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The TNF α locus is Altered in Monocytes from Patients with Systemic Lupus Erythematosus

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Abstract

In systemic lupus erythematosus, TNF α is elevated in the serum and correlates with disease activity and triglyceride levels. The stimuli that drive TNF α in this setting are incompletely understood. This study was designed to evaluate monocyte chromatin at the TNF α locus to identify semi-permanent changes that might play a role in altered expression of TNF α . SLE patients with relatively quiescent disease (mean Physician Global Assessment=0.6) and healthy controls were recruited for this study. TNF α expression was measured by intracellular cytokine staining of different monocyte subsets in patients (n=24) and controls (n=12). Histone acetylation at the TNF α locus was measured by chromatin immunoprecipitation using a normalized quantitative PCR in patients (n=46) and controls (n=24). There were no differences in the overall fractions of cells expressing CD14 in SLE patients compared to controls, however, the fraction of DR+/CD16+ cells expressing CD14 was slightly higher as was true in the monocyte subset defined by DR+/CD11b+. Within the monocyte population defined by physical characteristics and DR+/CD14+, TNF α expressing cells were more frequent in SLE patients compared to controls. Both the fraction of positive cells and the mean fluorescence intensity were higher in patients than controls. Consistent with this was the finding that monocytes from patients had increased TNF α transcripts and more highly acetylated histones at the TNF α locus compared to controls. Furthermore, patients with the highest levels of TNF α histone acetylation were more likely to have had consistently elevated erythrocyte sedimentation rates, and to have required cytotoxic use. Histone acetylation, associated with increased transcriptional competence of TNF α , may play a role in certain inflammatory aspects of the disease.

Keywords

lupus; epigenetics; histone; TNF α

Introduction

TNF α is a proinflammatory cytokine which is produced primarily by monocytes. Other immunologically important producers of TNF α are T cells and dendritic cells. Serum levels of TNF α are often elevated in systemic lupus erythematosus (SLE) patients and higher serum

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levels are associated with increased disease activity [1;2;3;4;5;6]. The role of TNF α in the disease process is controversial. While elevation of serum TNF α is a consistent finding in humans, several murine models suggest that diminished TNF α is associated with an increased risk of disease and/or increased severity of disease [7;8].

Regardless of the role of TNF α in the etiopathogenesis of SLE, increased expression of TNF α could contribute to certain inflammatory manifestations. For example, MRL/lpr mice massively overexpress TNF α . Treatment of MRL/lpr mice with a TNF α inhibitor ameliorates lung disease and skin disease [9]. Specifically in humans, TNF α is thought to contribute to the dyslipidemia of SLE and glomerular disease [10;11]. Serum levels of TNF α correlate with serum triglycerides and TNF α is a known inhibitor of lipoprotein lipase, the major enzyme that degrades triglyceride-rich VLDLs [12]. TNF α is overexpressed in the renal tissues of many murine lupus models [13] and also in the renal tissues of human SLE patients [14].

The regulation of TNF α is complex and active disease could stimulate TNF α through many potential mechanisms. TNF α is regulated at the levels of transcription, message turnover, translation, and protein processing. Transcriptional regulation is particularly complex, with tissue-specific regulation, tissue specific repression, and stimulus-specific regulation [15;16;17;18]. A core promoter region is sufficient to confer appropriate basal expression and responses to several stimuli. In addition, monocytes are “readied” for responses to stimuli by altering their chromatin conformation at the TNF α locus [19;20]. Increased histone acetylation is associated with monocyte maturation, responses to certain priming agents, and increased production of TNF α [20]. Increased histone acetylation is a general feature of increased chromatin accessibility and increased competence for transcription [19;21]. In general, the epigenetic changes required for competence for gene expression include DNA demethylation, localization into a euchromatin environment, and the establishment of a favorable chromatin environment. Many of these steps are often developmentally regulated such as demethylation of the perforin gene in cells capable of expressing perforin [22], localization of globin genes in euchromatin prior to expression [23], and localization of Th2 cytokine genes into a favorable spatial arrangement [24;25]. While often related to differentiation, these epigenetic may also be induced via a specific stimulus such as is seen with IL-2 DNA demethylation in T cells after activation [26]. Nevertheless, the more common epigenetic changes that reflect an acute stimulation are histone modifications. These may include acetylation, methylation, ubiquitination and phosphorylation. Each amino acid on the histone tail undergoes characteristic modifications and both the location and the type of modification are recognized by specific binding proteins which modulate transcription. In the case of IL-4, gene expression was closely regulated by a combination of DNA methylation and histone acetylation and individual clones derived from a common progenitor exhibited significant variation in expression, which in turn correlated with levels of histone acetylation and DNA methylation [27]. There are no comparable data for TNF α , and each individual gene is regulated by its own unique combination of epigenetic variables and its own timeframe for epigenetic alterations. In spite of this limitation, there is evidence to suggest that TNF α is substantially regulated at the level of epigenetics [19;20] and as these chromatin alterations are least partly heritable, can serve to mold the immune system and the response to subsequent stimuli.

This study examined whether patients with SLE exhibited fundamental changes at the TNF α locus which could contribute to its over-expression and perhaps lead to perpetuation of inflammation.

Methods

Patients

This study was approved by the Institutional Review Board and patients and controls consented to participation. Patients were recruited from an ongoing long term SLE cohort (n=46). Patient inclusion in this cohort is based on a clinical diagnosis of SLE [28]. A cumulative SLE database has been constructed for each patient. Patients are seen prospectively every three months after cohort entry. The specific patients in this study had quiescent disease with an average Physician Global Assessment of 0.6 and average ESRs throughout their time in the cohort of 27 mm/h. The average prednisone dose was 3.5 mg/d. Only 8% were positive for La antibodies. Controls (n=24) recruited from the community were required to have no ongoing health problems, nor acute illness, and to not be taking any medications.

Flow cytometry

Flow cytometry was performed utilizing a combination of antibodies to detect monocyte populations with intracellular cytokine staining with anti-TNF α (BD Biosciences, San Jose, CA). Monocytes were initially purified over Ficoll-Paque and subsequent adherence to plastic for 30 minutes. Purity was typically 85–90% with the major contaminant being T cells. On this fresh preparation, viability was always greater than 98%. Where indicated, cells were stimulated for 6 hours with 1 μ g/ml of LPS in the presence of brefeldin A. Surface staining with CD11b, CD16, CD14, and DR (BD Biosciences) was performed. Intracellular cytokine staining was performed using the reagents and methodology of the manufacturer (BD Biosciences). The FACSCalibur was set to define monocytes through the use of physical parameters and a combination of stains. The settings were conserved from sample to sample to allow comparisons of fluorescence intensity.

Transcription analyses

Chromatin immunoprecipitation (ChIP) assays were performed as per the protocol from Upstate Biotechnology (Lake Placid, NY) with some modifications [20]. Shear size was confirmed by gel analysis. Primer/probe combinations were optimized according to Applied Biosystems recommendations and competition was evaluated via serial titrations. No competition between TNF α primers and control GAPDH primers was seen at any concentration of primer. Antibodies were purchased from Upstate Biotechnology (anti-acetylated H3 and anti-acetylated H4) or Sigma-Aldrich (anti-BSA) (St. Louis, MO).

Real-time PCR was utilized to quantitate the amount of immunoprecipitated DNA from specific regions of the TNF α promoter and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal control. Each DNA sample was subjected to four PCR reactions for the four regions of the TNF α promoter [20]. The regions detected by each amplicon are the following: TNF1 (+99/–42), TNF2 (+32/–119), TNF3 (–100/–250), TNF4 (–195/–345) and GAPDH (–152/–211). Generally, duplicates or triplicates of samples were run and averaged separately prior to analysis. Known concentrations of genomic DNA were used as standards and positive controls with each Taqman run. Output was normalized to the GAPDH signal in each well according to the manufacturer's instructions.

Reverse transcription PCR was performed using RNA prepared with RNeasy plus DNase (Qiagen, Valencia, CA), followed by cDNA preparation using the Advantage RT for PCR method (BD Biosciences). Real-time PCR was used to quantitate the specific TNF α cDNA. Primers and probes for the human TNF α target and the 18S internal control were from Applied Biosystems (Foster City, CA).

Statistical analysis

Comparisons of flow cytometry results utilized t-tests, as did the overall comparisons of histone acetylation between controls and patients. Error bars on all graphs indicate Standard Deviation. Oneway ANOVA was used to identify clinical characteristics associated with the patients who exhibited the highest histone acetylation. The study design incorporated these clinical features and thus, correction for multiple comparisons was not performed.

Results

Monocyte characteristics in patients with SLE

We utilized flow cytometry to characterize monocyte subsets and their competence to produce TNF α . Monocyte subsets were identified through surface stains and TNF α was characterized by intracellular staining (Figure 1). The fraction of cells within the DR+/CD16+ subset co-expressing CD14 was similar between patients and controls ($p=0.26$). There was a trend towards a higher number of CD14+ cells within the DR+/CD11b+ subset although not statistically significant ($p=0.08$). These data suggest that the monocyte compartment is not grossly altered in SLE.

The monocytes were evaluated for their spontaneous and stimulated TNF α production. The unstimulated cells produced essentially no detectable TNF α in either patients or controls. The fraction of stimulated cells positive for TNF α within the DR+/CD16+ subset trended higher in patients than controls ($p=0.17$) but the fraction of stimulated cells positive for TNF α within the DR+/CD14+ subset was clearly higher in patients than controls ($p=0.02$). The mean fluorescence intensity of TNF α was also higher in patients than controls within this conventional subset of monocytes ($p=0.03$).

Histone acetylation at the TNF α locus

We utilized a ChIP assay to define histone acetylation at the TNF α locus in patients and controls. Purified monocytes were examined unstimulated to identify the resting characteristics of the locus. The TNF α promoter was divided into four regions for evaluation. The TNF1 region brackets the transcription initiation site and TNF4 corresponds to the distal promoter region. Increased histone acetylation is generally associated with increased competence to transcribe genes [21]. Acetylated histone H3 exhibits a characteristic pattern whereby the transcription initiation site (TNF1) is most highly acetylated. There is progressively less histone acetylation with transition to the distal upstream region (TNF4). Both patients and controls have the same pattern and have comparable levels of histone H3 acetylation (Figure 2). There is little variation between individuals with respect to H3 acetylation. Histone H4 acetylation has a different pattern with the highest levels of H4 acetylation in the distal promoter. The pattern is similar between patients and controls, however, there is far more histone H4 acetylation in patients than controls. This is particularly true for the distal TNF3 and TNF4 regions of the promoter ($p=0.003$ and $p=0.009$ respectively). There is greater variation among individual patients than in the controls. We analyzed the relationship of the TNF4 H4 acetylation with the production of TNF α in the conventional DR+/CD14+ monocyte population in 16 patients for which both data sets were available from the same specimen and 7 controls. There is a significant relationship between the expression TNF α and TNF4 H4 acetylation (Figure 3).

H3 and H4 acetylation generally is associated with enhanced competence for transcription. To determine whether the cells were producing increased transcripts, cDNA was quantitated from patient and control monocytes, before and after stimulation. Neither control nor patient cells produced substantial TNF α transcripts in the absence of stimulation. Patient cells produced substantially higher levels of TNF α upon stimulation compared to controls (Figure 4).

The H4 acetylation elevated above H3 acetylation at the TNF α locus has not been seen in *in vitro* model systems. To determine whether a soluble mediator present in the sera of patients with lupus could induce this alteration, twelve ChIP assays were performed on control human donor-derived monocytes incubated either with control sera or sera from lupus patients at 20% for sixteen hours. There were minimal differences between cells incubated with control sera vs. lupus sera, however, the pattern did not replicate that seen in fresh patient monocytes (Figure 5).

Elevated H4 acetylation and disease features

Seventeen patients with the highest H4 acetylation at the TNF4 region were compared to the other two thirds of patients. The mean H4 acetylation at the TNF4 site in this group was 2.0 with a range of 1.25–5.5. The middle third of patients had a mean H4 acetylation at the TNF4 site of 1.0 with a range of 0.84–1.2. The bottom third of patients had a mean H4 acetylation at the TNF4 site of 0.64 with a range of 0.33–0.8. These compare with the control group which had a mean H4 acetylation at the TNF4 site of 0.75 with a range of 0.3–1.3. Thus, the top one third of patient H4 acetylation levels were higher than the controls. The presence of features commonly associated with lupus were defined (Table 1). Oneway ANOVA was performed to compare the relationship of these features with high H4 acetylation (Table 1). High H4 acetylation was associated with an increased likelihood of cytotoxic drug use, and a higher erythrocyte sedimentation rate (on the day of the sample as well as over the course of the disease). In addition, it was noted that three of the five patients with a history of cerebro-vascular accident (CVA) had markedly elevated H4 acetylation. Due to the small number of patients, the findings were not statistically significant. The association of the elevated erythrocyte sedimentation rate and H4 acetylation is not surprising. TNF α typically drives the acute phase responses.

To determine whether medication use could alter the H4 acetylation, we compared current medication use in patients who were studied. There were no differences noted in the current medications (prednisone, non-steroidal anti-inflammatory agents, hydroxychloroquine, or anti-hypertensive agents) when the group with the highest TNF4 H4 acetylation was compared to the rest of the studied patients (Table 1).

Discussion

The rationale for this study was to investigate whether semipermanent changes in monocytes, as a consequence of the disease, could facilitate inflammatory gene expression and perhaps contribute to disease perpetuation. Over-expression of inflammatory genes could contribute to end organ manifestations such as the premature coronary artery disease seen in SLE [10;29;30;31;32]. This is the first study to specifically examine the chromatin conformation at the TNF α locus in patients with SLE. Epigenetic changes such as histone modifications have recently been demonstrated to predict chemotherapy responses in prostate cancer [33] and to correlate with COPD severity [34], suggesting that epigenetic changes arising during the course of the disease can alter outcome. Perhaps more significantly, interventions directed at histone acetylation have been shown to be beneficial in murine models of inflammation [35;36] and in human disease [37;38].

Monocytes in SLE patients have been infrequently evaluated previously. Traditional surface marker expression has not been markedly different in patients and controls in some studies, although receptors for immune complexes are down-regulated, perhaps due to internalization [39;40;41]. One study using single color flow cytometry, found decreased CD14 expression and decreased DR expression [41]. Increased monocyte apoptosis has been described and these results may reflect fewer monocyte cells rather than decreased expression of CD14 on an individual monocyte [42]. Interestingly, this same study identified increased spontaneous

TNF α production from patients compared to controls [41], which was not seen in the current study. Intracellular flow cytometry may not have been sufficiently sensitive to detect the spontaneous secretion.

The importance of this study is in the identification of a chromatin modification which is associated with increased TNF α transcripts in SLE patients. Although only one stimulus was used, the increased H4 acetylation suggests that responses to multiple stimuli would be increased. This is unlikely to be due to simple accelerated maturation because in previous studies, both the H3 acetylation and H4 acetylation have been increased with maturation [20]. Previous studies have determined that artificial histone acetylation at the TNF α locus is sufficient to increase the cells ability to respond to stimuli but does not lead in and of itself to transcription of TNF α [20]. Thus, we would hypothesize that the high serum levels of TNF α that are seen in SLE patients are due both to an increased competence to produce TNF α and to the presence of a stimulus, either or both of which may be a consequence of the disease process [43]. The immunology of SLE is extremely complex and there is evidence that histone acetylation at certain genetic loci may correlate with biochemical or phenotypic disease features while acetylation of other loci appears to be protective [35;44]. Thus, the effect of increased acetylation of H4 at the TNF α locus must be placed within the context of a complex network of immunologic relationships.

The finding of increased H4 acetylation specifically at the TNF4 site was unexpected. In previous studies, H3 and H4 acetylation were typically coordinately regulated [20]. The TNF4 site has recently been identified as a potential repressor binding location but has generally been felt to be transcriptionally silent based on transient transfection assays [15]. A known histone acetyltransferase, CBP/p300 has been shown to bind more proximally on the promoter, however, the TNF4 site is not known to bind any recognized histone acetyltransferases [18]. There is the potential for ATF2 to recognize two potential DNA motifs in the TNF4 region. ATF2 is a potent histone acetyltransferase, known to be activated in response to inflammatory stimuli and with a preference for H4 over H3 [45;46]. Future studies could address the role of ATF2.

This study demonstrates that monocytes from patients with lupus are altered such that they have increased competence to produce TNF α . Increased histone acetylation has been shown to independently [20] increase the competence of the cell to produce TNF α . Patients with a TNF α promoter wrapped around nucleosomes with increased histone acetylation would therefore respond more readily to any stimulus. This quality of the monocytes could predate the disease but is more likely a consequence of the disease and may contribute to the persistence of the disease.

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References

1. Malave I, Searles RP, Montano J, Williams RC. Production of tumor necrosis factor/cachectin by peripheral blood mononuclear cells in patients with systemic lupus erythematosus. *International Archives of Allergy and Applied Immunology* 1989;89:355–361. [PubMed: 2793223]
2. Robak E, Sysa-Jedrzejewska A, Dzikowska B, Torzecka D, Chojnowski K, Robak T. Association of Interferon γ , tumor necrosis factor α and interleukin 6 serum levels with systemic lupus erythematosus activity. *Archivum Immunologiae et Therapiae Experimentalis* 1998;46:375–380. [PubMed: 9883317]
3. Gabay C, Cakir N, Moral F, Roux-Lombard P, Meyer O, Dayer JM, Vischer T, Yazici H, Guerne PA. Circulating levels of tumor necrosis factor soluble receptors in systemic lupus erythematosus are

- significantly higher than other rheumatic diseases and correlate with disease activity. *Journal of Rheumatology* 1997;24:303–308. [PubMed: 9034987]
4. Swaak AJ, van den Brink HG, Aarden LA. Cytokine production (IL-6 and TNF alpha) in whole blood cell cultures of patients with systemic lupus erythematosus. *Scandinavian Journal of Rheumatology* 1996;25:233–8. [PubMed: 8792800]
 5. Al-Janadi M, Al-Balla S, Al-Dalaan A, Raziuddin S. Cytokine profile in systemic lupus erythematosus, rheumatoid arthritis, and other rheumatic diseases. *Journal of Clinical Immunology* 1993;113:58–67. [PubMed: 8445045]
 6. Studnicka-Benke A, Steiner G, Petera P, Smolen JS. Tumour necrosis factor alpha and its soluble receptors parallel clinical disease and autoimmune activity in systemic lupus erythematosus. *British Journal of Rheumatology* 1996;35:1067–74. [PubMed: 8948291]
 7. Brennan DC, Yui MA, Wuthrich RP, Kelley VE. Tumor necrosis factor and IL-1 in New Zealand black/white mice. *Journal of Immunology* 1989;143:3470–3475.
 8. Jacob CO, Lee SK, Strassmann G. Mutational analysis of TNF-alpha gene reveals a regulatory role for the 3'-untranslated region in the genetic predisposition to lupus-like autoimmune disease. *Journal of Immunology* 1996;156:3043–50.
 9. Kim N, Ussin L, Cheng X, Murali R, Sullivan KE. TNFalpha inhibition in MRL/lpr mice ameliorates pulmonary but not renal disease. *Journal of Autoimmunity* 2002;19:215–22. [PubMed: 12473242]
 10. Svenungsson E, Gunnarsson I, Fei GZ, Lundberg IE, Klareskog L, Frostegard J. Elevated triglycerides and low levels of high-density lipoprotein as markers of disease activity in association with up-regulation of the tumor necrosis factor alpha/tumor necrosis factor receptor system in systemic lupus erythematosus. *Arthritis & Rheumatism* 2003;48:2533–40. [PubMed: 13130473]
 11. Svenungsson E, Fei GZ, Jensen-Urstad K, de Faire U, Hamsten A, Frostegard J. TNF-alpha: a link between hypertriglyceridaemia and inflammation in SLE patients with cardiovascular disease. *Lupus* 2003;12:454–61. [PubMed: 12873047]
 12. Beutler B, Mahoney J, Le Trang N, Pekala P, Cerami A. Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. *Journal of Experimental Medicine* 1985;161:984–95. [PubMed: 3872925]
 13. Boswell JM, Yui MA, Burt DW, Kelley VE. Increased tumor necrosis factor and IL-1 beta gene expression in the kidneys of mice with lupus nephritis. *Journal of Immunology* 1988;141:3050–4.
 14. Herrerea-Esparza R, Barbosa-Cisneros O, Villalobos-Hurtado R, Avalos-Diaz E. Renal expression of IL-6 and TNF-alpha genes in lupus nephritis. *Lupus* 1998;7:154–158. [PubMed: 9607638]
 15. Suriano AR, Sanford AN, Kim N, Oh M, Kennedy S, Henderson MJ, Dietzmann K, Sullivan KE. GCF2/LRRFIP1 represses tumor necrosis factor alpha expression. *Mol Cell Biol* 2005;25:9073–81. [PubMed: 16199883]
 16. Falvo JV, Brinkman BM, Tsytsykova AV, Tsai EY, Yao TP, Kung AL, Goldfeld AE. A stimulus-specific role for CREB-binding protein (CBP) in T cell receptor-activated tumor necrosis factor alpha gene expression. *Proc Natl Acad Sci U S A* 2000;97:3925–9. [PubMed: 10760264]
 17. Goldfeld AE, Strominger JL, Doyle C. Human tumor necrosis factor alpha gene regulation in phorbol ester stimulated T and B cell lines. *Journal of Experimental Medicine* 1991;174:73–81. [PubMed: 2056282]
 18. Tsai EY, Falvo JV, Tsytsykova AV, Barczak AK, Reimold AM, Glimcher LH, Fenton MJ, Gordon DC, Dunn IF, Goldfeld AE. A lipopolysaccharide-specific enhancer complex involving Ets, Elk-1, Sp1, and CREB binding protein and p300 is recruited to the tumor necrosis factor alpha promoter in vivo. *Mol Cell Biol* 2000;20:6084–94. [PubMed: 10913190]
 19. Ramirez-Carrozzi VR, Nazarian AA, Li CC, Gore SL, Sridharan R, Imbalzano AN, Smale ST. Selective and antagonistic functions of SWI/SNF and Mi-2beta nucleosome remodeling complexes during an inflammatory response. *Genes Dev* 2006;20:282–96. [PubMed: 16452502]
 20. Lee JY, Kim NA, Sanford A, Sullivan KE. Histone acetylation and chromatin conformation are regulated separately at the TNF alpha promoter in monocytes and macrophages. *Journal of Leukocyte Biology* 2003;73:862–871. [PubMed: 12773519]
 21. Strahl BD, Allis CD. The language of covalent histone modifications. *Nature* 2000;403:41–5. [PubMed: 10638745]

22. Lu Q, Wu A, Ray D, Deng C, Attwood J, Hanash S, Pipkin M, Lichtenheld M, Richardson B. DNA methylation and chromatin structure regulate T cell perforin gene expression. *Journal of Immunology* 2003;170:5124–32.
23. Schubeler D, Francastel C, Cimborá DM, Reik A, Martin DI, Groudine M. Nuclear localization and histone acetylation: a pathway for chromatin opening and transcriptional activation of the human beta-globin locus. *Genes & Development* 2000;14:940–50. [PubMed: 10783166]
24. Spilianakis CG, Flavell RA. Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. *Nat Immunol* 2004;5:1017–27. [PubMed: 15378057]
25. Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA. T helper cell differentiation: regulation by cis elements and epigenetics. *Immunity* 2006;24:369–79. [PubMed: 16618596]
26. Bruniquel D, Schwartz RH. Selective, stable demethylation of the interleukin-2 gene enhances transcription by an active process. *Nat Immunol* 2003;4:235–40. [PubMed: 12548284]
27. Guo L, Hu-Li J, Zhu J, Watson CJ, Difilippantonio MJ, Pannetier C, Paul WE. In TH2 cells the IL4 gene has a series of accessibility states associated with distinctive probabilities of IL-4 production. *Proceedings of the National Academy of Sciences of the United States of America* 2002;99:10623–8. [PubMed: 12149469]
28. Tan EM, Cohen AS, Fries JF, Masi AT, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis and Rheumatism* 1982;25:1271–7. [PubMed: 7138600]
29. Vlachoyiannopoulos PG, Kanellopoulos PG, Ioannidis JP, Tektonidou MG, Moutsarakou I, Moutsopoulos HM. Atherosclerosis in premenopausal women with antiphospholipid syndrome and systemic lupus erythematosus: a controlled study. *Rheumatology* 2003;42:645–51. [PubMed: 12709540]
30. Manger K, Kusus M, Forster C, Ropers D, Daniel WG, Kalden JR, Achenbach S, Manger B. Factors associated with coronary artery calcification in young female patients with SLE. *Annals of the Rheumatic Diseases* 2003;62:846–50. [PubMed: 12922957]
31. Roman MJ, Shanker BA, Davis A, Lockshin MD, Sammaritano L, Simantov R, Crow MK, Schwartz JE, Paget SA, Devereux RB, Salmon JE. Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *New England Journal of Medicine* 2003;349:2399–406. [PubMed: 14681505]
32. Asanuma Y, Oeser A, Shintani AK, Turner E, Olsen N, Fazio S, Linton MF, Raggi P, Stein CM. Premature coronary-artery atherosclerosis in systemic lupus erythematosus. *New England Journal of Medicine* 2003;349:2407–15. [PubMed: 14681506]
33. Seligson DB, Horvath S, Shi T, Yu H, Tze S, Grunstein M, Kurdastani SK. Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* 2005;435:1262–6. [PubMed: 15988529]
34. Ito K, Ito M, Elliott WM, Cosio B, Caramori G, Kon OM, Barczyk A, Hayashi S, Adcock IM, Hogg JC, Barnes PJ. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. *N Engl J Med* 2005;352:1967–76. [PubMed: 15888697]
35. Mishra N, Reilly CM, Brown DR, Ruiz P, Gilkeson GS. Histone deacetylase inhibitors modulate renal disease in the MRL-lpr/lpr mouse. *J Clin Invest* 2003;111:539–52. [PubMed: 12588892]
36. Choi JH, Oh SW, Kang MS, Kwon HJ, Oh GT, Kim DY. Trichostatin A attenuates airway inflammation in mouse asthma model. *Clin Exp Allergy* 2005;35:89–96. [PubMed: 15649272]
37. Chang JG, Hsieh-Li HM, Jong YJ, Wang NM, Tsai CH, Li H. Treatment of spinal muscular atrophy by sodium butyrate. *Proceedings of the National Academy of Sciences of the United States of America* 2001;98:9808–13. [PubMed: 11504946]
38. Atweh GF, Sutton M, Nassif I, Boosalis V, Dover GJ, Wallenstein S, Wright E, McMahon L, Stamatoiyannopoulos G, Faller DV, Perrine SP. Sustained induction of fetal hemoglobin by pulse butyrate therapy in sickle cell disease. *Blood* 1999;93:1790–7. [PubMed: 10068649]
39. Szucs G, Kawai M, Suranyi P, Kiss E, Csipo I, Szegedi G. Correlations of monocyte phagocytic receptor expressions with serum immune complex level in systemic lupus erythematosus. *Scandinavian Journal of Immunology* 1994;40:481–4. [PubMed: 7973454]
40. Cairns AP, Crockard AD, Bell AL. The CD14+ CD16+ monocyte subset in rheumatoid arthritis and systemic lupus erythematosus. *Rheumatology International* 2002;21:189–92. [PubMed: 11958435]

41. Steinbach F, Henke F, Krause B, Thiele B, Burmester GR, Hiepe F. Monocytes from systemic lupus erythematosus patients are severely altered in phenotype and lineage flexibility. *Ann Rheum Dis* 2000;59:283–8. [PubMed: 10733475]
42. Shoshan Y, Shapira I, Toubi E, Frolkis I, Yaron M, Mevorach D. Accelerated Fas-mediated apoptosis of monocytes and maturing macrophages from patients with systemic lupus erythematosus: relevance to in vitro impairment of interaction with iC3b-opsonized apoptotic cells. *Journal of Immunology* 2001;167:5963–9.
43. Nagai T, Arinuma Y, Yanagida T, Yamamoto K, Hirohata S. Anti-ribosomal P protein antibody in human systemic lupus erythematosus up-regulates the expression of proinflammatory cytokines by human peripheral blood monocytes. *Arthritis Rheum* 2005;52:847–55. [PubMed: 15751081]
44. Nambiar MP, Warke VG, Fisher CU, Tsokos GC. Effect of trichostatin A on human T cells resembles signaling abnormalities in T cells of patients with systemic lupus erythematosus: a new mechanism for TCR zeta chain deficiency and abnormal signaling. *J Cell Biochem* 2002;85:459–69. [PubMed: 11967985]
45. Kawasaki H, Schiltz L, Chiu R, Itakura K, Taira K, Nakatani Y, Yokoyama KK. ATF-2 has intrinsic histone acetyltransferase activity which is modulated by phosphorylation. *Nature* 2000;405:195–200. [PubMed: 10821277]
46. Kawasaki H, Taira K, Yokoyama K. Histone acetyltransferase (HAT) activity of ATF-2 is necessary for the CRE-dependent transcription. *Nucleic Acids Symp Ser* 2000;259–60. [PubMed: 12903367]

Figure 1.

Comparison of monocyte subsets between patients (n=24) and controls (n=12). A) The surface phenotype of monocytes is not markedly different in patients compared to controls. CD14 cells within populations defined by DR + CD11b and DR + CD16 were enumerated. B) Cells were evaluated from cultures that were either mock stimulated or stimulated with 1 μ g/ml of LPS for six hours in the presence of brefeldin A. The fraction of stimulated cells expressing TNF α within the DR+/CD14+ monocytes is greater in patients than controls (p=0.02). C) The mean fluorescence intensity of TNF α expression within the stimulated TNF α positive cells is higher in the patients than the controls (p=0.03). D) A representative flow plot from a patient for intracellular cytokine staining. The light grey plot is an isotype control, the dark grey plot represents unstimulated cells stained for TNF α , and the black plot represents stimulated cells. All were gated on monocytes, DR and CD14. Note the clear distinction between positive and negative cells.

A

Histone Acetylation in SLE Patients and Controls

B **Figure 2.**

A) H3 or H4 histone acetylation was measured in resting monocytes by ChIP analysis. Real time PCR was performed using an internal standard and the results are reported as normalized units. DNA quantitation is expressed as the ratio of TNF DNA to GAPDH promoter DNA. Acetylated H3 and acetylated H4 at the GAPDH promoter were not different between patients and controls. Patients (n=46) and controls (n=24) were compared. Histone H3 acetylation was comparable for patients and controls. Histone H4 acetylation is greater in the patients than the controls, particularly in the TNF3/4 region, the distal promoter. Specifically, the p value comparing the TNF1 region was 0.62, the p value comparing the TNF2 region was 0.11, the p value comparing the TNF3 region was 0.003, and the p value comparing the TNF4 region

was 0.009. B) A map of the TNF probes utilized in the Real Time PCR analyses. The bent arrow represents the transcription initiation site. Transcription factor binding sites are indicated in boxes.

Correlation of TNF alpha expression and H4 acetylation

Figure 3.

Higher acetylated H4 levels at the TNF4 region are seen in the patients (n=16, solid circles) with the highest frequency of TNF α producing cells within the CD14/DR positive population (y-axis). Seven controls (grey squares) are included for comparison. Their H4 acetylation levels tend to be lower than the patients, although there is overlap. Importantly, there is an overall relationship between H4 acetylation and the ability to produce TNF α . The R^2 value is 0.61, the p value is <0.002.

Figure 4.

SLE patients (n=11) produce higher levels of TNF α message in their peripheral blood monocytes than controls (n=11). Approximately 10 million monocytes were purified by adherence to plastic and either mock stimulated or stimulated with 1 μ g/ml of LPS for 3 hours. RNA and cDNA were prepared and real-time PCR was used to quantitate the cDNA. 18S RNA was used as an internal control and the expressed values represent TNF α normalized to 18S RNA with unstimulated control signal acting as the calibrator.

Figure 5.

Control human monocytes incubated with sera from patients with systemic lupus erythematosus do not have the same pattern of increased H4 acetylation. The primer/probe combinations define the same regions as shown in Figure 2b. There is a trend towards higher H4 acetylation in monocytes treated with sera from lupus patients, however, it reached statistical significance for the TNF2 region only ($P=0.02$). None of the p values for the H3 acetylation approached significance. For H4, the relevant p values are TNF1; $p=0.15$, TNF2; $p=0.02$, TNF3; $p=0.06$, TNF4; $p=0.35$.

Table 1
Clinical Features of Patients with the Highest TNF4 H4 Acetylation

Clinical Feature	Number of patients affected	P value
Average Hematocrit	N=46	0.1972
Hematocrit at sample date	N=46	0.2071
Low CH50	N=4	0.2799
ANA positive	N=44	0.5237
Average ESR	N=46	0.0449
Maximum ESR	N=46	0.0291
ESR at sample date	N=46	0.0283
Cerebrovascular accident	N=5	0.3429
Physician Global Assessment at sample date	N=46	0.4374
Cytotoxic use (ever)	N=26	0.0348
Prednisone use (current)	N=46	0.5063
Plaquenil use (current)	N=32	0.1949
Non-steroidal use (current)	N=18	0.1254

* The p value represents a comparison of the features within the 17 patients with the highest H4 acetylation versus the 29 patients with lower H4 acetylation. Significant p values are bolded. The number of patients affected represents the number in the entire cohort with that feature.