HHS Public Access

Author manuscript

Toxicol Lett. Author manuscript; available in PMC 2017 October 17.

Published in final edited form as:

Toxicol Lett. 2016 October 17; 260: 1–7. doi:10.1016/j.toxlet.2016.08.017.

Chronic exposure to trichloroethylene increases DNA methylation of the *Ifng* promoter in CD4+ T cells

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Abstract

CD4$^+$ T cells in female MRL+/+ mice exposed to solvent and water pollutant trichloroethylene (TCE) skew toward effector/memory CD4$^+$ T cells, and demonstrate seemingly non-monotonic alterations in IFN-γ production. In the current study we examined the mechanism for this immunotoxicity using effector/memory and naïve CD4$^+$ T cells isolated every 6 weeks during a 40 week exposure to TCE (0.5 mg/ml in drinking water). A time-dependent effect of TCE exposure on both *Ifng* gene expression and IFN-γ protein production was observed in effector/memory CD4$^+$ T cells, with an increase after 22 weeks of exposure and a decrease after 40 weeks of exposure. No such effect of TCE was observed in naïve CD4$^+$ T cells. A cumulative increase in DNA methylation in the CpG sites of the promoter of the *Ifng* gene was observed in effector/memory, but not naïve, CD4$^+$ T cells over time. Also unique to the *Ifng* promoter was an increase in methylation variance in effector/memory compared to naïve CD4$^+$ T cells. Taken together, the CpG sites of the *Ifng* promoter in effector/memory CD4$^+$ T cells were especially sensitive to the effects of TCE exposure, which may help explain the regulatory effect of the chemical on this gene.

Graphical Abstract

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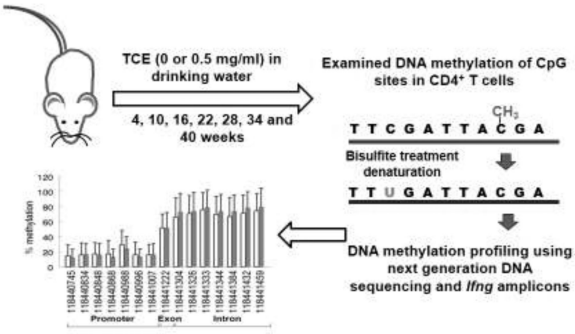

Keywords

trichloroethylene; next-generation sequencing; epigenetics; immunotoxicity; CD4$^+$ T cells

Introduction

Mouse models which combine genetic susceptibility and an environmental stressor can be utilized to study toxicant-induced autoimmune disease etiology. Using such a model we have shown that chronic exposure to the industrial solvent and common water pollutant trichloroethylene (TCE) generates a slow-developing autoimmune disease that closely resembles human autoimmune hepatitis (AIH).[1,2] Several autoimmune diseases and hypersensitivity disorders have been linked to human TCE exposure.[3,4,5,6,7,8,9,10,11,12,13,14] In our mouse model TCE appeared to predominantly impact CD4$^+$ T cells, resulting in increased percentages of effector/memory CD4$^+$ T cells and increased production of IFN-γ by CD4$^+$ T cells. However, the effect of TCE on IFN-γ production by CD4$^+$ T cells was inconsistently detected depending upon the length of the TCE exposure.[1,15]

Autoimmune disease development is a lengthy process with both linear and non-linear components. For example, human autoimmune pancreatitis appears to involve a biphasic mechanism in which the early stage involves release of IFN-γ, while the chronic stage is instead mediated by Th2-like cytokines.[16] Similar to many human autoimmune diseases, studies in rodent models have shown that levels of specific cytokines such as IFN-γ can wax and wane during the different stages (e.g. onset, peak and regression) of disease development.[17] Expression kinetics ranging from bell-shaped, biphasic, and linear have been noted for different cytokines and chemokines in specific tissues in a model of adjuvant arthritis in rats.[18] A biphasic cytokine response in which an early increase in IFN-γ in the blood is followed by an increase in IL-17 and TNF-α was found in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis.[19] Similarly, IFN-γ was shown to promote the early induction phase of murine collagen-induced arthritis, but to suppress later stages of the arthritic process.[20]

Generation of IFN-γ by CD4$^+$ T cells is regulated at several levels, including differential activation of transcription factors such as STAT4 and STAT6.[21] Expression of *Ifng* has also been shown to be under epigenetic control.[22] Unlike the promoters of *Il2* and *Il4*, the *Ifng* promoter is already hypomethylated in naïve murine CD4$^+$ T cells and remains so when

naïve CD4$^+$ T cells differentiate into IFN-γ-secreting Th1 cells.[23] However, during differentiation of naïve CD4$^+$ T cells to Th2 cells silencing of *Ifng* expression is achieved by repressive DNA methylation on different CpG sites in the promoter.[21] Regulation is somewhat different in human CD4$^+$ T cells in which methylation of the *IFNG* promoter in naïve cells is in the mid/high range, and requires demethylation to permit expression of *IFNG* by effector/memory CD4$^+$ T cells. Even though the *IFNG* promoter in human naïve T cells is not hypomethylated, it can undergo increased methylation associated with gene silencing following exposure to xenobiotics, as demonstrated by CD4$^+$ T cells from patients with diisocyanate asthma [24], in the cord white blood cells children of mothers exposed to polycyclic aromatic hydrocarbons [25], and in the T cells of children exposed to secondhand smoke.[26] These studies point out the potential impact of xenobiotics on *Ifng* methylation, and therefore *Ifng* expression.

We have previously reported that chronic TCE exposure can alter DNA methylation; CD4$^+$ T cells from mice exposed to TCE for 17 weeks showed increased expression of *Iap* (intracisternal A particle) retrotransposons and decreased global DNA methylation in CD4$^+$ T cells.[15] The time-dependent effects of TCE on gene-specific DNA methylation was not evaluated. Since directional changes in global DNA methylation are often quite different from what occurs at the level of gene-specific CpG regions, the current study was designed to examine the impact of chronic adult TCE exposure on methylation of CpG sites in the *Ifng* promoter and exon/intron. In view of the seemingly non-linear IFN-γ production profile by CD4$^+$ T cells in our mouse models, this examination encompassed a 40 week exposure to TCE.

Lastly, since DNA methylation patterns can be cell type specific both naïve and effector/memory CD4$^+$ T cells isolated from MRL+/+ mice every 6 weeks during were assessed. The evaluation revealed time-dependent subset-specific TCE-induced alterations in DNA methylation of CpG sites flanking the *Ifng* transcription start site, and help explain the non-monotonic effect of TCE on CD4$^+$ T cell IFN-γ production. This evaluation is also the first to examine the impact of a toxicant on gene-specific epigenetic drift.

Materials and Methods

Mouse treatment

Eight week-old female MRL+/+ mice (Jackson Laboratories; Bar Harbor, ME) were exposed to TCE as previously described.[15] The mice (8–9 mice/treatment group/time point) received 0 or 0.5 mg/ml TCE in their drinking water for 4, 10, 16, 22, 28, 34 or 40 weeks. Mice received drinking water and food (Harlan 7027) *ad libitum*. TCE exposure from water consumption averaged 40–50 mg/kg/day, in comparison to the Permissible Exposure Limit [established by the Occupational Safety and Health Administration (OSHA)] for TCE of approximately 76 mg/kg/day. All studies were approved by the Animal Care and Use Committee at the University of Arkansas for Medical Sciences.

CD4+ T cells

Mice were sacrificed at different time points, and splenic CD62L^{lo} and CD62L^{hi} CD4$^+$ T cell populations were isolated as described.[27] The resulting CD62L^{hi} CD4$^+$ T cells (naïve CD4$^+$) or CD62L^{lo} CD4$^+$ T cells (effector/memory CD4$^+$ T cells) were stimulated with immobilized anti-CD3 antibody and anti-CD28 antibody for 20 hours.[28] The resulting culture supernatants were collected and evaluated for IFN-γ by ELISA, and the activated CD4$^+$ T cells themselves were frozen in RLT (Qiagen) for subsequent examination of DNA methylation. To ensure sufficient cell numbers for the analyses each sample of CD4$^+$ T cells used in the study originated as an equal number of pooled spleen cells from 2 – 3 mice resulting in 4 samples per time point within each treatment group.

DNA methylation

Targeted bisulfite next-generation sequencing (NGS) was performed at the Molecular Cytogenetics and Epigenetics facility of Columbia University. Amplicons were generated on a Fluidigm Access Array and sequenced using an Illumina MiSeq platform. Sequence alignment to chromosomal locations (mouse genome build mm10) and DNA methylation levels were determined using Bismark (http://www.bioinformatics.babraham.ac.uk/projects/bismark/).[29] The degree of methylation is expressed as the percentage of total cytosines methylated at individual CpG sites. For most CpG sites, hundreds of reads per sample were used for each percentage methylation determination. In all cases, at least 10 reads/CpG site/sample were used.

qRT-PCR

Fluorescence-based quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was conducted using RNA isolated from naïve or effector/memory CD4$^+$ T cells as described.[2] Fold differences ($\log_2$) in expression were determined using expression levels of resting (unactivated) CD4$^+$ T cells of the appropriate subset of control mice as the control (1×) expression level.

Gene Arrays

This assessment was conducted by the Genomics Core at the University of Arkansas for Medical Sciences. All RNA samples extracted from CD4$^+$ T cells had RIN (RNA integrity number) values of 8.0 or above. Total RNA (500 ng) was converted to cDNA, amplified and biotinylated by use of the Ambion Illumina TotalPrep™-96RNA Amplification Kit (Life Technologies, Carlsbad, CA) . Gene expression profiling was performed using the Expression BeadChip System from Illumina (Illumina Inc, San Diego, CA) following the manufacturer's instructions. Raw data were $\log_2$ transformed and normalized to the median intensity signal of 47,231 genes on the array. After normalization and filtering of low intensity spots, 2-sample Student's t-tests were performed and these data were plotted against fold-change measurements. Statistical significance was set at false discovery rate (FDR) <0.05. Ingenuity Pathway Analysis software (Redwood City, CA) was used for network identification

Statistics

The data are presented as means ± standard deviations. Most assays were conducted using naïve or effector/memory $CD4^+$ T cells isolated from equal numbers of pooled spleen cells from 2–3 mice resulting in 4 samples/treatment group/time point. The threshold for statistical significance was set at $p< 0.05$. Differences between experimental groups were tested first with analysis of variance (ANOVA), and where the F test was significant, subsequent pairwise contrasts were tested using a two-sample t-test. $CD4^+$ T cell concentration and gene expression values were right-skewed, and therefore these data were log-transformed for statistical analyses. Adjusting for multiple comparisons, p-values from pairwise comparisons that were smaller than the Bonferroni-adjusted significance level indicated statistical significance.

Results

TCE alters $CD4^+$ T cell gene expression

A microarray approach was used to obtain a more comprehensive assessment of TCE-induced alterations in $CD4^+$ T cell gene expression. This analysis used effector/memory ($CD62L^{lo}$) $CD4^+$ T cells collected from mice exposed to TCE at the 22 week time point. This time point was selected because it was temporally mid-range, when TCE-induced alterations in effector/memory $CD4^+$ T cells are evident. Gene expression was examined 20 hours after activation of the $CD4^+$ T cells *in vitro*. At a cutoff of FDR<0.05 and a fold-change >1.25, the expression of over 400 genes was found to be significantly altered in the activated effector/memory $CD4^+$ T cells of mice exposed to TCE compared to similarly activated effector/memory $CD4^+$ T cells from control mice. Approximately 75% of the gene changes observed represented decreased expression in the TCE-exposed mice. Altered expression of immune-associated genes in the effector/memory $CD4^+$ T cells after 22 weeks of TCE exposure is listed in Table 1. A network evaluation suggested that gene expression increases induced after 22 weeks of TCE exposure centered on *Ifng* (data not shown).

To confirm and extend the microarray results regarding *Ifng*, the effects of TCE on the expression of *Ifng* were examined by qRT-PCR at 10-, 22-, and 40-week time points in both naïve and effector/memory $CD4^+$ T cells. Gene expression was examined 24 hours after activation of the $CD4^+$ T cells *in vitro*, and compared to resting $CD4^+$ T cells of the appropriate subset from control mice. In effector/memory $CD4^+$ T cells from control mice the activation-induced expression of *Ifng* remained fairly constant over time (Figure 1A). In contrast, exposure to TCE caused a peak-and-valley pattern of *Ifng* expression in effector/memory $CD4^+$ T cells with an increase at 22 weeks and a decrease at 40 weeks. This result recapitulated the 22-week TCE-induced increase in *Ifng* expression found in the array analysis. It also supported the apparent plasticity of the TCE effect on *Ifng* in the effector/memory $CD4^+$ T cells. Unlike effector/memory $CD4^+$ T cells naïve $CD4^+$ T cells did not demonstrate TCE-induced changes in *Ifng* expression. In addition to gene expression, protein levels of IFN-γ secreted by the different subsets of $CD4^+$ T cells at the 3 time points were evaluated. This result mirrored the qRT-PCR results, showing that effector/memory $CD4^+$ T cells from TCE-treated mice produced more IFN-γ than controls at 22 weeks, and less IFN-γ than controls at 40 weeks (Figure 1B). Taken together, it appeared that exposure

to TCE had non-monotonic effects on IFN-γ at both the gene and protein level. These effects were confined to effector/memory $CD4^+$ T cells, and eventually resulted in a downregulation of the cytokine.

DNA methylation of CpG sites associated with cytokines in $CD4^+$ T cells

Targeted bisulfite NGS was used to test whether the decrease in *Ifng* expression in effector/memory $CD4^+$ T cells after 40 weeks of TCE exposure was associated with specific changes in DNA methylation. Both naïve and effector/memory $CD4^+$ T cells were evaluated at each of the seven time points. The analysis targeted the region of the *Ifng* promoter where methylation of individual CpGs has been shown to regulate gene expression[23]. The CpG sites we examined are in an 800 bp region approximately centered on the transcription start site (TSS) and included seven CpG sites in the upstream (promoter) region and eight CpG sites in the downstream (exon and intron) region[27]. CpG sites in the *Ifng* promoter displayed consistently low-to-medium (under 40%) levels of DNA methylation regardless of $CD4^+$ T cell subset, mouse age, or TCE exposure. In contrast, CpG sites in the exon/intron uniformly demonstrated intermediate-to-high (over 60%) levels of DNA methylation. Interestingly, within individual samples, we observed an inverse correlation between the cumulative methylation of the CpG sites in the promoter and the cumulative methylation of the CpG sites in the exon/intron (Figure 2). Thus, when methylation was increased at the CpG sites in the promoter of a sample it was decreased at the CpG sites in the exon/intron of that sample, and vice versa. This correlation was most evident in the effector/memory $CD4^+$ T cells from control ($R^2 = 0.599$) and TCE-treated ($R^2=0.706$) mice. A similar correlation, albeit less robust, was observed when comparing the CpG methylation in the promoter to the exon/intron region of *Ifng* in naïve $CD4^+$ T cells. The interconnected methylation levels flanking the *Ifng* TSS suggested the presence of a mechanism to stabilize DNA methylation in this gene region.

The effect of TCE exposure on the mean DNA methylation levels of the 15 individual CpG sites are illustrated in Figure 3. The results represent the average DNA methylation of individual CpG sites measured over all seven time points. When the CpG sites in the *Ifng* promoter were examined individually at specific time points, none of the TCE-induced changes in DNA methylation reached statistical significance. However, when examined cumulatively, the CpG sites in the *Ifng* promoter showed a significant increase in DNA methylation in effector/memory $CD4^+$ T cells from TCE-treated mice. This cumulative TCE-induced increase in DNA methylation in the *Ifng* promoter was not found in naïve $CD4^+$ T cells. There was no consistent cumulative effect of TCE on the methylation status of CpG sites in the exon/intron region of *Ifng* in either effector/memory or naïve $CD4^+$ T cells. This finding showed that despite the tight control of DNA methylation in CpG sites flanking the *Ifng* TSS, TCE could alter that pattern in effector/memory, but not naïve $CD4^+$ T cells.

Effects of TCE on epigenetic drift

TCE has been shown to have subtle effects on interindividual variance in DNA methylation (epigenetic drift) in $CD4^+$ T cells.[27] We extended that finding to examine epigenetic drift specifically associated with *Ifng*. Interesting, variance in the CpG sites in the *Ifng* promoter increased when $CD4^+$ T cells differentiated from naïve to effector/memory $CD4^+$ T cells

(Figure 4). This was true in effector/memory $CD4^+$ T cells from both control and TCE-treated mice. Variance in the DNA methylation of CpG sites in the exon/intron region of *Ifng* was not increased by differentiation from naïve to effector/memory $CD4^+$ T cell status. Taken together these findings suggest that methylation of the *Ifng* promoter is unique in several ways, including the fact that differentiation from naïve to effector/memory $CD4^+$ T cells results in an increase in the otherwise tightly controlled methylation variance, and that this variance may help make the effector/memory $CD4^+$ T cells susceptible to TCE-induced changes in DNA methylation.

Discussion

Most studies of autoimmune disease, whether in humans or animal models, represent a snapshot of a limited number of events at a single time point. However, it is clear that longitudinal investigations are needed to generate the most accurate picture of the events that initiate and maintain disease development. Using a long-term multi-endpoint evaluation this study demonstrated a non-monotonic effect of TCE exposure on IFN-γ production by effector/memory $CD4^+$ T cells. Other investigators have similarly shown temporal differences in IFN-γ expression and/or production over the course of autoimmune disease development. For example, it has been reported that EAE disease pathology was preceded by an increase in IFN-γ production by splenic T cells, followed by a decrease in splenic IFN-γ production.[30] Similarly, the kinetics of IFN-γ production by lymph node cells in experimental autoimmune arthritis was shown to be a critical part of disease pathology, and to determine whether treatment with exogenous IFN-γ at different times in disease development was protective.[31] However, since these studies measured IFN-γ using whole splenic T cells, or whole lymph node populations it is difficult to known how they reflect activity in individual $CD4^+$ T cell subsets. Our study shows that even within the effector/memory $CD4^+$ T cell subset, IFN-γ production was not linear.

As a pleotropic regulator it is not surprising that IFN-γ levels can change over the course of disease development to reflect both pro- and anti-inflammatory effects. IFN-γ is dichotomous in the regulation of distinct chemokine genes in the livers of mice with fulminant autoimmune hepatitis.[32] Similarly, although known primarily as an autoimmune-promoting cytokine IFN-γ can inhibit the development of experimental autoimmune uveitis [33], experimental allergic encephalomyelitis [34], and autoimmune arthritis.[31] Especially relevant to our study in MRL+/+ mice was the finding that exogenous IFN-γ has dichotomic effects on the development of spontaneous lupus in closely-related MRL lpr/lpr mice, both inhibiting and worsening disease progression depending on the stage of disease process.[35]

It has been reported that DNA methylation in a relatively small region of the *Ifng* promoter (that includes the −53, −205 and −297 CpG sites) controls promoter-driven *Ifng* expression during $CD4^+$ T cell differentiation.[36,23] This region of the *Ifng* promoter proximal to the TSS is hypomethylated in naïve and effector/memory murine $CD4^+$ T cells, while downstream regions proximal to the TSS are hypermethylated.[22] $CD4^+$ T cells skewed toward a Th1 phenotype retain their relatively hypomethylated promoter sites (10 to 19% 5MC in three promoter sites), while the same sites demonstrated 30 to 56% 5MC in Th2

polarized cells.[23] Generally consistent with this methylation pattern, Th1 cells generated *IFN-γ* protein at 50 times the level of the Th2 polarized cells. In addition to the cytokines secreted during Th2 cell differentiation, certain environmental events such as adult exposure to diesel exhaust or developmental exposure to polycyclic aromatic hydrocarbons can alter epigenetic marks in the *Ifng* promoter.[37,38,25] Low to moderate levels of methylation on the *Ifng* promoter such as we report here following TCE exposure may allow the gene to remain "poised" for varying levels of transcription. However, it appears that long-term exposure to TCE can increase the variance of that methylation, and eventually silence that transcription via increased DNA methylation. Effector/memory $CD4^+$ T cells isolated after a 40-week TCE exposure demonstrated lower levels of both *Ifng* gene expression and IFN-γ protein secretion when compared to effector/memory $CD4^+$ T cells from control mice. Thus, although in general effector/memory $CD4^+$ T cells express more *Ifng* than naïve $CD4^+$ T cells, on a per cell basis long-term exposure to TCE inhibited *Ifng* expression in the effector/memory $CD4^+$ T cell subset. It appears that TCE-induced expansion of effector/memory $CD4^+$ T cells is distinct from TCE-induced increased cytokine production by $CD4^+$ T cells. The cumulative effects of long-term TCE exposure on effector/memory $CD4^+$ T cells, combined with the differential IFN-γ production by naïve and effector/memory $CD4^+$ T cells, may account for the seemingly non-linear IFN-γ profile measured in unseparated populations of $CD4^+$ T cells at different times during TCE exposure.

Our previous study showed that CpG sites for most genes displayed either low 0–20% methylation or high 80–100% methylation.[27] This pattern was very stable over time, and variation in the DNA methylation of a particular site was largely due to stochastic effects related to mean methylation of the site. Thus, variance for sites that averaged 0–20% or 80–100% methylation was uniformly low, while variance was highest for CpG sites where the average methylation was intermediate. This mathematical phenomenon has been described by others.[39] In comparison to the CpG sites for the other genes examined, the CpG sites in the *Ifng* promoter did not conform well to the model in which methylation variability was linked to mean methylation levels [27]. This suggested that compared to several other genes methylation of CpGs in the *Ifng* promoter was less tightly controlled, and more susceptibility to changes induced by external factors such as exposure to TCE.

It should be noted that one limitation of this study was the need to pool spleen cells from 2–3 mice in order to obtain sufficient numbers of both effector/memory and naïve $CD4^+$ T cells. Although this eliminated possible confounding effects that might result from different percentages of the two subsets in a sample, it may have decreased the ability to detect subtle consistent changes in DNA methylation of individual CpG sites. It should also be noted that we do not know whether the decrease in IFN-γ observed in memory $CD4^+$ T cells from mice exposed to TCE for 40 weeks was an important part of disease progression or a compensatory mechanism designed to protect against the pro-inflammatory effects of the cytokine.

Acknowledgments

Thanks to Rachel Lee and Dustyn Barnette for excellent technical assistance.

Funding

This work was supported by grants from the Arkansas Biosciences Institute, the National Institutes of Health (R01ES017286, R01ES021484), the Organic Compounds Property Contamination class action settlement (CV 1992-002603), and the UAMS Translational Research Institute (National Institutes of Health UL1RR029884).

Abbreviations

TCE	trichloroethylene
TSS	transcription start site
AIH	autoimmune hepatitis

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Highlights

- Chronic TCE decreased IFN-γ production by effector/memory $CD4^+$ T cells
- TCE increased DNA methylation of *Ifng* promoter in effector/memory $CD4^+$ T cells
- *Ifng* promoter of effector/memory $CD4^+$ T cells susceptible to epigenetic drift

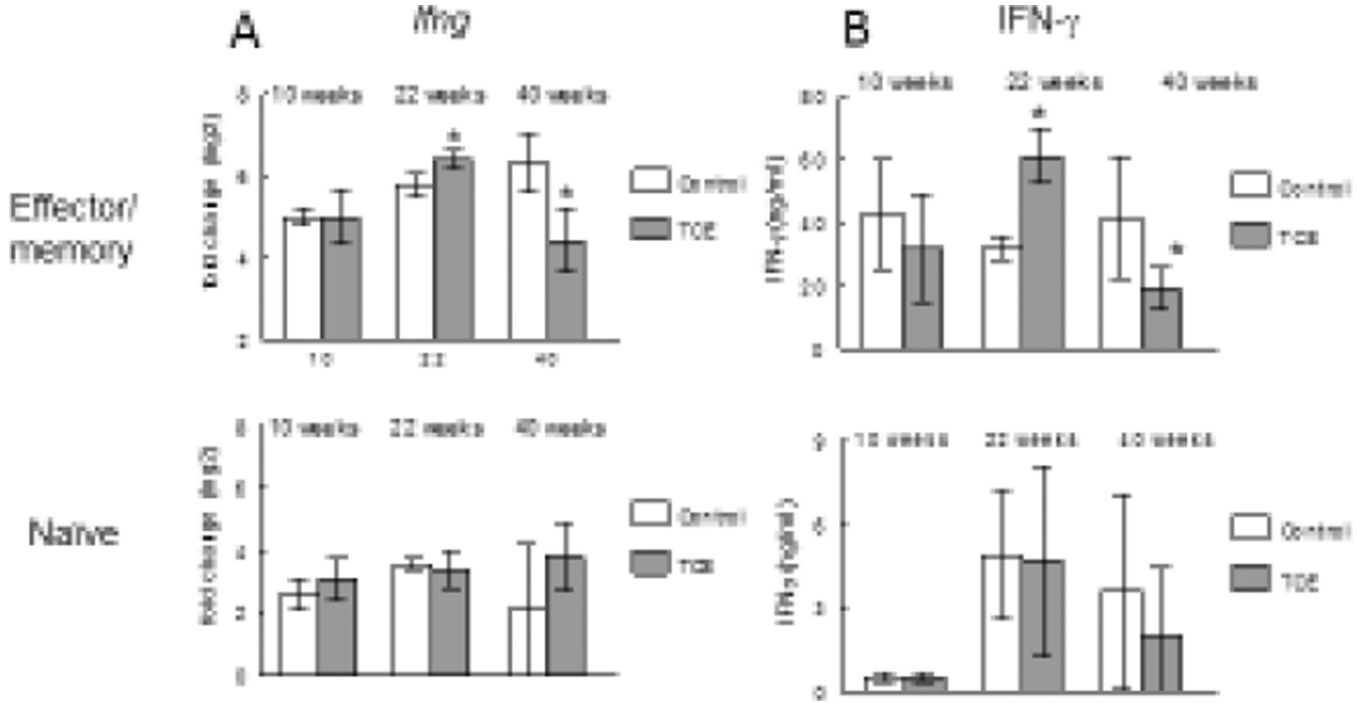

Figure 1. Chronic TCE exposure induced non-monotonic effects on IFN-γ

Female MRL+/+ mice were exposed to TCE (0.05 mg/ml) for 4, 22 or 40 weeks. Effector/memory ($CD62L^{lo}$) and naïve ($CD62l^{hi}$) $CD4^+$ T cells were isolated from the spleens and activated ex vivo. Both *Ifng* expression (A) and secreted IFN-γ (B) was measured. *Significantly different ($p<0.05$) compared to control values. At the 40 week time point, the number of $CD62L^{lo}$ effector/memory $CD4^+$ T cells per mouse spleen was significantly different between control mice ($5.6 \pm 2.3 \times 10^7$) and TCE-treated mice ($8.9 \pm 2 \times 10^7$).

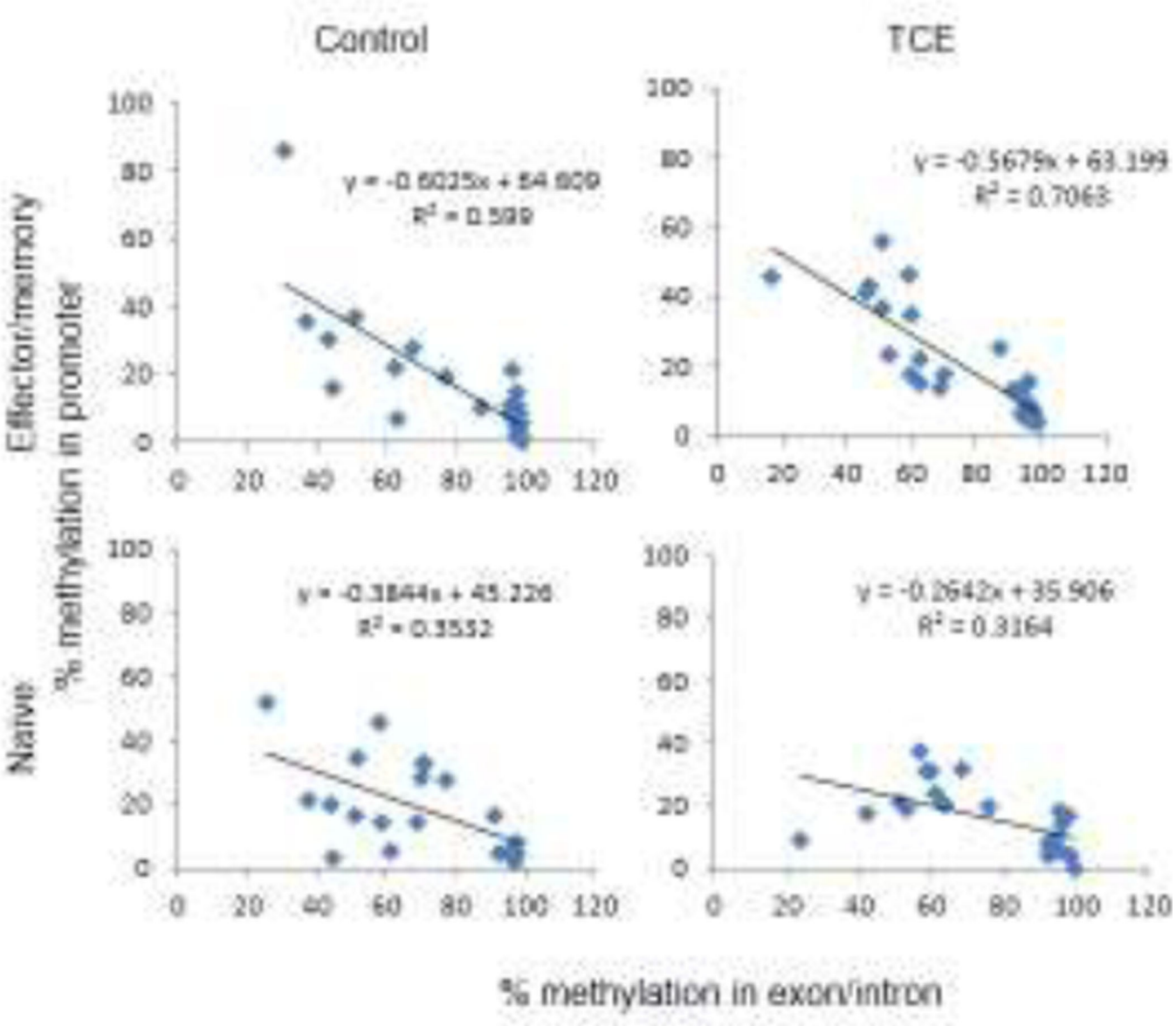

Figure 2. Relationship of methylation of CpG sites in the promoter and exon/intron of *Ifng*

Scatter plots show the correlation between the mean DNA methylation of CpG sites in the promoter in a single sample and the mean DNA methylation of the CpG sites in the exon/intron of the same sample. Data was collected from naïve and effector/memory $CD4^+$ T cells across all time points, and each point represents a single sample.

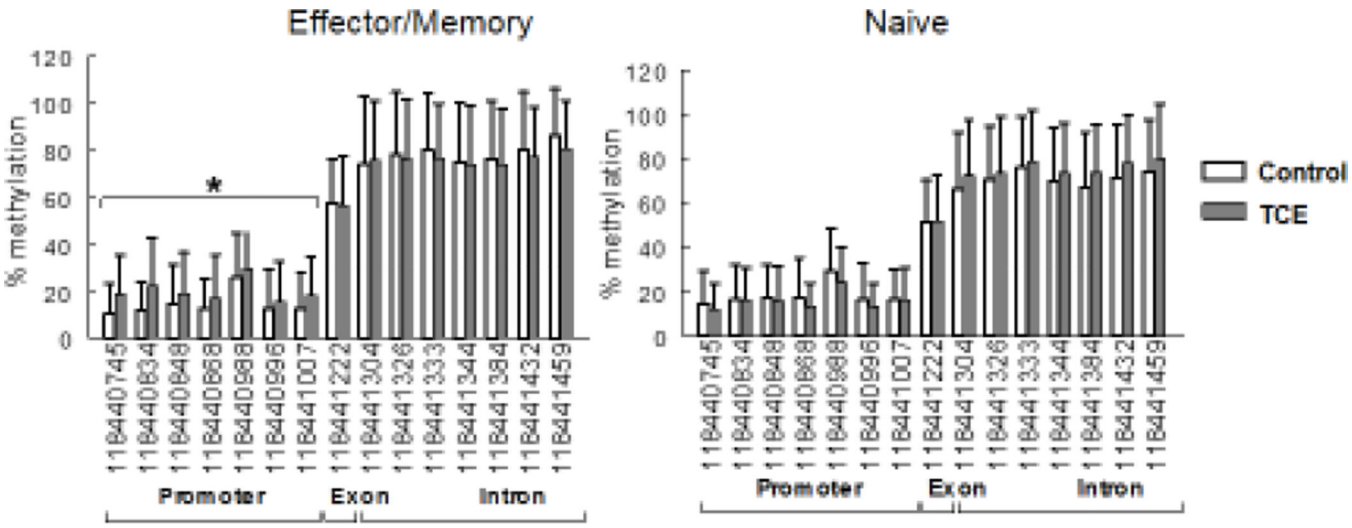

Figure 3. Mean methylation of *Ifng*-associated CpG sites in both effector/memory and naïve CD4$^+$ T cells

DNA methylation at CpG sites in both the promoter and exon/intron region of *Ifng* in effector/memory and naïve CD4$^+$ T cells from control or TCE-treated mice was determined. Shown is the mean methylation of the data collected at all 7 time points. The CpG sites are designated by chromosome position. *Significantly different ($p<0.05$) compared to control values.

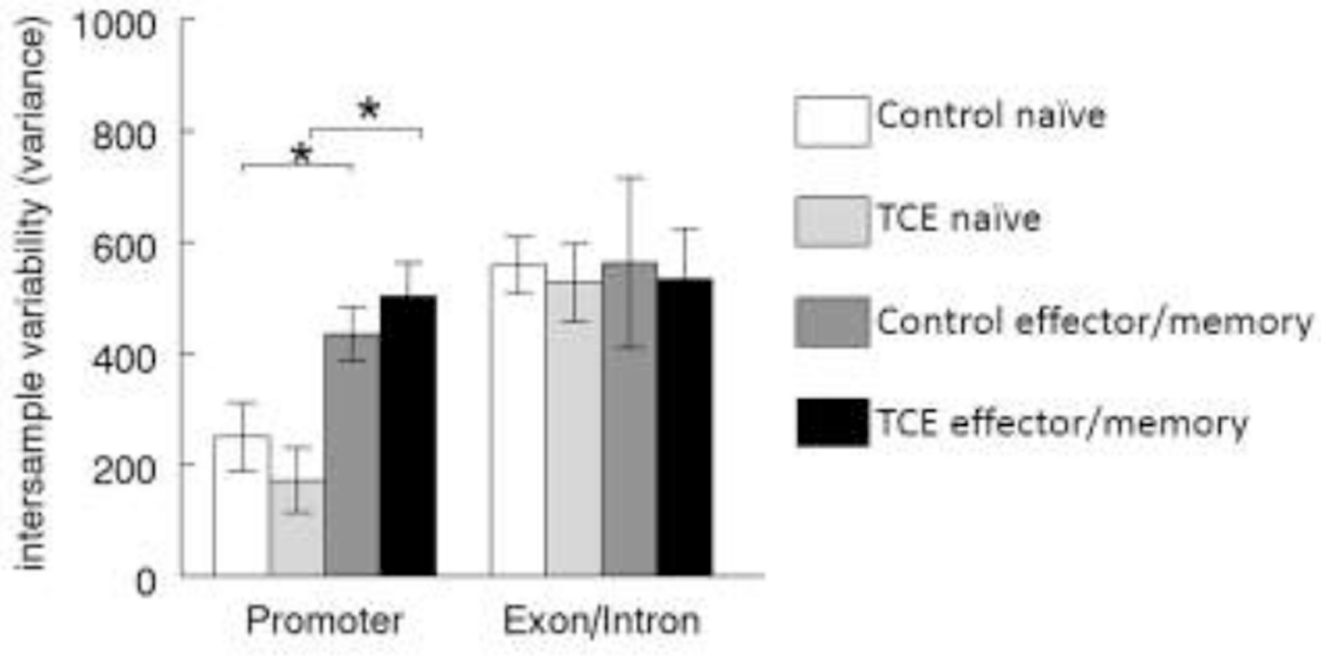

Figure 4. Alterations in methylation variance in CpG sites of *Ifng* promoter in effector/memory CD4$^+$ T cells

Methylation variance was measured across all time points for CpG sites in the promoter or exon/intron regions of *Ifng* in both naïve and effector/memory CD4$^+$ T cells from control and TCE-treated mice. *Significantly different ($p<0.05$) compared to control values.

Table 1

Immune gene expression altered in effector/memory $CD4^+$ T cells from mice exposed to TCE for 22 weeks compared to controls

Genebank	Gene symbol	Gene name
Increased gene expression		
Cytokines/Chemokines		
NM_007720.2	Ccr8	Chemokine (C-C motif) receptor 8
NM_009969.4	Csf2	Colony stimulating factor 2 (granulocyte-macrophage)
NM_007913.5	Egr1	Early growth response 1 transcription factor
NM_008337.1	Ifng	Interferon gamma
NM_008366.2	Il2	Interleukin 2
NM_010556.4	Il3	Interleukin-3
NM_010558.1	Il5	Interleukin-5
NM_001039537.1	Lif	Leukemia inhibitory factor
NM_010735.1	Lta	Lymphotoxin A
NM_013693.2	Tnf	Tumor necrosis factor
NM_011612.2	Tnfrsf9	Tumor necrosis factor receptor superfamily, member 9
NM_011613.3	Tnfsf11	Tumor necrosis factor (ligand) superfamily, member 11
NM_009424.2	Traf6	Tnf receptor-associated factor 6
Decreased gene expression		
Cytokines/Chemokines		
NM_008332.2	Ifit2	Interferon-induced protein with tetratricopeptide repeats 2
NM_010501.2	Ifit3	Interferon-induced protein with tetratricopeptide repeats 3
NM_010511.2	Ifngr1	Interferon gamma receptor 1
NM_008390.1	Irf1	Interferon regulatory factor 1
NM_016850.2	Ifr7	Interferon regulatory factor 7
NM_008394.2	Irf9	Interferon regulatory factor 9
Apoptosis		
NM_026976.2	Faim3	Fas apoptotic inhibitory molecule 3
NM_011050.3	Pdcd4	Programmed cell death 4
Signal transduction		
NM_019963.1	Stat2	Signal transducer and activator of transcription 2
Integrin		
NM_010581.3	Cd47	Integrin-associated signal transducer
NM_007799.2	Cd180	CD180 antigen
NM_010545.3	Cd74	Major histocompatibility complex, class II antigen-associated
NM_010741.2	Ly6c1	Lymphocyte antigen 6 complex, locus C1
NM_010742.1	Ly6d	Lymphocyte antigen 6 complex, locus D
NM_008529	Ly6e	Lymphocyte antigen 6 complex, locus E