such as lipopolysaccharide (LPS) or the inflammatory cytokines are modest inducers of MT, stimulating a 10- to 20-fold increase in MT levels (Brady 1982; Cousins and Leinart 1988; Min et al 1992). In vivo administration of LPS or turpentine, compounds which induce inflammatory responses, produced a 12-fold and 15-fold increase, respectively, in hepatic MT (Min et al 1992). As with heavy metal cations, induction of MT is both tissue- and isoform-specific (Karin 1985; Karin et al 1985; Choudhuri et al 1993). Limited information exists about the cis-acting DNA sequences responsive to acute phase response lymphokines and inflammatory molecules although an element required for induction by endotoxin has been defined (Durnam et al 1984; Hamer 1986).

MT expression is also upregulated in response to free radicals and ionizing radiation although induction appears to be mediated by secondary events. X-irradiation, for example, has been demonstrated to increase MT in liver (Shiraishi et al 1986) and this effect was observed only in intact animals. Irradiation of isolated cells did not induce MT, suggesting an indirect pathway of induction (Sato and Bremner 1993). Adrenalectomized animals still demonstrated elevated hepatic MT after irradiation, indicating that this MT increase was not via glucocorticoid effects (Shiraishi et al 1986; Sato and Bremner 1993). Both X- and UV-irradiation have been demonstrated to generate activated oxygen species including hydroxyl radical, superoxide anions and hydrogen peroxide which can cause tissue damage leading to release of inflammatory mediators and onset of the acute phase response (Halliwell and Aruoma 1991; Sato and Bremner 1993). In these circumstances, MT may be induced by the acute phase lymphokines.

Induction of MT by free radicals, organics, alkylating agents and oxidants is also thought to be mediated through the acute phase response that is induced by frank hepatotoxicity or the onset of inflammation (Daston et al 1991; Min et al 1991; DiSilvestro and

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Carlson 1992). Toxins such as CCl4, paraquat and benzene were found to elicit hepatic MT. Induction of MT by these agents paralleled increases in serum fibrinogen and ceruloplasmin, proteins characteristic of the acute phase response. Adrenalectomy did not diminish the degree of MT induction by these hepatotoxins indicating that induction of MT in these situations does not depend upon a glucocorticoid intermediary (Min et al 1991). CCl4 has been demonstrated to initiate hepatic lipid peroxidation (DiSilvestro and Carlson 1992) which can cause widespread membrane damage. Thus, inflammation induced by hepatoxins may elicit MT production.

In summary, inducible synthesis of MT-I and -II is regulated by indicators of biological stress. Some of these inducing agents (e.g. heavy metal cations or glucocorticoids) directly influence gene expression while other agents (e.g. irradiation, alkylating agents) alter MT expression by causing a physiological injury that subsequently promotes inflammatory- or hormone-mediated MT expression. In contrast, MT-III and -IV are not responsive to the traditional inducers of MT. MT-IV is responsive to zinc but not cadmium while MT-III is not induced by cadmium, zinc, dexamethasone or endotoxin (Palmiter et al 1992; Quaife et al 1994). Other agents to which these isoforms are responsive have not been identified, suggesting unique roles for these tissue-specific MTs.

MT PROTEIN TRAFFICKING

In hepatocytes, MT mRNA is predominantly associated with free polysomes. Less than 10% of the MT message is associated with membrane-bound ribosomes in either basal or induced hepatocytes, suggesting that MT is a largely intracellular protein (Shapiro and Cousins 1980; Palmiter et al 1992). Once translated, MT is found in almost every subcellular compartment including mitochondria, endoplasmic reticulum, nucleus and cytoplasm (Banerjee et al 1982; Goering and Klaassen 1983). However, MT is also found in significant quantities in normal (uninduced) physiologic fluids (human serum 0.01–1.0 ng/ml; human urine 1–10 ng/ml) and is present in elevated levels after stress (metal or restraint stress) (Garvey and Chang 1981; Garvey 1984; Hidalgo et al 1988). Metal-stressed individuals have been found to have significant increases in both serum and urine MT (Falck et al 1983; Dudley et al 1985; Chan et al 1992; Koyama et al 1992). These observations suggest that MT may be a secreted (or released) protein even though most MT mRNA is associated with free polysomes. It may that MT is released from cells via a pathway distinct from the common secretory pathway, as has been suggested for other stress proteins (Hightower and Guidon 1989). Alternatively, MT may be released either by passing through a damaged plasma membrane or from apoptitic cells that have avoided or exceeded the normal processing capacity of the organism.

PHYSIOLOGICAL FUNCTIONS OF MT

Even though MT is well characterized biochemically, the principal physiologic function of this protein remains unclear. As already noted, MT has been suggested to serve as a reservoir of essential metals such as zinc and copper, which are required for normal cell metabolism. MT is found in high concentration in rapidly dividing tissues (Brady 1982; Andersen et al 1983; Webb 1987; Gallant and Cherian 1989) and transformed cells undergoing rapid growth frequently have elevated levels of MT (Waalkes and Goering 1990; Cherian et al 1993). During murine fetal development, increases in MT precede thymic outgrowths and MT levels are reduced during involution (Olafson 1985). In the adult, MT levels are maximal preceding the regeneration process after partial hepatectomy (Margeli et al 1994; Tsujikawa et al 1994). Taken together, these data suggest that MT contributes to cell proliferation and differentiation.

The recently discovered isoforms, MT-III and -IV (which are not responsive to many of the traditional inducers of MT-I and -II), may have a role specific to cell maturation. Squamous epithelial cells have been shown to undergo a switch in expression from MT-I to MT-IV during differentiation, suggesting that MT-IV may promote events necessary for epithelial-specific development (Quaife et al 1994). In contrast to the growth-promoting effects of MT-I, -II and -IV, MT-III has been shown to inhibit neurotrophic activity in the brain, indicating a negative role for this MT isoform (Erickson et al 1994; Masters et al 1994b; Sewell et al 1995).

Another proposed role for MT is that of a cellular defense mechanism. Due to a high thiol content, MT is a strong nucleophile that can bind not only metal cations but also reactive oxygen intermediaries (ROI) and organic radicals (Chubatsu and Meneghini 1993; Sato and Bremner 1993). Preinduction of MT expression correlates with cell survival subsequent to exposure to otherwise lethal doses of metal cations, alkylating agents and free radicals and overexpression of MT has been demonstrated to protect cells from the toxic side-effects of several antineoplastic agents. In view of MT's nuclear and cytoplasmic locations, MT can confer protection to both DNA and cytoplasmic components (Banerjee et al 1982; Goering and Klaassen 1983; Kuo et al 1994). Damage to MT itself can be repaired (in vitro) by glutathione (Thornalley and Vasak 1985). It is possible that MT released to the extracellular environment may also have the capacity to serve as a biological nucleophile. Whether detoxification is the primary biological role of MT remains to be established.

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NON-PROTECTIVE EFFECTS OF MT

Although MT has the capacity to scavenge metal cations, free radicals and other biological toxicants, there are some instances in which MT does not play a protective role. For example, Cd,Zn-MT has been shown to actually promote genotoxicity (single-strand DNA damage) (Muller et al 1991, 1994). MT is also associated with the onset of metal-mediated nephropathy (Chan et al 1992; Wang et al 1993). Degradation of Cd-MT in the kidney caused localized release of the metals to the S1 and S2 segments of the proximal tubule, the primary sites of nephrotoxicity (Dorian et al 1995). MT can also cause physiologic damage by altering essential metal availability. MT induced by exposure of pregnant dams to styrene, a maternal but not developmental toxin, caused redistribution of zinc from the circulatory system to the maternal liver. Reduction in available zinc led to profound developmental toxicity in the fetus (Daston et al 1991).

DISORDERS ASSOCIATED WITH MISREGULATION OF MT

Several pathological disorders are associated with altered regulation of MT including Indian Childhood Cirrhosis (ICC), Menkes' disease and Wilson's disease. Individuals with ICC are uniquely sensitive to copper poisoning and manifest symptoms of toxicity upon exposure to environmental copper (e.g. via domestic copper piping). Hahn et al (1994) demonstrated that, while copper transport into cells was not altered, both basal and inducible expression of hMT-IIA (mRNA and protein) was markedly reduced in these patients. No mutations were found in the regulatory region of this gene, suggesting a defect in a trans-acting factor.

Menkes' disease is also marked by impaired copper regulation (Hunt and Clarke 1983; Leone et al 1985). Like ICC, copper uptake by cells is normal; however, individuals afflicted by Menkes' have an overabundance of MT. The synthesis of MT in these patients is sensitive to 4-fold lower concentrations of copper. Basal expression, zinc-, or glucocorticoid-induced MT were not different than controls, indicating a copper-specific defect. Again, a trans-acting mechanism is implicated as the disease is X-linked and thus the defect does not appear to lie within the MT genes themselves (Riordan and Jolicoeur-Paquet 1982; Leone et al 1985).

Patients with Wilson's disease have elevated copper levels in liver, brain, kidney and urine leading to eventual hepatic and neurological impairment. Although MT mRNA is only slightly elevated, these individuals have a substantial increase in MT protein. Research in the murine model of Wilson's disease, the toxic milk (tx) mutation, indicates that the increases in protein levels may actually be due to an increase in the half-life of hepatic MT (Hunt et al 1986; Koropatnick and Cherian 1993; Stephenson et al 1994). Whether the defect is due to structural alteration rendering the MT protease resistant or is a defect with the degradative enzymes themselves is not known. It has also been suggested that the primary defect lies in aberrant copper transport that leads secondarily to elevations in MT (Koropatnick and Cherian 1994; Mercer et al 1994).

In order to further explore the functions of MT, Palmiter et al (1993) have engineered mice that overexpress MT-I while two other research groups have independently developed mice lacking expression of both MT-I and -II (Michalska and Choo 1993; Masters et al 1994a). While maintained under ideal colony conditions, none of these mice display overt abnormalities. However, the mice deficient in MT were extremely susceptible to the effects of in vivo metal exposure and cells isolated from these mice were sensitive to the toxic effects of oxidative stress and anticancer agents (Kondo et al 1995; Lazo et al 1995). The MT knockout and MT transgenic mouse strains will each serve as an interesting model in which to study the physiologic roles of MT.

MT AND THE IMMUNE RESPONSE

In light of the rather dramatic changes to immune functioning that can be elicited by heavy metals and other inducers of MT, it is reasonable to consider the potential role of MT as a mediator of some forms of immunomodulation. Our laboratory has been engaged in a study of the ways in which MT might act to alter immunity, both in the context of metal- (and other toxicant-) induction of MT, and in the context of MT synthesized as a consequence of normal cellular responses to other inflammatory agents. One of our original observations showed that MT was capable of provoking murine lymphocyte proliferation, either when added alone to splenocyte cultures, or when added in the context of a T cell-specific mitogen (Con A) or B cell-specific mitogen (LPS). MT-mediated lymphoproliferation in the presence of mitogen was synergistic: MT plus mitogen was able to elicit dramatically higher levels of proliferation than was mitogen or MT alone (Lynes et al 1990). This proliferation appears to depend in part on binding to the lymphocyte plasma membrane. Binding of MT can be observed to occur with purified populations of T and B lymphocytes (Borghesi et al 1996), as well as to adherent macrophages (Youn et al 1995). Despite the binding of MT to the T cell plasma membrane, MT was unable to initiate proliferation in purified T cells unless added in the context of T cell mitogen (Fig. 2). In contrast, B cells were capable of responding to MT added alone, or in the

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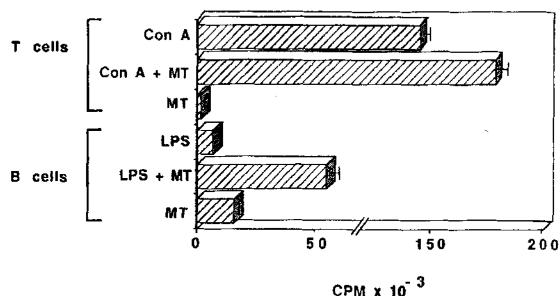
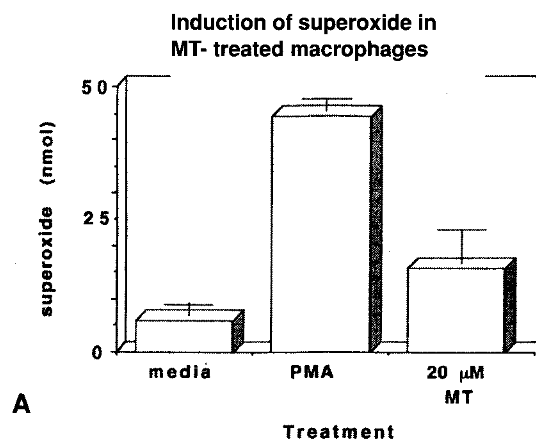


Fig. 2 Effects of MT on lymphocyte proliferation. Purified splenic T or B cells isolated from C3H/HeJ mice were cultured with mitogen (1 μg/ml Concanavalin A or 10 μg/ml lipopolysaccharide), MT (10 μM), or mitogen plus MT for 3 days. Proliferation was assessed by tritiated thymidine incorporation in triplicate cultures.



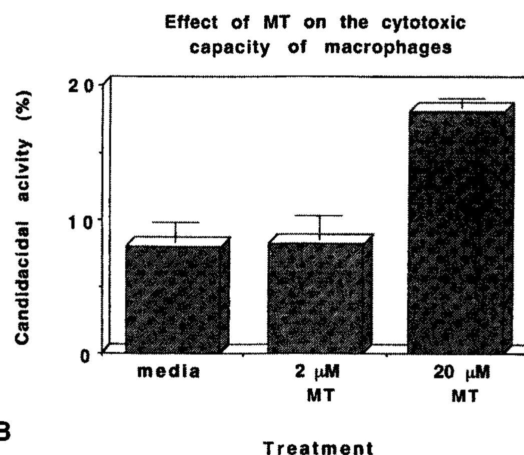


Fig. 3 Stimulatory effects of MT on macrophage activity. (A) Cultures of adherent BALB/cByJ macrophages were incubated with 20 μM MT or 80 nM PMA at 37°C. Generation of superoxide radicals was assessed by measuring the capacity of each sample to reduce the substrate, ferricytochrome C, in the prescence or absence of superoxide dismutase. (B) Macrophages were incubated with opsonized *Candida albicans* in the presence or absence of MT for 3 h. Following cytocentrifugation and fixation, the number of intracellular, dead yeast was assessed using Giemsa stain. Candidacidal activity was calculated as (number of intracellular dead yeast/total number of intracellular yeast) x 100.

context of mitogen. This may suggest that targets of MT binding on B cells include receptors that can mediate lymphoproliferative signals, but that the targets bound on the T cell plasma membrane are not involved in the lymphoproliferative response. The ability of MT to enhance the proliferation elicited by Con A in these T cell preparations appears to depend upon the potential of MT to serve as a scavenger of the oxidative by-products of metabolism. In support of this conclusion, MT can block the suppressive effects of N-ethylmaleimide, a thiol-specific agent that ordinarily is capable of suppressing the proliferative response of T cells to Con A. Intriguingly, MT is also able to induce lymphoproliferation in C3H/HeJ B cells in the presence of LPS (Fig. 2) (Borghesi et al 1996). This is somewhat surprising since B cells from this strain are otherwise refractive to stimulation by LPS alone, due to a defect in PKC translocation (Shinji et al 1994). This suggests that MT overcomes the PKC defect in these cells, or alternatively, circumvents the consequences of this defect.

MT also contributes to the functioning of macrophages. Leibbrandt and Koropatnick (1994) demonstrated that addition of LPS to the monocytic line THP-1 leads to a reduction in MT synthesis. This decrease in MT production is paralleled by an inhibition in the ability of the THP-1 cells to undergo an LPS-induced respiratory burst. Liebbrandt et al (1994) also used anti-sense RNA to specifically down-regulate MT production in THP-1 cells. Again, LPS-induced hydrogen peroxide production was significantly decreased in these cultures, as was the ability of these cells to adhere to a substratum or to invade a reconstituted basement membrane. These data suggest that intracellular MT may contribute to increases in monocyte activation, and that reduction in MT expression can limit the efficacy of this arm of the immune response. As shown in Figure 3A, extracellular MT can induce a modest but significant superoxide burst in peritoneal macrophages (Youn et al 1995). This increase in metabolic activity parallels an increase in the ability of these cells to kill phagocytized yeast, but there is no effect on attachment or engulfment of the yeast by these macrophages (Fig. 3B). It may be that increased release of MT synthesized after an encounter with a stressor serves to activate macrophages in ways that subsequently cause bystander damage to surrounding tissue. If this is the case, a blockade of cellular interactions with extracellular MT would

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have important value in mitigating the damage associated with inflammation.

MT also has effects on the in vivo humoral immune response. Mice immunized with either ovalbumin (OVA) or sheep erythrocytes (sRBC) in the presence of MT developed significantly lower antibody responses to either antigen. For example, mice co-injected with OVA in the presence of MT had a 20–30% decrease in circulating anti-OVA IgG as compared to animals injected with OVA alone, even though the kinetics of the response was similar in both treatment groups (Lynes et al 1993). A monoclonal antibody selected to be specific for MT was able to block this immunosuppression. In the MT-treated animals, total serum IgG levels were unchanged, indicating that MT did not cause a global immunosuppression. In an in vitro system of antigen presentation, MT was found to be capable of dramatic inhibitory effects on the capacity of macrophages to stimulate T cell proliferation.

The molecular mechanisms by which MT mediates changes in the immune system remain to be clarified. Some of the interactions described above depend upon thiols within the protein backbone. If the MT is alkylated, or treated in a fashion that allows oxidation of the thiols, the ability to stimulate lymphoproliferation can be eliminated. Moreover, addition of 2-mercaptoethanol can also reduce the mitogenic potential of MT. While it is possible that some of the effects attributed to MT depend upon the associated metal cations, and that the thionein serves simply to deliver those cations to target tissues, certain of these immunomodulatory effects cannot be ascribed to the metals. We know, for example, that Cd added to cells as a salt, in amounts equimolar to that added with MT, will be toxic to these cells. Moreover, experiments with apothionein have shown that the protein backbone in the absence of metal cations is capable of some of these same immunomodulatory activities. Finally, the observation that anti-MT antibody can suppress the effects of MT on humoral responses supports the idea that it is not simply the presence of the metal cations that cause MT-mediated immunosuppression.

SUMMARY

The stress response proteins each have somewhat unique characteristics that enable them to function under conditions of cellular stress, and to contribute to cellular survival in difficult times. The immune response is, by definition, a mechanism that often operates in times of cellular stress, and even creates stress during its operation. Cells called upon to respond to tissue damage caused by inflammation can have extraordinary demands placed upon them and surrounding tissue may suffer damaging conditions that were originally established to eliminate the source of the inflammation. Stress proteins may be released from some of these damaged cells as a programmed response to the stress, or as a simple consequence of excessive damage to the plasma membrane. In either instance, there is the opportunity for these stress proteins to interact with cells and proteins in the extracellular environment. It may be that those same characteristics that enable stress proteins to interact with structures within the cell also enable interactions outside the cell, but with dramatically different results. As has been found with MT, interference with these extracellular interactions may decrease the consequences of stress on the immune response, and may enable more effective immunity. It may also be possible to employ the various stress proteins to manipulate normal immune function.

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