RESULTS
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The present study was carried out in four distinct parts – (I) evaluation of clinical profile of patients whose blood or serum were undertaken in this study was carried out, (II) characterization of monocytes from patients with SLE, TB and SLE-TB whose untreated / treated monocytes in turn would be utilized as valuable immuno-reagents, (III) regulation / modulation of above monocytes and (IV) immunological investigations of the above characterized / modulated monocytes and clinically evaluated sera’s.

Thus, first of all, prior to any study, evaluation of clinical profile of patients whose blood or serum were undertaken in this study was carried out, which are described as under:

(I): CLINICAL PROFILE OF PATIENTS

A. Classification of patients

A total of 100 subjects were selected for clinical evaluation, where 10 were healthy normal subjects that served as controls. The remaining ninety study group patients (100%) comprised of 30 SLE patients (33.3%), 30 TB patients (33.3) and 30 SLE-TB patients (33.3%).

B. Distribution of sex in clinically classified patients

Next, the patients and control subjects undertaken in this study were distributed with respect to sex. Thus, out of 10 healthy normal subjects, 5 were males (50%) and 5 females (50%). The 30 SLE patients comprised of 10 males (33.3%) and 20 females (66.6%). Similarly, out of 30 TB patients, 10 were males (33.3%) and 20 females (66.6%). The same is true for SLE-TB patients, where out of 30 such patients, 10 were males (33.3%) and 20 were females (66.6%).

C. Distribution of Age

The above clinically classified patients were of 4 age groups, and are summarized in Table 3. Briefly, the 4 types of age groups were: Group 1= 20-35
yrs, Group 2 = 36-50 yrs, Group 3 = 51-65 yrs and Group 4 = 66-80 yrs. In control subjects, 20% subjects were from age group 1, whereas 30% each were from age group 2 and 3 respectively. Finally, there were 20% subjects from age group 4.

D. Montoux Test

The initial detection of TB infection was detected by Montoux test. The following Table IV shows the distribution of Montoux Test (+ve/-ve) in control, TB and SLE-TB patients. None of the healthy control subjects or SLE patients responded towards Montoux test. On the contrary, 100% TB patients as well as SLE-TB selected in this study showed a positive Montoux test. The observation of positive Montoux test in TB and SLE-TB patients selected in this study indicates the presence of bacilli infection in the selected host. The distribution of Montoux test is depicted in Table 1.

TABLE I

Distribution of Montoux Test in control, TB, SLE and SLE-TB patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of cases</th>
<th>Montoux Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Normal control</td>
<td>healthy</td>
<td>10</td>
</tr>
<tr>
<td>SLE</td>
<td>30</td>
<td>Nil</td>
</tr>
<tr>
<td>TB</td>
<td>30</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>SLE-TB</td>
<td>30</td>
<td>30 (100%)</td>
</tr>
</tbody>
</table>
E. Blood Glucose levels in SLE, TB and SLE-TB patients

All the patients and control samples undertaken in this study were subjected to evaluation of blood glucose levels. Healthy normal subjects showed normal blood glucose levels. Interestingly, 16% SLE patients of age group 51-65 yrs (both sex) exhibited a slightly augmented blood glucose level (mean range: 167-183 mg%). Similarly, 19% TB patients of age group 66-80 yrs showed to have elevated blood glucose (mean range: 171-187 mg%). Also, 20% patients having both SLE along with TB (SLE-TB) in the age group of 51-65 yrs and 66-80 yrs showed an enhanced blood glucose level falling under the mean range of 173-193 mg%.

F. Distribution of Anti-dsDNA

Direct binding ELISA analyzed patients and control samples undertaken in this study for anti-dsDNA evaluation. The observations made are summarized in Table VI. Healthy normal subjects showed negative anti-dsDNA results as the titre of anti-dsDNA was found to be less that 1:100. It is to be pointed out here that any anti-dsDNA titre, which is equal to or greater than 1:1600, is considered to be positive. Similarly, all the TB patients selected in this study exhibited an anti-dsDNA titer of <1:800, thereby suggesting for the fact that none of the TB patient's sera had any anti-dsDNA antibody in it. On the contrary, both SLE and SLE-TB patient's sera showed an anti-dsDNA titre that was greater than 1:128000, thereby indicating for the presence of high titer of anti-DNA autoantibodies (P<0.001).

G. Chest X-Ray

SLE patients had no evidence of cavity formation/fluffy shadow on chest X-ray to suggest pulmonary kochs. On the contrary, both TB and TB-SLE groups of patients exhibited pulmonary kochs. The summary is depicted below in Table II.
### TABLE II

Distribution of Chest X-ray in TB, SLE-TB and SLE patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>%Age</th>
<th>Chest X-ray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>10</td>
<td>100</td>
<td>Mean observation</td>
</tr>
<tr>
<td>TB</td>
<td>30</td>
<td>33.3</td>
<td>No cavity / fluffy shadow</td>
</tr>
<tr>
<td>TB-SLE</td>
<td>30</td>
<td>33.3</td>
<td>Cavity/fluffy shadow in all cases</td>
</tr>
<tr>
<td>SLE</td>
<td>30</td>
<td>33.3</td>
<td>No cavity/fluffy shadow</td>
</tr>
</tbody>
</table>

(II) CHARACTERIZATION OF MONOCYTES FROM NORMAL HEALTHY AND PATIENTS WITH SLE, TB AND SLE-TB:

1. Comparative study on the human housekeeping gene of human monocytes from normal controls versus patient with SLE, TB and SLE-TB:

Prior to any investigation, an attempt was made to probe the adverse effect of disease activity in monocytes of patients with SLE (n=3; P<0.001), TB (n=3; P<0.001) and SLE-TB (n=3; P<0.001) in comparison to normal healthy controls (n=3), if any, on the human housekeeping gene. It was observed that disease activity in 24 hr monocyte cultures failed to exert any adverse effect on the host housekeeping genes like R18 gene (18S rRNA) as revealed by quantitative real-time RT-PCR (Fig. 1).

2. Basal levels of TNF-α expressions at the gene and protein levels in 24 hr monocyte cultures from normal healthy controls and patients with SLE, TB and SLE-TB.
Thereafter, we investigated the basal levels of TNF-α mRNA expressions in 24 hr monocyte cultures from normal healthy controls (n=8) and patients with SLE (n=8) TB (n=8) and SLE-TB (n=8). TNF-α mRNA was corrected to host internal control, i.e., 18S rRNA, in the same sample. As evident from Fig. 2, the basal levels of TNF-α mRNA copy numbers in patients with SLE, TB and SLE-TB were recorded as 6.6 logs (P<0.001), 6.3 logs (P<0.001) and 7.7 logs (P<0.001) respectively. No or negligible TNF-α mRNA expression was observed in normal healthy controls (Fig. 2).

Thus, the magnitude of TNF-α mRNA expression in monocytes was of the order of SLE-TB > SLE > TB patients respectively.

Next, we further probed the basal secreted TNF-α expressions in culture supernatants of the above monocyte cultures that were harvested after 24 hrs. As evident from Fig. 3, the concentration of soluble TNF-α secreted in supernatants was observed as 194.3 (P<0.001), 183.2 (P<0.001) and 221.2 (P<0.001) pg/ml from patients with SLE (n=8), TB (n=8) and SLE-TB (n=8) respectively. Again, no or negligible TNF-α protein expression was observed in normal healthy controls (n=8) (Fig. 3).

3. Basal levels of iNOS mRNA expressions in 24 hr monocyte cultures from normal healthy controls and patients with SLE, TB and SLE-TB.

Next, we investigated the basal levels of iNOS mRNA expressions in 24 hr monocyte cultures from normal healthy controls (n=8) and patients with SLE (n=8) TB (n=8) and SLE-TB (n=8). iNOS mRNA was corrected to host internal control, i.e., 18S rRNA, in the same sample. As evident from Fig. 4, the basal levels of iNOS mRNA copy numbers in patients with SLE, TB and SLE-TB were recorded as 5.3 logs (P<0.001), 5.4 logs (P<0.001) and 5.9 logs (P<0.001) respectively. No or negligible iNOS mRNA expression was observed in normal healthy controls (Fig. 4). Thus, the magnitude of iNOS mRNA expression in monocytes was of the order of SLE-TB > SLE > TB patients respectively.
4. Basal levels secreted IFN-γ protein expressions in 24 hr monocyte cultures from normal healthy controls and patients with SLE, TB and SLE-TB.

Next, we further probed the basal secreted IFN-γ expressions in culture supernatants of the above monocyte cultures that were harvested after 24 hrs. In comparison to normal healthy control cultures (167.85 pg/ml), the concentration of soluble IFN-γ was found to be down-regulated to 43.56 (P<0.001), 41.23 (P<0.001) and 38.26 (P<0.001) pg/ml in cultures from patients with SLE (n=8), TB (n=8) and SLE-TB (n=8) respectively (Fig. 5).

5. Glutathione peroxidase (GPx) activity in 24 hr monocyte cultures from normal healthy controls and patients with SLE, TB and SLE-TB.

Next, an attempt was made to probe the glutathione peroxidase (GPx) activities in culture supernatants of the above monocyte cultures that were harvested after 24 hrs. Normal healthy control cultures exhibited a GPx activity of 57.22 U/mg proteins (Fig. 6). As expected, GPx activities were suppressed to the order of 29.25 (P<0.001), 25.14 (P<0.001) and 20.48 (P<0.001) U/mg protein in cultures from patients with SLE (n=8), TB (n=8) and SLE-TB (n=8) respectively (Fig. 6).

(III) REGULATION STUDY:

1. Dose response effect of NAC on the expressions of secreted TNF-α protein in 24 hr monocyte cultures from normal healthy controls and patients with SLE, TB and SLE-TB.

Monocytes obtained from patients with SLE (n=3), TB (n=3) and SLE-TB (n=3) were co-cultured in RPMI-1640 media with varying doses of NAC (0-10 mM) for
24 hrs at 37C/5%CO₂. Thereafter, the cells were harvested and subjected to secreted TNF-α protein expression analysis by ELISA. As expected, normal healthy control cells failed to show any effect of NAC as no or negligible TNF-α was recorded in untreated cells. However, NAC exhibited an appreciable dose-dependent suppression in secreted TNF-α protein expressions in 24 hr monocyte cultures from patients with SLE (n=3), TB (n=3) and SLE-TB (n=3) respectively (Fig. 7; P<0.001 for all three groups).

2. Toxicity assessment of EGCG:
Prior to probing the effect of EGCG, an attempt was made to check the toxic effect, if any, monocytes from patients with SLE (n=3), TB (n=3) and SLE-TB (n=3) respectively. For this purpose, we chose a dose in between 0 – 25 μg/ml of EGCG (dose-response assay) as this dose has previously been established in our laboratory in healthy monocytes. The results depicted in Fig. 8 (P<0.001), failed to show any toxic effect of EGCG (0 – 25 μg/ml) on monocytes as revealed by MTT (Fig. 8) or trypan blue exclusion assays. Cell viability is expressed as percent (mean ± SEM) viable cells compared to untreated cells (taken as 100% viable). Also, no effect was observed on housekeeping genes like R18 (18S rRNA) by quantitative real-time RT-PCR (Fig. 9), thereby indicating that EGCG did not non-specifically affect human TNF-α transcription.

3. Dose response effect of EGCG on the expressions of secreted TNF-α protein in 24 hr monocyte cultures from normal healthy controls and patients with SLE, TB and SLE-TB.
Next, monocytes from patients with SLE (n=5), TB (n=5) and SLE-TB (n=5) were co-cultured with varying doses of EGCG (0-25 μg/ml) for 24 hrs at 37C/5%CO₂. After harvesting, the cells were subjected to analysis of secreted TNF-α protein expression by ELISA.
As expected, normal healthy control cells failed to show any effect of EGCG as no or negligible TNF-α was recorded in untreated cells. However, EGCG exhibited an appreciable dose-dependent suppression in TNF-α expressions in 24 hr monocyte cultures from patients with SLE, TB and SLE-TB respectively (Fig. 10; P<0.001 for all). The IC_{50} was computed out to be ~ < 5 μg/ml.

4. Effect of SN50 and SN50M on the expressions of secreted TNF-α in 24 hr monocyte cultures from normal healthy controls and patients with SLE, TB and SLE-TB in order to probe the role of NF-κB:

Induction of TNF-α as a consequence of cellular activation is mediated via NF-κB (Toossi et al., 1997; Fan et al., 2002, Hasan et al., ?006). Reports indicate that TNF-α induced nuclear translocation of NF-κB was inhibited in SN50 peptide-treated human monocytic cell lines as demonstrated in EMSA (Lin et al., 1995). In view of it, we employed SN50, an inhibitor of NF-κB, to assess the role of NF-κB in activation of TNF-α protein expression in M. tuberculosis-infected monocytes. SN50 (100 μg/ml) was added to monocytes 3 minutes prior to M. tuberculosis infection. Control cultures did not receive SN50. At 24 hours, SN50 suppressed secreted TNF-α protein expression by around 1.96-folds (i.e. suppressed from 194.3 pg/ml to 98.9 pg/ml), 2.11-folds (i.e. suppressed from 183.2 pg/ml to 86.8 pg/ml) and 2.04-folds (i.e. suppressed from 212.2 pg/ml to 103.6 pg/ml) in 24 hr monocyte cultures from patients with SLE (n=5; P<0.001), TB (n=5; P<0.001) and SLE-TB (n=5; P<0.001) respectively (Fig. 11). Furthermore, in order to ensure that cellular inhibition was not non-specific, we compared the effect of SN50 with its inactive analogue, SN50/M at the same concentration. SN50/M did not affect secreted TNF-α protein expression (P<0.001) (Fig. 11). Therefore, it can be inferred that the increased expression of TNF-α in 24 hr monocyte cultures from patients with SLE, TB and SLE-TB respectively is mediated mainly via NF-κB.
5. Effect of NAC and EGCG on GPx activity in 24 hr monocyte cultures from normal healthy controls and patients with SLE, TB and SLE-TB.

Next, an attempt was made to probe the modulatory effect of NAC and EGCG on glutathione peroxidase (GPx) activities in culture supernatants of the above monocyte cultures that were harvested after 24 hrs. As described above in Fig. 6, normal healthy control cultures exhibited a GPx activity of 57.22 U/mg proteins, whereas GPx activities were suppressed to the order of 29.25, 25.14 and 20.48 U/mg proteins in cultures from patients with SLE (n=8), TB (n=8) and SLE-TB (n=8) respectively (Fig. 6). On the contrary, modulation with 10 mM NAC and 5 µg/ml of EGCG showed interesting results. Thus, as evident from Fig. 12, NAC (10 mM) showed amelioration in GPx activities from 29.25, 25.14 and 20.48 U/mg proteins to 44.23, 42.01 and 40.88 U/mg proteins in cultures from patients with SLE, TB and SLE-TB respectively (Fig. 12). Similarly, EGCG (5 µg/ml) exhibited showed amelioration in GPx activities from 29.25, 25.14 and 20.48 U/mg proteins to 49.23, 46.01 and 45.88 U/mg proteins in cultures from patients with SLE, TB and SLE-TB respectively (Fig. 13) (P<0.001 for all).

6. Determination of MDA levels in 24 hr culture filtrate of monocytes of patients with SLE, TB and SLE-TB

Thereafter, an attempt was also made to evaluate the MDA levels in culture supernatants of 24 hr monocyte cultures from patients with SLE (n=3), TB (n=3) and SLE-TB (n=3) respectively. As shown in Fig. 14, normal healthy control (n=3) cultures exhibited an MDA level to the order of 8.12 ng/ml (Fig. 14). On the contrary, augmented MDA levels in 24 hr monocyte cultures supernatants from patients with SLE (n=3), TB (n=3) and SLE-TB (n=3) were found, which were of the order 18.22 ng/ml (2.24-folds; P<0.001), 17.34 ng/ml (2.12-folds; P<0.001) and 22.26 ng/ml (2.74-folds; P<0.001) respectively (Fig. 14).
7. NAC and EGCG-induced modulation of MDA levels in 24 hr culture filtrate of monocytes of patients with SLE, TB and SLE-TB

To have further in-sight, an attempt was also made to probe the modulatory effect of NAC and EGCG on augmented levels of MDA in culture supernatants of the above monocyte cultures that were harvested after 24 hrs. As described above in Fig. 14, normal healthy control cultures exhibited 8.12 ng/ml of MDA, and augmented to the order of 18.22 ng/ml, 17.34 ng/ml and 22.26 ng/ml in cultures from patients with SLE (n=3), TB (n=3) and SLE-TB (n=3) respectively. On the contrary, modulation with 10 mM NAC showed down-regulation in MDA levels to 10.32 ng/ml, 9.34 ng/ml and 9.01 ng/ml in cultures from patients with SLE, TB and SLE-TB respectively (Fig. 15). Similarly, EGCG (5 ug/ml) exhibited down-regulation in MDA levels to 8.23 ng/ml, 7.89 ng/ml and 7.12 ng/ml in cultures from patients with SLE, TB and SLE-TB respectively (Fig. 16, P<0.001 for all three groups of patients).

(IV) IMMUNONOLICAL STUDY:

1. Immuno-binding of dsDNA antigens by antibodies present in sera of patients with SLE by direct binding and inhibition-ELISA:

The binding of naturally occurring SLE autoantibodies with a variety of antigens, ranging from native calf thymus purified DNA (free of proteins and single stranded regions) to mycobacterial protein antigens was probed by direct binding and inhibition ELISA. Prior to any study, SLE sera (n = 40) were screened for their activity. Only those SLE sera exhibiting a titer of greater than 1:6400 (n=20) against native DNA (Fig. 17) were selected for determining the reactivity against mycobacterial protein antigens. It is to be pointed out that apart from showing high magnitude specificity towards native DNA, the selected SLE sera having anti-DNA antibody also showed appreciable degree of reactivity towards a wide spectrum of antigens. Also, it's worth mentioning out here that prior to any ELISA experiments, it was ensured that the secreted mycobacterial proteins were free
from any contaminants of DNA due to possible leakage from ruptured bacilli, which in turn, may yield false positive test. Colorimetric estimations showed absence of any DNA contaminations in the culture filtrate having secreted proteins (data not shown). Thereafter, direct binding ELISA on microtiter plates coated with intracellular and secreted mycobacterial protein antigens exhibited an anti-DNA antibody titer of greater than 1:12800 (Fig. 18, 19; P<0.001 for all). Normal human sera, which acted as corresponding controls failed to show any appreciable reactivity. Surprisingly, the binding curves showed augmented reactivity of SLE anti-DNA antibodies against mycobacterial protein antigens than against native dsDNA.

Next, after observing high binding with SLE sera, an attempt was also made to check the binding with Protein-A-Sepharose purified SLE IgG. The binding curves by direct binding ELISA on plates coated with secreted mycobacterial protein antigens against SLE IgG (n=4) were of similar pattern observed with SLE sera (Fig. 20; P<0.001).

After determining the reactivity of SLE anti-DNA antibodies by direct binding ELISA, an attempt was also made to determine the specificity of the above autoantibodies in SLE sera by employing competition-inhibition ELISA. A variety of inhibitors ranging from nucleic acids to mycobacterial proteins were employed in inhibition ELISA. As evident from Fig. 21, double stranded DNA showed a maximum inhibition in the anti-DNA antibody binding to the order of 73.11 percent (P<0.001) at a maximum inhibitor concentration of 10 µg/ml. Here, fifty percent inhibition in the antibody activity was observed at 2.1 ug/ml. Similar maximum inhibition in antibody activity was observed with heat denatured single stranded DNA (71.33%; P<0.001), but fifty percent inhibition in antibody activity was recorded a much lower ssDNA inhibitor concentration (1.2 ug/ml). Next, no appreciable inhibition in antibody activity was observed when RNA was employed as an inhibitor (Fig. 22; P<0.05).
Thereafter, secreted mycobacterial protein antigens(s), were employed as inhibitors. As evident from Fig. 23, 82.13 percent inhibition in SLE IgG activities respectively were recorded at maximum inhibitor concentrations of 10 µg/ml (P<0.001). Fifty percent inhibition in SLE IgG activities was recorded at an inhibitor concentration of 0.01 µg/ml. Next, the above observations were further substantiated from results obtained by employing immunoaffinity purified (DNA-polylysyl-sepharose 4B column) anti-DNA antibody (SLE IgG) in the binding studies (Fig. 24). It may be pointed out here that the lower the inhibitor concentration at which fifty percent inhibition is achieved, the greater the specificity of the concerned antibody towards the concerned antigen/inhibitor. Thus, judged on the basis of fifty percent inhibition, the binding curves indicate the presence of highly specific autoantibodies in SLE sera for *Mycobacterium tuberculosis* H$_{37}$Rv secreted protein antigen(s). Thus, the above results show that the binding of SLE anti-DNA autoantibodies were of greater magnitude towards mycobacterial secreted protein antigens as compared to native DNA.

Next, in sharp contrast to the above, when anti-TB antibodies (sera from TB patients, n=30) were employed on plates coated with dsDNA, then no binding was observed (Fig. 25) in direct binding ELISA. Since, DNA binding with TB sera was not observed in direct binding ELISA, thus, inhibition ELISA was not carried out.

2. Immuno-binding of MTB 30kDa antigens by antibodies present in sera of patients with SLE and TB by inhibition-ELISA:

All the TB sera's (n=30) undertaken in this study were subjected to specificity determination against respective antigens and inhibitors i.e. tuberculosis 85B antigen (30kDa) for TB sera and dsDNA for SLE patients respectively, by employing competition-inhibition ELISA. Inhibition ELISA on plates coated with mycobacterium tuberculosis 85B antigen (30kDa) against sera of patients (n=30) with TB indicated interesting results.
Nearly all the TB sera employed in this study exhibited a maximum of 84 percent inhibition (P<0.001) in anti-TB antibody activity at a maximum inhibitor (i.e. MTB 30kDa) concentration of 10 µg/ml (Fig. 26). Fifty percent inhibition here was achieved at an inhibitor concentration of 0.1 µg/ml, thereby indicating the high specificity of TB antibodies in TB sera against MTB 30kDa.

Next, after selecting high specificity sera’s of TB and SLE patients as described above, an attempt was made to evaluate the correlation, if any, between TB and autoimmune SLE. For this purpose, we investigated the (a) reactivity/specificity of anti-DNA antibodies found in SLE sera (n=20) against mycobacterial Ag85B (30 kDa) in comparison to dsDNA, (b) reactivity/specificity of anti-TB antibodies found in TB sera (n=20) against dsDNA in comparison mycobacterial Ag85B (30 kDa), and (c) comparative reactivity of dsDNA versus MTB 30 kDa against sera from patients having both TB and SLE (n=20).

As evident from Fig. 27, sera of patients with SLE (n=30) exhibited a maximum of 87.7% (P<0.001) inhibition with dsDNA on microtiter ELISA plates coated with dsDNA, where 50% inhibition in anti-DNA antibody activity was recorded at an inhibitor concentration of 0.08 ug/ml (Fig. 27). Interestingly, when MTB 30kDa was employed as an inhibitor on plates coated with dsDNA, then a magnitude of binding was observed with anti-DNA Abs found in SLE sera. A maximum of 78.2% inhibition (P<0.001) in anti-DNA activity was recorded by 10 ug/ml of mycobacterial 30kDa antigen/inhibitor, where 50% inhibition was achieved at an inhibitor concentration of 0.09 µg/ml (Fig. 27). On the contrary, anti-TB antibodies found in TB sera (n=30) showed low magnitude recognition with dsDNA on plates coated with MTB 30kDa. Here only 29% (P>0.05) inhibition in anti-TB antibodies was recorded with dsDNA (Fig. 28), and that, 50% inhibition could not be achieved. However the same anti-TB antibodies on the same antigen (30 kDa) coated plates showed high magnitude antibody recognition (83%; P<0.001) when MTB 30kDa was employed as an inhibitor (Fig. 28). Thus, the results are indicative for an appreciable immunological correlation between SLE and TB. To
have further insight, inhibition-ELISA against dsDNA and MTB 30 kDa was carried out by using antibodies present in sera of patients having both SLE and TB. Here, a maximum of 95% and 55% (P<0.001) inhibition was observed with dsDNA and MTB 30kDa inhibitors respectively on plates coated with MTB 30 kDa antigen against Abs found in sera of patients having both the diseases i.e. SLE and TB (TB-SLE) (n=30) (Fig. 29). Fifty percent inhibition in both cases was recorded at very low inhibitor concentrations (0.07 µg/ml for both). The data depicted in Fig 29 are again indicative for the close immunological relationship between SLE and TB.

3. Immuno-recognition of antigens found in human monocytes of SLE and TB patients by anti-dsDNA Abs and anti-TB Abs found in patients with SLE and TB

Next, we also carried out a number of inhibition-ELISA's to probe the binding effects by employing antigen / inhibitors obtained from cell lysates of human monocytes from TB and SLE patients. Figures 30 and 31 illustrates the binding of anti-TB antibodies and anti-DNA Abs found in sera of patients with TB (n=5) and SLE (n=5) respectively with protein lysates obtained from monocytes (0.5 x 10^6/well) from blood of respective TB and SLE patients that were cultured for 24 hrs in 12-well tissue culture plates.

Here, a maximum of 91.8% (P<0.001) and 95.9% (P<0.001) inhibitions in anti-TB and anti-dsDNA Ab activity respectively were recorded at a maximum inhibitor concentration of 10 µg/ml, where 50% inhibitions in the anti-TB Ab activity was achieved at a low inhibitor concentration of 0.08 µg/ml (Fig. 30), whereas that for anti-DNA from SLE sera was achieved at an inhibitor concentration of 0.07 µg/ml (Fig. 31). A further augmented inhibition (~ 97%; P<0.001) in anti-TB antibody activity was observed with sera of patients having both TN and SLE (n=5) (Fig. 32) at a maximum inhibitor concentration of only 1 µg/ml, and that, 50% inhibition
in this case was recorded at a much low inhibitor concentration of 0.01 μg/ml (Fig. 32).

4. Reduced Glutathione-induced immuno-suppression studies

In view of the role of reactive oxygen species (ROS) in both autoimmune SLE and tuberculosis (TB) being well documented, an attempt was made in the present study to neutralize/suppress the ROS induced effects in monocytes of patients with TB and SLE by employing reduced glutathione (r GSH), which is a known in vivo antioxidant. Thus, for this purpose, monocytes from blood of patients with SLE (n=5), TB (n=5) and SLE-TB (n=5) were isolated as described in methods and were adhered onto 12-well tissue culture plates and cultured with or without 10 nM of reduced glutathione for 24 hrs at 37 °C / 5%CO₂. Thereafter, treated/untreated monocytes were lysed in 1 ml/well of protein lysis buffer and were subsequently used as antigen / inhibitor in inhibition ELISA against antibodies in sera with SLE or TB or SLE-TB.

As evident from Fig. 33, co-culturing of monocytes obtained from TB patients with 10 nM of reduced glutathione for 24 hrs resulted in the tremendous amount of down-regulation of recognition of anti-TB antibodies by cell lysates of the above cultured monocytes. Here, only a maximum of 32% (P>0.01) inhibition in anti-TB antibody activity was recorded at a maximum inhibitor concentration of 20 μg/ml, and that, 50% inhibition could not be achieved here (Fig. 33). Thus, when compared to the data in Fig 30, reduced glutathione suppressed the anti-TB antibody activity (Fig. 33) by around 58%. Similarly, in comparison to binding results depicted in Fig. 31, reduced glutathione down-regulated the anti-DNA antibody activity in monocytes of SLE patients by ~ 53% (P<0.01) (Fig. 34). On the other hand, reduced glutathione suppressed the anti-TB antibody activity in monocytes of patients with SLE-TB by ~ 36% (P<0.01) (Fig. 35) in comparison to untreated monocytes of TB-SLE patients whose data are depicted in Fig. 32). Thus, the results indicate the high magnitude suppression in respective antibody
binding (i.e. abs in sera of patients with SLE or TB or SLE-TB) to corresponding antigens by reduced glutathione.

5. Binding studies with anti-TNF-α antibody

In view of the fact that ROS activates the production of autocrine proinflammatory cytokine, namely TNF-α, which in turn, is involved in the pathogenesis of TB and SLE, thus an attempt was made in the present study to evaluate the binding of anti-TNF-α antibody with various antigens prepared and used in the above-mentioned modulatory studies. Figures 36-38 demonstrates the binding of anti-TNF-α antibody with protein lysates of monocytes from patients with TB (n=5), SLE (n=5) and TB-SLE (n=5).

A maximum of 68.9% (P<0.001) inhibition in anti-TNF-α antibody activity was recorded at a maximum inhibitor (i.e. lysates of monocytes from TB patients) concentration of 1 µg/ml, where 50% inhibition was achieved at an inhibitor concentration of 0.08 µg/ml (P<0.001) (Fig. 36). On the other hand, a maximum of 76.5% (P<0.001) inhibition in anti-TNF-α antibody activity was recorded at a maximum inhibitor (i.e. lysates of monocytes from SLE patients) concentration of 10 µg/ml, where 50% inhibition was achieved at an inhibitor concentration of 0.09 µg/ml (P<0.001) (Fig. 37). It is noteworthy to observe that a maximum of 80.09% (P<0.001) inhibition in anti-TNF-α antibody activity was achieved at a maximum inhibitor (i.e. lysates of monocytes from SLE-TB patients) concentration of only 0.1 µg/ml, where 50% inhibition was recorded at an inhibitor concentration of 0.05 µg/ml (P<0.001) (Fig. 38).

Co-culturing of monocytes obtained from patients having TB (n=5), SLE (n=5) and TB-SLE (n=5) with reduced glutathione (10 nM) for 24 hrs resulted in the suppression of recognition of anti-TNF-α Ab. Here, only a maximum of 17.26%, 28.03% and 32.01% (P>0.01 for all) inhibition in anti-TNF-α antibody activity was recorded as is evident from Figs. 39-41, respectively.
6. Measurement of secreted TNF-α in 24 hr culture supernatants of monocytes treated with reduced glutathione.

Level of secreted TNF-α in culture supernatants was found to be greatest in cultures of monocytes from patients with TB-SLE, followed by cultures of monocytes of SLE and TB patients respectively (P<0.001 for all) (Fig. 42). The order of reduced glutathione-induced suppression in secreted TNF-α in monocyte culture supernatants was TB > SLE > TB-SLE (P<0.001) (Fig. 42). It is noteworthy to observe that secreted TNF-alpha was highest in culture supernatants of patients belonging to category III (Age: 51 yrs to 65 yrs), followed by category II (Age: 36 yrs to 50 yrs) and category I (Age: 20 yrs to 35 yrs) respectively (Fig. 42).

7. Binding of anti-DNA antibodies against dsDNA antigen/inhibitor isolated from monocytes of SLE patients that were co-cultured for 24 hr with NAC, EGCG, SN50 or SN50M.

DNA was isolated by Qiagen Kit from untreated / treated monocytes from patients with SLE (n=5), and were subsequently employed as coating antigen / inhibitor in inhibition-ELISA. As evident from Fig. 43, the specificity of anti-DNA antibodies against dsDNA isolated from SLE monocytes that were co-cultured for 24 hr with 10 mM NAC was found to decrease markedly to 41.2 percent (P<0.001) when compared to control untreated monocytes (73.45 percent) (Fig. 43). Here, fifty percent inhibition could be achieved with any of the inhibitor concentrations employed. Next, dsDNA isolated from SLE monocytes (n=5) that were co-cultured for 24 hr with 5 ug/ml EGCG was employed as coating antigen / inhibitor, then a maximum of only 32.45 percent inhibition (P<0.001) was observed (Fig. 44). Again, here fifty percent inhibition could not be achieved. The results thus indicate the positive involvement of reactive oxygen species (ROS) in SLE. which was appreciably neutralized / inhibited by NAC and EGCG.
Thereafter, for reasons as described in modulation studies earlier, an attempt was also made to probe the immuno-binding of anti-DNA antibodies against dsDNA isolated from SLE monocytes (n=5) treated with SN50 and SN50M. As evident from Fig. 45, anti-DNA antibodies exhibited a non-significant binding with SN50 treated SLE monocytes (39.22 percent). On the contrary, treatment with control peptide SN50M exhibited high binding as is evident by 69.44 percent inhibition in anti-dsDNA antibody activity (Fig. 45, P<0.001). Thus, the activation of NF-kB and in turn, its involvement in SLE is substantiated by the above immuno-binding data.

8. Anti-DNA antibody binding against dsDNA antigen/inhibitor isolated from monocytes of tuberculosis (TB) patients that were co-cultured for 24 hr with NAC, EGCG, SN50 or SN50M.

As mentioned above, DNA was isolated from untreated / treated monocytes from patients with TB (n=5), and were subsequently employed as coating antigen / inhibitor in inhibition-ELISA. As evident from Fig. 46, the specificity of anti-DNA antibodies against dsDNA isolated from TB monocytes that were co-cultured for 24 hr with 10 mM NAC was found to decrease markedly to 46.56 percent (P<0.001) when compared to control untreated monocytes (79.33 percent) (Fig. 46). Fifty percent inhibition could not be achieved with any of the inhibitor concentrations employed. Similarly, dsDNA isolated from monocytes of TB patients (n=5) that were co-cultured for 24 hr with 5 µg/ml EGCG, and in turn, were employed as coating antigen / inhibitor in ELISA. The results show a maximum of only 29.45 percent inhibition in comparison to cells devoid of EGCG (maximum inhibition to the order of 79.33%) (P<0.001) (Fig. 47). Again, here fifty percent inhibition could not be achieved. The results thus indicate the positive involvement of reactive oxygen species (ROS) in TB, which was appreciably neutralized / inhibited by NAC and EGCG. The results also indicate involvement of mycobacterial antigen(s) in SLE as is evident from high binding of anti-DNA antibodies with mycobacterial DNA.
Thereafter, as described in modulation studies earlier, an attempt was also made to probe the immuno-binding of anti-DNA antibodies (n=5) against dsDNA isolated from TB monocytes (n=5) treated with SN50 and SN50M. As evident from Fig. 48, anti-DNA antibodies exhibited a maximum inhibition of only 35.39 percent with SN50 treated TB monocytes (P<0.005). On the contrary, DNA isolated from monocytes treated with control peptide SN50M exhibited high binding as is evident by 71.42 percent inhibition in anti-dsDNA antibody activity (Fig. 48, P<0.001). Thus, the activation of NF-kB and in turn, its involvement in TB is substantiated by the above immuno-binding data. This is in accordance with earlier reports from our laboratory at the molecular level (Hasan et al., 2006).

9. Cross reactivity of anti-TB antibodies against dsDNA antigen/inhibitor isolated from monocytes of SLE patients that were co-cultured respectively for 24 hr with NAC, EGCG, SN50 or SN50M.

Next, after ascertaining the immuno-binding of anti-DNA antibodies from SLE patients, an attempt was made to evaluate the specificity / binding of anti-TB antibodies found in patients with TB against DNA isolated from untreated/treated monocytes of patients with (i) SLE, (ii) TB and (iii) SLE-TB.

Inhibition-ELISA results show no or negligible binding of anti-TB antibodies found in sera of patients with TB (n=5) against dsDNA isolated from untreated / treated monocytes of SLE patients (n=5) that were cultured for 24 hr. As evident from the binding curves, a maximum inhibition in anti-TB antibody activity to the order of only 10.23 percent was observed (Fig. 49). Similarly, negligible inhibitions in anti-TB antibody activity of the order of 8.34 percent, 7.12 percent, 9.42 percent, 8.19 percent and 13.44 percent was observed with ds DNA isolated from control untreated, 10 mM NAC treated, 5 μg/ml EGCG treated, 100 μg/ml SN50 treated and 100 μg/ml SN50M treated monocytes respectively from SLE patients (Fig. 50).
Fig. 1. Expression of human house keeping gene R18 rRNA in monocytes of patients with SLE (n=3; P<0.001), TB (n=3; P<0.001) and SLE-TB (n=3; P<0.001). Monocytes from normal healthy individuals served as controls (n=3). Data represents mean ± SEM of 3 experiments.
Fig. 2. Expression of human TNF-α mRNA in monocytes of patients with SLE (n=8; P<0.001), TB (n=8; P<0.001) and SLE-TB (n=8; P<0.001). Monocytes from normal healthy individuals served as controls (n=3). Data represents mean ± SEM of 8 experiments.
Fig. 3. Expression of secreted TNF-α protein in 24 hr culture supernatants of monocytes of patients with SLE (n=8; P<0.001), TB (n=8; P<0.001) and SLE-TB (n=8; P<0.001). Monocytes from normal healthy individuals served as controls (n=8). The concentration of secreted TNF-α was in pg/ml. Data represents mean ± SEM of 8 experiments.
Fig. 4. Expression of iNOS mRNA in monocytes of patients with SLE (n=8; P<0.001), TB (n=8; P<0.001) and SLE-TB (n=8; P<0.001). Monocytes from normal healthy individuals served as controls (n=8). Data represents mean ± SEM of 8 experiments.
Fig. 5. Expression of secreted IFN-γ protein in 24 hr culture supernatants of monocytes of patients with SLE (n=8; P<0.001), TB (n=8; P<0.001) and SLE-TB (n=8; P<0.001). Monocytes from normal healthy individuals served as controls (n=8). The concentration of secreted IFN-γ was in pg/ml. Data represents mean ± SEM of 8 experiments.
Fig. 6. Determination of glutathione peroxidase (GPx) activity in monocytes of patients with SLE (n=8; P<0.001), TB (n=8; P<0.001) and SLE-TB (n=8; P<0.001). Monocytes from normal healthy individuals served as controls (n=8). The unit of GPx activity was determined as U/mg protein. Data represents mean ± SEM of 8 experiments.
Fig. 7. Dose response effect of NAC (0-10 mM) on the expression of secreted TNF-α in 24 hr culture filtrates of monocytes of patients with SLE (black bars; n=3; P<0.001), TB (square bars; n=3; P<0.001) and SLE-TB (ladder bars; n=3; P<0.001). Monocytes from normal healthy individuals served as controls (empty bars; n=3). Data represents mean ± SEM of 8 experiments.
Fig 8: MTT cell viability assay for dose-response (0 – 25 ug/ml) effect of EGCG on 24 hr monocytes cultures of patients with SLE (black bars; n=3), TB (square bars; n=3), SLE-TB (ladder type bars; n=3) and normal healthy controls (empty bars; n=3). After 24 hrs of culture, the cells were harvested and processes as described in methods. Data represents the analysis of three independent experiments in duplicates, which are expressed as mean viable cells (±S. E.) percentage of controls. (P<0.001).
Fig. 9. Expression of human housekeeping gene R18 r18 mRNA in 24 hr monocyte cultures treated with 5 μg/ml of EGCG. The monocytes were of patients with SLE (n=3; P<0.001), TB (n=3; P<0.001) and SLE-TB (n=3; P<0.001). Monocytes from normal healthy individuals served as controls (n=3). Data represents mean ± SEM of 3 experiments.
Fig. 10. Dose response effect of EGCG (0-25 μg/ml) on the expression of secreted TNF-α protein in 24 hr culture supernatants of monocytes of patients with SLE (black bars; n=5; P<0.001), TB (square bars; n=5; P<0.001) and SLE-TB (ladder bars; n=5; P<0.001). Monocytes from normal healthy individuals served as controls (empty bars; n=5; P<0.001). The concentration of secreted TNF-α was in pg/ml. IC$_{50}$ was computed out to be < 5 μg/ml. Data represents mean ± SEM of 5 experiments.
Fig. 11. Effect of SN50 (black bars; 100 µg/ml SN50M) and control peptide SN50M (square bars; 100 µg/ml) on the expression of secreted TNF-α protein in 24 hr culture supernatants of monocytes of patients with SLE (n=5; P<0.001), TB (n=5; P<0.001), SLE-TB (n=5; P<0.001) and normal healthy individuals (n=5). Untreated cultures served as controls (empty bars). The concentration of secreted TNF-α was in pg/ml. Data represents mean ± SEM of 5 experiments.
Fig. 12. NAC (10 mM)-induced modulation of glutathione peroxidase (GPx) activity (U/mg protein) in 24 hr monocytes cultures of patients with SLE (black bars; n=8; P<0.001), TB (square bars; n=8; P<0.001) and SLE-TB (ladder bars; n=8; P<0.001). Monocytes from normal healthy individuals served as controls (empty bars; n=8). Data represents mean ± SEM of 8 experiments.
Fig. 13. EGCG (5 μg/ml)-induced modulation of glutathione peroxidase (GPx) activity (U/mg protein) in 24 hr monocytes cultures of patients with SLE (black bars; n=8; P<0.001), TB (square bars; n=8; P<0.001) and SLE-TB (ladder bars; n=8; P<0.001). Monocytes from normal healthy individuals served as controls (empty bars; n=8). Data represents mean ± SEM of 8 experiments.
Fig. 14. Expression MDA in 24 hr culture supernatants of monocytes of patients with SLE (n=3; P<0.001), TB (n=3; P<0.001) and SLE-TB (n=3; P<0.001). Monocytes from normal healthy individuals served as controls (n=3). The concentration of MDA measured was in pg/ml. Data represents mean ± SEM of 3 experiments.
Fig. 15. Modulation of the expression MDA levels by 10 mM NAC in 24 hr culture supernatants of monocytes of patients with SLE (n=3; P<0.001), TB (n=3; P<0.001) and SLE-TB (n=3; P<0.001). Monocytes from normal healthy individuals served as controls (n=3; P<0.001). The concentration of MDA measured was in pg/ml. Data represents mean ± SEM of 3 experiments.
Fig. 16. Modulation of the expression MDA levels by 5 ug/ml EGCG in 24 hr culture supernatants of monocytes of patients with SLE (n=3; P<0.001), TB (n=3; P<0.001) and SLE-TB (n=3; P<0.001). Monocytes from normal healthy individuals served as controls (n=3). The concentration of MDA measured was in pg/ml. Data represents mean ± SEM of 3 experiments.
Fig. 17. Direct binding ELISA on microtitre ELISA plates that were coated with purified calf thymus dsDNA (100 µl/well from 250 ng/ml stock) against anti-DNA antibodies (▲; SLE sera; n=20; P<0.001). Normal human sera served as controls (Δ; NHS) Data represents mean ± SEM of number of experiments.
Fig. 18. Direct binding ELISA on microtitre ELISA plates that were coated with Mycobacterial total sonic extract (100 ul/well from 20 ug/ml stock) against anti-DNA antibodies (-▲--; SLE sera; n=20; P<0.001). Normal human sera served as controls (-●--; NHS) Data represents mean ± SEM of number of experiments.
Fig. 19. Direct binding ELISA on microtitre ELISA plates that were coated with Mycobacterial total culture filtrate (MTCF) (100 μl/well from 20 μg/ml stock) against anti-DNA antibodies (-▲-; SLE sera; n=20; P<0.001). Normal human sera served as controls (-△-; NHS; n=10). Data represents mean ± SEM of number of experiments.
Fig. 20. Direct binding ELISA on microtitre ELISA plates that were coated with Mycobacterial total culture filtrate (MTCF) (100 μl/well from 20 μg/ml stock) against Sepharose 4B purified SLE IgG i.e. purified anti-DNA antibodies (-▲--; IgG from SLE sera; n=4; P<0.001). Normal human sera served as controls (-△--; IgG from NHS; n=4). Data represents mean ± SEM of 4 experiments.
Fig. 21. Inhibition ELISA on microtitre ELISA plates that were coated with dsDNA (†) (100 ul/well from 2.5 ng/ml stock) and ssDNA (■) (100 ul/well from 2.5 ng/ml stock) against Sepharose 4B purified SLE IgG i.e. purified anti-DNA antibodies (n=4; P<0.001). The inhibitors were dsDNA and ssDNA. The immune complex prepared was used in place of antibody as described in methods. Fifty percent inhibition in anti-DNA antibody activity (IC$_{50}$) for dsDNA and ssDNA was computed out to be 2.2 μg/ml and 1.2 ug/ml respectively. Data represents mean ± SEM of 4 experiments.
Fig. 22. Inhibition ELISA for RNA on microtitre ELISA plates that were coated with dsDNA (- -) (100 ul/well from 2.5 ng/ml stock) against Sepharose 4B purified SLE IgG i.e. purified anti-DNA antibodies (n=4; P<0.001). The inhibitor used to prepare immune complex was RNA. The immune complex was used in place of antibody as described in methods. Fifty percent inhibition in antibody activity (IC$_{50}$) could not be achieved. Data represents mean ± SEM of 4 experiments.
Fig. 23. Inhibition ELISA for MTCF on microtitre ELISA plates that were coated with dsDNA (100 μl/well from 2.5 ng/ml stock) against Sepharose 4B purified SLE IgG i.e. purified anti-DNA antibodies (n=4; P<0.001). The inhibitor was MTCF. The immune complex prepared with varying doses of MTCF was used in place of antibody as described in methods. Fifty percent inhibition in anti-DNA antibody activity (IC₅₀) was computed out to be 0.01 ug/ml. (MTCF= Mycobacterial total culture filtrate i.e. secretory proteins). Data represents mean ± SEM of 4 experiments.
Fig. 24. Inhibition ELISA for MTCF on microtitre ELISA plates that was coated with dsDNA (100 ul/well from 2.5 ng/ml stock) against immunoaffinity purified anti-DNA antibodies (on DNA-polylysylsepharose 4B column) i.e. immunoaffinity purified SLE IgG (n=4; P<0.001). The inhibitor was dsDNA (■■) and MTCF (■■). The immune complex prepared with varying doses of dsDNA or MTCF, and was used in place of antibody as described in methods. Fifty percent inhibition in anti-DNA antibody activity (IC₅₀) was computed out to be 0.01 ug/ml for both dsDNA and MTCF. (MTCF= Mycobacterial total culture filtrate i.e. secretory proteins). Data represents mean ± SEM of 4 experiments.
Fig. 25. Direct binding ELISA on microtitre ELISA plates that were coated with dsDNA (100 µl/well from 2.5 ng/ml stock) against TB antibodies (TB sera) (-□-). Normal human sera served as controls (■-NHS). No binding was observed, as the titer was <1:100. Data represents mean ± SEM of 8 experiments.
Fig. 26. Inhibition ELISA against antibodies in sera of patients with TB (n=30) on plates coated with 30 kDa Ag85B isolated from mid-logarithmic phase cultures of *Mycobacterium tuberculosis* H₃₇Rv. The inhibitor used here was varying concentrations of 30 kDa Ag85B. The concentrations were 0.01, 0.1, 1.0, 10 and 20 µg/ml. Data are mean ±SD.
Fig. 27. Comparative Inhibition ELISA against autoantibodies in Patients with SLE (n=30) on plates coated with native dsDNA (non mycobacterial), where the inhibitors used were varying concentrations of native dsDNA versus mycobacterial 30 kDa Ag85B. The concentrations for both the inhibitors were 0.01, 0.1, 1.0, 10 and 20 µg/ml. dsDNA (■■) and mycobacterial 30kDa Ag85B (▲▲). Data are mean ±SD.
**Fig. 28.** Comparative Inhibition ELISA against antibodies in patients with TB (n=30) on plates coated with mycobacterial 30 kDa Ag85B, where the inhibitors used were varying concentrations of mycobacterial 30 kDa Ag85B versus non-mycobacterial native dsDNA. The concentrations for both the inhibitors were 0.01, 0.1, 1.0, 10 and 20 µg/ml. Mycobacterial 30 kDa (Black bars) and non-mycobacterial native dsDNA (shaded bars). Data are mean ±SD.
Fig. 29. Comparative Inhibition ELISA against antibodies in patients with TB-SLE (n=30) on plates coated with mycobacterial 30 kDa Ag85B, where the inhibitors used were varying concentrations of mycobacterial 30 kDa Ag85B versus non-mycobacterial native dsDNA. The concentrations for both the inhibitors were 0.01, 0.1, 1.0, 10 and 20 µg/ml. Mycobacterial 30 kDa Ag85B (Black bars) and non mycobacterial native dsDNA (white bars). Data are mean ±SD.
Fig. 30. Inhibition ELISA against antibodies in sera of patients with TB (n=5) on plates coated with total protein lysates obtained from human monocytes isolated from blood of TB patients where the inhibitor used were varying concentrations of the above mentioned total protein lysates obtained from monocytes of TB patients. The inhibitor concentrations were 0.01, 0.1, 1.0, 10 and 20 μg/ml. Data are mean ±SD.
Fig. 31. Inhibition ELISA against antibodies in sera of patients with SLE (n=5) on plates coated with non-mycobacterial native dsDNA and the inhibitors used were lysates obtained from monocytes isolated from blood of SLE patients where the varying inhibitor concentrations were 0.01, 0.1, 1.0, 10 and 20 μg/ml. Data are mean ±SD.
**Fig. 32.** Inhibition ELISA against antibodies in sera of patients with TB-SLE (n=5) on plates coated with non-mycobacterial native dsDNA and the inhibitors used were lysates obtained from monocytes isolated from blood of SLE patients where the varying inhibitor concentrations were 0.01, 0.1, 1.0, 10 and 20 µg/ml. Data represents mean ± SEM of 5 experiments.
Fig. 33. Modulation of TB monocytes with reduced glutathione: Inhibition ELISA against antibodies in sera of patients with TB (n=5) on plates coated with total protein lysates obtained from TB patient monocytes that were co-cultured for 24 hrs with 10 nM of reduced glutathione where the varying inhibitor concentrations used of the above mentioned total protein lysates were 0.01, 0.1, 1.0, 10 and 20 μg/ml. Data represents mean ± SEM of 5 experiments.
Fig. 34. Modulation of SLE monocytes with reduced glutathione: Inhibition ELISA against antibodies in sera of patients with SLE (n=5) on plates coated with total protein lysates obtained from SLE patient monocytes that were co-cultured for 24 hrs with 10 nM of reduced glutathione where the varying inhibitor concentrations used of the above mentioned total protein lysates were 0.01, 0.1, 1.0, 10 and 20 μg/ml. Data are mean ±SD. Data represents mean ± SEM of 5 experiments.
Fig. 35. Modulation of TB-SLE monocytes with reduced glutathione: Inhibition ELISA against antibodies in sera of patients with TB-SLE (n=5) on plates coated with total protein lysates obtained from TB-SLE patient monocytes that were co-cultured for 24 hrs with 10 nM of reduced glutathione where the varying inhibitor concentrations used of the above mentioned total protein lysates were 0.01, 0.1, 1.0, 10 and 20 μg/ml. Data represents mean ± SEM of 5 experiments.
Fig. 36. Anti-TNF-α antibody Activity: Inhibition ELISA against monoclonal anti-TNF-α antibody on plates coated with total protein lysates obtained from human monocytes isolated from blood of TB patients (n=5) where the inhibitor used were varying concentrations of the above mentioned total protein lysates obtained from monocytes of TB patients. The inhibitor concentrations were 0.01, 0.1, 1.0, 10 and 20 µg/ml. Data represents mean ± SEM of 5 experiments.
Fig. 37. Anti-TNF-α antibody activity: Inhibition ELISA against monoclonal anti-TNF-α antibody on plates coated with total protein lysates obtained from monocytes isolated from blood of SLE patients (n=5) where the inhibitor used were varying concentrations of the above mentioned total protein lysates obtained from monocytes of SLE patients. The inhibitor concentrations were 0.01, 0.1, 1.0, 10 and 20 µg/ml. Data represents mean ± SEM of 5 experiments.
Fig. 38. Anti-TNF-α antibody activity: Inhibition ELISA against monoclonal anti-TNF-α antibody on plates coated with total protein lysates obtained from monocytes isolated from blood of TB-SLE patients (n=5) where the inhibitor used were varying concentrations of the above mentioned total protein lysates obtained from monocytes of TB-SLE patients. The inhibitor concentrations were 0.01, 0.1, 1.0, 10 and 20 µg/ml. Data represents mean ± SEM of 5 experiments.
Fig. 39. Modulation of TB monocytes with reduced glutathione versus Anti-TNF-α antibody Activity: Inhibition ELISA against antibodies in sera of patients with TB (n=5) on plates coated with total protein lysates obtained from TB patient monocytes that were co-cultured for 24 hrs with 10 nM of reduced glutathione where the varying inhibitor concentrations used of the above mentioned total protein lysates were 0.01, 0.1, 1.0, 10 and 20 μg/ml. Data represents mean ± SEM of 5 experiments.
Fig. 40. Modulation of SLE monocytes with reduced glutathione versus Anti-TNF-α antibody Activity: Inhibition ELISA against antibodies in sera of patients with SLE (n=5) on plates coated with total protein lysates obtained from SLE patient monocytes that were co-cultured for 24 hrs with 10 nM of reduced glutathione where the varying inhibitor concentrations used of the above mentioned total protein lysates were 0.01, 0.1, 1.0, 10 and 20 μg/ml. Data represents mean ± SEM of 5 experiments.
Fig. 41. Modulation of TB-SLE monocytes with reduced glutathione versus Anti-TNF-α antibody Activity: Inhibition ELISA against antibodies in sera of patients with TB-SLE (n=5) on plates coated with total Protein lysates obtained from TB-SLE patient monocytes that were co-cultured for 24 hrs with 10 nM of reduced glutathione where the varying inhibitor concentrations used of the above mentioned total protein lysates were 0.01, 0.1, 1.0, 10 and 20 µg/ml. Data represents mean ± SEM of 5experiments.
Fig. 42. Expression of secreted pro-inflammatory cytokine TNF-α in supernatants of monocytes that were cultured for 24 hrs with and without 10 nM of reduced glutathione. The different monocytes cultured were obtained from blood of patients having TB (n=5), SLE (n=5) and TB-SLE (n=5). Also in each group, three categories were undertaken in this study. They were: Category I: Patients in the age group of 20 yrs to 35 yrs (black bars), Category II: patients in the age group of 36 yrs to 50 yrs (white bars) and Category III: patients in the age group of 51 yrs to 65 yrs (shaded bars). Data represents mean ± SEM of 5 experiments.
Fig. 43. Inhibition ELISA against anti-DNA antibodies in sera of patients with SLE (n=5) on plates coated with dsDNA obtained from SLE patient monocytes (n=5) that were co-cultured for 24 hrs with or without (-■-) or with 10 mM of NAC (-□-), where the varying inhibitor concentrations used were 0, 0.01, 0.1, 5 and 10 µg/ml. Data represents mean ± SEM of 5 experiments. P<0.001 for all.
Fig. 44. Inhibition ELISA against anti-DNA antibodies in sera of patients with SLE (n=5) on plates coated with dsDNA obtained from SLE patient monocytes (n=5) that were co-cultured for 24 hrs with without (-■-) or with 5 µg/ml of EGCG, (-□-), where the varying inhibitor concentrations used were 0, 0.01, 0.1, 5 and 10 µg/ml. Data represents mean ± SEM of 5 experiments. P<0.001 for all.
Fig. 45. Inhibition ELISA against anti-DNA antibodies in sera of patients with SLE (n=5) on plates coated with dsDNA obtained from SLE patient monocytes (n=5) that were co-cultured for 24 hrs with without (-■-) or with 100 ug/ml SN50 (-▲-) or 100 ug/ml SN50M (-□-), where the varying inhibitor concentrations used were 0, 0.01, 0.1, 5 and 10 µg/ml. Data represents mean ± SEM of 5 experiments. P<0.001 for all.
Fig. 46. Inhibition ELISA against anti-DNA antibodies in sera of patients with SLE (n=5) on plates coated with dsDNA obtained from TB patient monocytes (n=5) that were co-cultured for 24 hrs with without (- ■ -) or with 10 mM of NAC (- □ -), where the varying inhibitor concentrations used were 0, 0.01, 0.1, 5 and 10 μg/ml. Data represents mean ± SEM of 8 experiments. P<0.001 for all.
Inhibition ELISA against anti-DNA antibodies in sera of patients with SLE (n=5) on plates coated with dsDNA obtained from TB patient monocytes (n=5) that were co-cultured for 24 hrs with without (-•-) or with 5 ug/ml EGCG (-○-), where the varying inhibitor concentrations used were 0, 0.01, 0.1, 5 and 10 µg/ml. Data represents mean ± SEM of 5 experiments. P<0.001 for all.
Fig. 48. Inhibition ELISA against anti-DNA antibodies in sera of patients with SLE (n=5) on plates coated with dsDNA obtained from TB patient monocytes that were co-cultured for 24 hrs with without (■) or with 100 ug/ml SN50M (○) or 100 ug/ml SN50 (▲), where the varying inhibitor concentrations used were 0, 0.01, 0.1, 5 and 10 µg/ml. Data represents mean ± SEM of 5 experiments. P<0.001 for all.
Fig. 49. Inhibition ELISA against anti-TB antibodies in sera of patients with TB (n=5) on plates coated with dsDNA obtained from untreated SLE patient monocytes (n=5) that were cultured for 24 hrs, where the varying inhibitor concentrations used were 0, 0.01, 0.1, 5 and 10 µg/ml. Data represents mean ± SEM of 5 experiments. P<0.05 for all.
Fig. 50. Inhibition ELISA against anti-TB antibodies in sera of patients with TB (n=5) on plates coated with dsDNA obtained from SLE patient monocytes (n=5) that were co-cultured for 24 hrs with either 10 mM NAC (empty bars), 5 µg/ml EGCG (black bars), 100 µg/ml SN50 (square bars) and 100 µg/ml SN50M (ladder bars). The varying inhibitor (above respective dsDNA from treated cells) concentrations used were 0, 0.01, 0.1, 5 and 10 µg/ml. Data represents mean ± SEM of 5 experiments. P<0.05 for all.