DISCUSSION
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The immune system has tremendous discrimination potential of self and non-self. However, this enormous recognition potential includes possible interaction with self components, some of which may appear to be essential for the regulation of the immune functions (Alam et al., 1992) whereas others can be pathogenic, particularly if expressed at high level, leading to autoimmune diseases.

Systemic Lupus Erythematosus (SLE) is a chronic, usually life-long, potentially fatal prototype autoimmune disease characterized by unpredictable exacerbations and remissions with protean clinical manifestations. In SLE there is a predilection for clinical involvement of the joints, skin, kidney, brain, serosa, lung, heart and gastrointestinal tract. SLE is a complex disorder affecting a predominately young population and shares similarities with HIV infection as regards the propensity for multiple organ involvement, potentially life-threatening episodes, and need for sophisticated monitoring. The clinical features of SLE are protean and may mimic infectious mononucleosis, lymphoma, or other systemic disease. The origin of autoantibody production in SLE is unclear but a role has been suggested for an antigen driven process, spontaneous B-cell hyper-responsiveness, or impaired immune regulation. Thus, the etiology of SLE remains unknown and warrants in-depth investigation for the management of the disease. Evidences exist for the involvement of mycobacterial protein antigens in the pathogenesis of autoimmune diseases (Tasneem et al., 2001). Despite current treatment regimens, tuberculosis continues to confound attempts at control, fuelling an urgent need for developing novel therapeutic strategies.

Although attenuation of the biological activity of TNF-α has lately become an important therapeutic intervention in the management of a wide variety of chronic inflammatory diseases (Shanahan and St Clair, 2002), a growing body of clinical evidence indicates that neutralization of TNF-α is associated with an increased risk of opportunistic infections, including mycobacterial diseases (Dinarello,
2003), and autoimmune disorders. In view of this, modulation of TNF-α release is being proposed as the basis for novel therapeutic approaches (Warwick-Davies et al., 2001). Focus has now shifted to development of compounds from natural sources that have anti-inflammatory and antimycobacterial activity. By boosting host immunologic responsiveness, these compounds may be particularly useful in the treatment of autoimmune disorders as well as drug-resistant tuberculosis. Our study involves the incorporation of such a compound, namely EGCG – a green tea polyphenol, as the natural herbal component for SLE and SLE-TB management.

Reports indicate TNF-α exerting its effects through its receptors namely TNFR-I and TNFR-II is a central mediator of inflammation (Islam et al., 2004), immunity and autoimmunity and that; it plays a crucial role in host defense. Inhibition of TNF-α clearly predisposes to certain infections, such as granulomatous infections like TB. Inhibition of TNF-α may also play a role in autoimmunity (Malemud et al., 2003; Islam et al., 2002) although the pathophysiologic mechanisms are uncertain. Furthermore, cases of TB have been reported in patients treated with TNF-α antagonists. The risk for TB in RA/SLE patients is associated with multiple other factors including age, country of origin or current residence, exposure history to persons with TB, concomitant therapy with other immunomodulators including corticosteroids, and disease activity. TNF-α antagonists have been associated with an increase in the percentage of RA patients with positive serologies (ANA and anti-ds-DNA) and lupus-like syndromes.

Cellular signalling by TNF-α is mediated mainly through activation of NF-κB (Baeuerele and Baltimore, 1996). In turn, activation of NF-κB and other pathways sustain TNF-α activity (Ropert et al., 2001). During characterization studies in order to assess the role of NF-κB in the expression of TNF-α in SLE, TB and SLE-TB monocytes, we employed SN50, an inhibitor of NF-κB. From our results,
it was apparent that the induction of TNF-α expression was mediated through activation of NF-κB, because TNF-α was suppressed when SN50 was present in cultures. The inactive analogue of SN50 (i.e. SN50M) did not have any effect. Thus, as previously reported in macrophages (Islam et al., 2004), this study also shows that cellular activation is associated with augmentation of expression of both TNF-α in monocytes of patients with SLE, TB and SLE-TB.

Putative and effective host defense mechanisms by innate immune cells to *M. tuberculosis* within the phagolysosomes of activated macrophages include the production of reactive oxygen (ROI) and reactive nitrogen intermediates (RNI). Phagocytosis of microbes as well as cellular activation activates ROIs (Takao et al., 1995), and H2O2, a product of the ROI pathway, activates the expression of iNOS and production of NO (Han et al., 2001). Both ROI and RNI are downstream mediators of macrophage-activating cytokines and are thought to be microbicidal. Activation of iNOS and production of NO may be important in the final containment of *M. tuberculosis* by macrophages (MacMicking et al., 1997). However, *M. tuberculosis* has evolved resistance mechanisms against both, ROI (Hillas et al., 2000) and RNI (St John et al., 2001).

Cell viability and potential cytotoxicity of EGCG, if any, on monocytes obtained from patients with SLE, TB and SLE-TB were determined for the concentrations (0-25 μg/ml) employed in this study using trypan blue and MTT assays, where viability of ~98-99% was observed. Interestingly, no effect of EGCG was observed on human housekeeping genes like R18 rRNA, thereby demonstrating that the effect of EGCG was not mediated by cellular death, but rather by specific inhibition of expression as well as secretion of TNF-α.

To the best of our understanding, in our characterization studies, we show for the first time that EGCG exerts potent anti-inflammatory effects on host mononuclear cells obtained from patients of SLE, TB and SLE-TB, as evidenced by a strong inhibition of the pro-inflammatory cytokine TNF-α. The results indicate an
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GPx catalyzes the removal of hydrogen peroxide (Mesiter and Anderson, 1983). Decrease in GPx activity indicates impairment of hydrogen peroxide-neutralizing mechanisms (Rukmii: et al., 2004). In the present study, reduced / suppressed GPx activity in patient monocytes that were untreated or treated with SN50/M was recorded, thereby concurring with earlier reports that substantial amounts of ROS are being generated in patient cells due to cellular activation (Islam et al., 2004). Enhancement of GPx activity in monocyte cultures from patients with SLE, TB and SLE-TB after addition of NAC, a precursor of the *in vivo* antioxidant glutathione, indicates reversal of impaired neutralizing mechanisms. Surprisingly, GPx activity was observed to be further augmented when EGCG was co-cultured instead of NAC, indicating EGCG to be an effective natural antioxidant combating ROS, generated as a consequence of cellular activation in mononuclear phagocytes. Moreover, in continuation to the above, our data further shows the lipid peroxidation-induced augmented MDA levels in culture supernatants of monocytes from patients with SLE, TB and SLE-TB were appreciably suppressed or down-regulated with EGCG.

Our results are strongly indicative for the appreciable correlation between autoimmune diseases like SLE and *Mycobacterium tuberculosis* infection. Inhibition ELISA results strongly suggests that all the TB positive sera selected in this study were having a high degree of specificity towards MTB Ag85B (30kDa). Similarly, our data show high degree of recognition of anti-DNA antibodies found in SLE sera by native dsDNA. The achievement of 50% inhibition in antibody activity in both the above diseases i. e. TB and SLE at a very low inhibitor concentrations are indicative for the presence of highly specific respective antibodies in all the sera selected for this study.

One of the interesting findings of this study was that the SLE patients were found to be more susceptible to TB development than the vice-versa case. This is evident from the data indicating that autoantibodies found in SLE sera strongly recognized both native dsDNA as well as mycobacterial Ag85B 30kDa, whereas
anti-TB antibodies found in TB sera strongly recognized only Ag85B 30kDa, whereas it exhibited low or negligible recognition with native dsDNA. However, antibodies found in sera of patients suffering with both TB along with SLE (SLE-TB) revealed high specificity for both the antigens i.e. native dsDNA and MTB 30 kDa.

An attempt was also made to evaluate the immuno-binding by generating in-vivo conditions i.e. by using cultured monocytes that were infected with bacilli (TB patients) as well as monocytes from SLE patients. The high percent maximum inhibitions as well 50% inhibitions in anti-TB and anti-DNA activities at extremely low inhibitor concentrations are suggestive for the appreciably high affinity immuno-interaction occurring between anti-TB antibodies and anti-DNA autoantibodies with antigens in monocyte protein lysates as well as respective dsDNA of TB patients and SLE patients respectively.

A striking finding in the present study is the EGCG as well as reduced glutathione-induced down-regulation in binding of anti-TB antibodies and anti-DNA antibodies with antigens present in protein lysates prepared from monocyte of TB patients and SLE patients respectively. Similar actions of reduced glutathione in monocytes obtained from blood of patients having both TB along with SLE further substantiates the antioxidant-induced suppression in immuno-binding. Furthermore, these results are correlating appreciably with the amount of TNF-α secreted in different monocyte cultures undertaken in this study. The importance of glutathione and its involvement in the above actions in monocytes of TB and SLE patients undertaken in our study could be best viewed by the following mentioned relevance of glutathione. Glutathione (g-glutamylcysteinylglycine, GSH) is a sulphydryl (-SH) antioxidant, antitoxin, and enzyme cofactor, which often attains millimolar levels inside cells, which makes it one of the most highly concentrated intracellular antioxidants. The reducing power of GSH is a measure of its free-radical scavenging, electron-donating, and sulphydryl-donating capacity. Reducing power is also the key to the multiple
actions of GSH at the molecular, cellular, and tissue levels, and to its effectiveness as a systemic antitoxin (Meister et al., 1994). Experimental GSH depletion can trigger suicide of the cell by a process known as apoptosis (Slater et al., 1995). Antioxidants are the body's premier resource for protection against the diverse free radical and other oxidative stressors to which it invariably becomes exposed (Cross et al., 1987). The antioxidant defense system is sophisticated and adaptive, and GSH is a central constituent of this system. Nowhere is its presence more important than in the mitochondria. The liver seems to have two pools of GSH; one has a fast turnover (half-life of 2-4 hours), while the other is avidly retained with a half-life of about 30 hours (Meister et al., 1995). The first corresponds to cytosolic GSH, the second mainly to mitochondrial GSH which is known to be more tightly held.

Being directly in the path of airborne materials, the lung tissue is particularly at risk from oxidative stressors such as cigarette smoke, atmospheric pollutants, and other inhaled environmental toxins (Kidd, 1985). GSH and GSH-associated enzymes present in the epithelial lining fluid (ELF) of the lower respiratory tract may be the first line of defense against such challenges (Deleve and Kaplowitz, 1990; Pacht et al., 1991). Sustained oxidative challenge to the lung results in depletion of GSH and other antioxidants from the lungs.

As with other cell types, the proliferation, growth, and differentiation of immune cells is dependent on GSH. Both the T and the B-lymphocytes require adequate levels of intracellular GSH to differentiate, and healthy humans with relatively low lymphocyte GSH were found to have significantly lower CD4 counts (Kinscherf et al., 1994). Intracellular GSH is also required for the T-cell proliferative response to mitogenic stimulation, for the activation of cytotoxic T "killer" cells (Droge et al., 1994), and for many specific T-cell functions, including DNA synthesis for cell replication, as well as for the metabolism of interleukin-2, which is important for the mitogenic response (Wu et al., 1994).
Experimental depletion of GSH inhibits immune cell functions, sometimes markedly (Fidelus and Tsan, 1987), and in a number of different experimental systems the intracellular GSH of lymphocytes was shown to determine the magnitude of immunological capacity (Droge et al., 1994). Thus intracellular GSH status plays a central role in the functioning of immune cells. In the auto-immune diseases of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), and as seen in aging, T lymphocytes demonstrate depressed responsiveness to antigens and mitogens, perhaps because of insufficient IL-2 production (Fidelus and Tsan, 1987). Patients with RA have low blood sulfhydryl (-SH) status (Fidelus and Tsan, 1987), as did patients with Type II diabetes or with ulcerative colitis (Fidelus and Tsan, 1987).

Chronic viral infections may also trigger GSH depletion in circulating immune cells. Patients chronically infected with hepatitis C virus have low GSH in their circulating monocytes. Monocyte GSH levels are abnormal in early HIV-1 disease (Droge et al., 1997), then in patients with advanced disease the GSH levels normalized in monocytes but the GSH/GSSG ratio became abnormal. Significant decreases in the plasma levels of both cysteine and cystine has also been documented in subjects with HIV-1 infection (Droge et al., 1997). Since cysteine is a rate-limiting precursor for GSH synthesis, an associated decrease of GSH in the lung ELF was highly suggestive of a systemic GSH insufficiency in these subjects (Buhl et al., 1989). The most marked GSH decreases occurred in subjects who were asymptomatic but had CD-4 counts below 400. Both the abnormal cytokine expression and the progression to weight loss seen in HIV-1 disease may be linked (at least in part) to abnormalities in the uptake of GSH precursors by immune cells of HIV-1 subjects, and/or to abnormalities in their synthesis of GSH.

To have further in-sight, the present study involves utilization of dsDNA isolated from monocyte cultures of SLE, TB and SLE-TB patients that were either untreated or treated with EGCG, NAC, SN50 and SN50M, an in turn, employed
as antigens / inhibitors in immunoassays against anti-DNA SLE antibodies and anti-TB antibodies respectively. Thus, interesting important observations were made. We observed that purified anti-DNA antibodies from SLE patients exhibited high degree of recognition / specificity against dsDNA isolated from monocytes of patients with SLE, TB and SLE-TB respectively. However, this high magnitude specificity / binding of purified anti-DNA antibodies from SLE patients was reduced or suppressed appreciably towards respective dsDNA isolated from monocytes of patients with SLE, TB and SLE-TB respectively that were treated with EGCG, NAC or SN50. On the contrary, anti-TB antibodies exhibited high binding only with dsDNA isolated from monocytes of patients with TB, but failed to show any significant recognition / binding with dsDNA isolated from monocytes of patients with SLE. Furthermore, when monocyte cultures of SLE, TB and SLE-TB patients that were treated with EGCG, NAC and SN50, and in turn, respective dsDNA isolated and employed in ELISA, reduced or insignificant binding was observed against anti-dsDNA antibodies from SLE patients or anti-TB antibodies from TB patients. Thus, in view of the fact that native DNA is a poor immunogen, and that, the exact trigger of anti-DNA production in SLE still remains poorly understood, where DNA has been thought to act only as a cross-reacting antigen, the results indicate possible involvement of Mycobacterium tuberculosis protein(s) / nucleic acid antigens(s) in providing an alternate trigger / origin for autoantibody production in systemic lupus erythematosus (SLE). Moreover, the data generated in the present study is suggestive for the fact that reactive oxygen species (ROS) generated as a consequence of stress of any kind in autoimmune SLE results in the activation of proinflammatory cytokine TNF-α, which in turn results in the production of anti-DNA auto antibodies. These SLE patients having high ROS levels become highly susceptible to MTB infection. Upon MTB infection, the ROS and TNF-α potentiates or activates the MTB 85B replication in SLE-TB patients. Such an activation of ROS or TNF-α and in turn the SLE and TB proliferation could be arrested or limited by the usage of EGCG- a green tea polyphenol and reduced glutathione as revealed by our
data. In conclusion, based on characterization studies, followed by immunological data, it can be inferred from the present study that:

- There exist high basal levels of TNF-α in sera as well as in monocyte cultures of patients with SLE, TB and SLE-TB.
- Activation of monocytes by *M. tuberculosis* infection in SLE patients induces the expression of both TNF-α at both the gene and protein levels.
- Both RNI and ROI, induced early after infection of SLE monocytes, increases expression of TNF-α.
- Activation of monocytes by *M. tuberculosis* initiates a cascade of events whereby a vicious circle may exist in which expression of host inflammation and mycobacterial products amplify one another.
- EGCG (0-25 µg/ml) exhibited no toxic effect on the viability of human monocytes.
- EGCG inhibits the expression of TNF-α protein production in a dose-dependent manner in 24 hr monocyte cultures from patients with SLE, TB and SLE-TB, and that, it is mediated mainly via NF-κB.
- EGCG ameliorates the glutathione peroxidase activity in monocytes from patients with SLE, TB and SLE-TB.
- EGCG suppresses the augmented MDA levels in monocytes from patients with SLE, TB and SLE-TB.
- All the *M. tuberculosis* and SLE sera involved in this study showed a high degree of specificity for Ag85B (30 kDa) and native dsDNA respectively.
- Mycobacterial 30 kDa protein antigen (Ag85B) as well as protein
lysates prepared from monocytes of *M. tuberculosis* patients were recognized appreciably by anti-tuberculosis antibodies present in *M. tuberculosis* sera, whereas non-mycobacterial native dsDNA showed poor recognition with the same anti-tuberculosis antibodies.

- On the contrary, both non-mycobacterial native dsDNA and protein lysates prepared from monocytes of SLE patients as well as mycobacterial 30 kDa protein antigen (Ag85B) were found to be recognized appreciably by anti-DNA autoantibodies present in SLE sera.

- Furthermore, co-culturing of monocytes obtained from *M. tuberculosis*, SLE or *M. tuberculosis*-SLE with 10 nM of reduced glutathione showed amelioration of ROS and TNF-α induced actions, which in turn, subsequently suppressed the immuno-bindings observed in monocytes of *M. tuberculosis* and SLE patients cultured without glutathione.

- Our data shows that SLE patients are more susceptible to *M. tuberculosis* development, as ROS and TNF-α in SLE patients could activate the replication of Ag85B (30 kDa) after bacilli infection.

- Finally, immunoaffinity purified anti-DNA antibodies from SLE patients recognized dsDNA isolated from monocytes of both SLE and TB patients, but on the contrary, anti-TB antibodies recognized dsDNA only from monocytes from TB patients but not SLE patients.