Chapter 4
Trichloroethylene-Induced Oxidative Stress and Autoimmunity

M. Firoze Khan and Gangduo Wang

Abstract Trichloroethylene (trichloroethene, TCE) is a widely used organic solvent and a common environmental and occupational contaminant. Apart from diseases like cancer and heart defects, TCE exposure has also been implicated in the development of various autoimmune diseases (ADs), such as systemic lupus erythematosus (SLE), systemic sclerosis and fasciitis, both from occupational and environmental exposures. Experimental studies using MRL+/+ mice as an animal model also support an association between TCE exposure and autoimmunity. Increasing evidence suggests that free radical-mediated reactions could play a potential role in the pathogenesis of ADs, and TCE exposure is known to cause oxidative stress both in vivo and in vitro. Recent studies have contributed to the understanding of the role of oxidatively modified proteins, especially lipid peroxidation-derived aldehyde (LPDA)-modified proteins in TCE-induced autoimmune response. These studies support that oxidative modification of endogenous proteins leads to structural alterations, resulting in the formation of neoantigens which elicit autoimmune responses by stimulating T and/or B lymphocytes, particularly Th1 and Th17 lymphocytes. More detailed studies to understand the distinct pathways by which oxidative stress contributes to autoimmunity, especially mapping of gene expression, analyzing proteome, blocking/inhibiting specific signal transduction pathways will also unravel critical mechanisms in TCE-mediated autoimmunity.

Keywords Trichloroethylene • Autoimmunity • Oxidative stress • MDA/HNE-protein adducts • Th1 cells • Th17 cells

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4.1 Introduction

Trichloroethylene (trichloroethene, TCE) is a widely used organic solvent and a common environmental and occupational contaminant (Diot et al. 2002; Hardin et al. 2005; Bakke et al. 2007; Moran et al. 2007; ATSDR 2010; Purdue et al. 2011). About 3.5 million people are occupationally exposed to TCE in the United States mainly through its use in degreasing operation, but also through dry cleaning, textile scouring, and in handling adhesives, drugs, paints, leather and other products (Wu and Schaum 2000; Bakke et al. 2007; ATSDR 2010).

Environmental exposure to TCE occurs through air, contaminated ground water and drinking water. Most TCE used in the United States is released to the atmosphere from vapor degreasing operations, and its release to air also occurs at sewage treatment and disposal facilities, water treatment facilities, and landfills (Wu and Schaum 2000; Bakke et al. 2007; ATSDR 2010). TCE has been detected in the air throughout the United States, and the 1998 air levels across all 115 monitors ranged between 0.01–3.9 μg/m^3 with a mean of 0.88 μg/m^3 (Wu and Schaum 2000; Bakke et al. 2007; ATSDR 2010). TCE was detected in 28 % of 9,295 surface water reporting stations nationwide, and it is the most frequently reported organic contaminant in groundwater with up to 34 % of the drinking water supplies in USA contaminated with TCE (IARC 1995; Wu and Schaum 2000; ATSDR 2010).

TCE has also been identified in 72 food items in the US Food and Drug Administration's Total Diet Study, including fruits, beverages and many foods prepared with oils and fats (Wu and Schaum 2000; ATSDR 2010). Because of its widespread commercial use and improper disposal, TCE has become a major occupational and environmental toxicant, and is one of the most abundant organic contaminants (NTP 1990; Bourg et al. 1992; Ashley et al. 1994; Hardin et al. 2005; Moran et al. 2007; ATSDR 2010). Therefore, there is clearly a need to extensively study the potential adverse health effect of TCE.

4.2 TCE Exposure and Autoimmune Response: Human Studies

TCE exposure has been associated with a variety of human diseases. Apart from diseases like cancer and heart defects (Boyer et al. 2000; Rhomberg 2000; Caldwell and Keshava 2006; Drake et al. 2006; Purdue et al. 2011), TCE has also been implicated in the development of various autoimmune diseases (ADs), such as systemic lupus erythematosus (SLE), systemic sclerosis and fasciitis, both from occupational (Phoon et al. 1984; Flindt-Hansen and Isager 1987; Lockey et al. 1987; Yáñez Díaz et al. 1992; Waller et al. 1994; Nietert et al. 1998; Cooper et al. 2009) and environmental exposures (Haustein and Ziegler 1985; Byers et al. 1988; Kilburn and Warshaw 1992; Hayashi et al. 2000 ; Albert et al. 2005 ; Cooper et al. 2009). The involvement of TCE exposure in ADs was first reported as early as in 1957 (Reinl 1957), and in recent

years an increasing number of reports have further implicated TCE in the development of various ADs. Kilburn and Warshaw (1992) examined the prevalence of connective tissue disease symptoms and ANA, by comparing 362 residents of Tucson to 158 residents of another area of Southwest Arizona. The prevalence of some self-reported symptoms (malar rash, arthritis/arthalgias, Raynaud syndrome, skin lesions, and seizure or convulsion) and ANA levels were higher in Tucson residents (Kilburn and Warshaw 1992). Reports have shown that occupational TCE exposure is also associated with scleroderma (Flindt-Hansen and Isager 1987; Lockey et al. 1987; Yáñez Díaz et al. 1992; Nietert et al. 1998; Diot et al. 2002; Pralong et al. 2009) and fasciitis (Waller et al. 1994). Some case-control studies provided data specifically about TCE exposure, based on industrial hygienist review of job history data. Three of these studies are of scleroderma (Nietert et al. 1998; Diot et al. 2002; Garabrant et al. 2003), one is of undifferentiated connective tissue disease (Lacey et al. 1999), and one is of small vessel vasculitis involving anti-neutrophil cytoplasmic autoantibodies (Beaudreuil et al. 2005). Occupational TCE-induced Stevens-Johnson syndrome and other skin disorders have also drawn attention (Phoon et al. 1984; Huang et al. 2006; Kamijima et al. 2008; Jia et al. 2012).

4.3 TCE Exposure and Autoimmune Response: In Vivo Studies

Khan and his colleagues were first to propose and use MRL+/+ mice as an animal model to provide direct evidence of an association between TCE exposure and autoimmunity (Khan et al. 1995). This association was further substantiated by their subsequent studies and reports from other laboratories using MRL+/+ mice (Gilbert et al. 1999; Griffin et al. 2000a; Khan et al. 2001; Wang et al. 2007a, b, 2008, 2009, 2012a; Cai et al. 2008). MRL+/+ mice, therefore, have been the most often used animal models in experimental studies of TCE exposure.

Several studies in MRL+/+ mice have reported autoimmunity-related effects following exposure to TCE via drinking water (Blossom et al. 2004, 2007; Cai et al. 2008; Gilbert et al. 1999; Griffin et al. 2000a, b, c; Wang et al. 2007a) or ip injection (Cai et al. 2006; Khan et al. 1995; Wang et al. 2007b, 2008, 2009, 2012a). The initial drinking water studies used relatively high TCE concentrations of 2.5 and 5 mg/mL, with serologic measurements of ANA and IgG levels and assays for the activation of $CD4^+$ T cells from spleen (Gilbert et al. 1999; Griffin et al. 2000a). Subsequent studies focused on examining TCE effects at lower exposure levels (0.1, 0.5, and 2.5 mg/mL) (Griffin et al. 2000b; Wang et al. 2007a, 2012a; Cai et al. 2008), also showed an accelerated autoimmune response. The effects observed by Griffin et al. (2000a) with respect to formation of TCE-protein adducts and $CD4^+$ T cell activation was blocked by inhibiting CYP2E1 metabolic pathway (Griffin et al. 2000b), suggesting the role of TCE activation and its metabolites. In another chronic exposure study (0.5 mg/mL TCE in drinking water), Cai et al. (2008) found evidence of systemic inflammation as determined by serum cytokines measured after 36–48 weeks of exposure.

Some chronic oral exposure studies in the MRL+/+ mice, with exposure periods of 32–48 weeks, reported the presence of distinct clinical effects in exposed mice. One of these effects was characterized as an autoimmune hepatitis (Griffin et al. 2000b; Cai et al. 2008). Griffin et al. (2000b) found an inflammatory focal areas in the 0.5- and 2.5-mg/mL TCE-treated mice, with a dose-related effect on severity hepatic infiltrate in the portal tracts and lobular scores seen at 32 weeks. Cai et al. (2008) found similar liver lymphocytic infiltrates at 36 and 48 weeks in a study using 0.5 mg/mL TCE exposure through drinking water, and also infiltrates in the pancreas, lungs, and kidneys at 48 weeks. Wang et al. (2007a) observed increased autoantibodies in another study using 0.5 mg/mL TCE via drinking water for 48 weeks. In a 40-week study using trichloroacetaldehyde hydrate, Blossom et al. (2007) reported diffuse alopecia and skin inflammation and ulceration. Studies with other animal models also demonstrated the potential of TCE in inducing autoimmune response. For example, a chronic (26-week) drinking water exposure study in NZB × NZW mice reported increased level of proteinuria and prevalence of renal pathology with TCE exposure of 10,000 ppb via drinking water (Gilkeson et al. 2004). Production of anti-dsDNA and other antibodies was increased following 1,400 ppb TCE exposure for 19 weeks.

Several studies also evaluated the involvement of one or more metabolites of TCE in the induction of an autoimmune response observed in MRL+/+ mice. These include studies of dichloroacetyl chloride (Khan et al. 1995, 2001; Cai et al. 2006), trichloroacetaldehyde hydrate (Blossom et al. 2004, 2007; Blossom and Gilbert 2006; Gilbert et al. 2006), and trichloroacetic acid (Blossom et al. 2004). Effects were similar to those found with TCE in terms of accelerated autoantibody expression, T cell activation, and secretion of inflammatory cytokines.

4.4 Role of Oxidative Stress in Autoimmune Diseases

ADs such as SLE, rheumatoid arthritis and scleroderma are chronic and life-threatening disorders that affect ~3 % of United States population, and contribute disproportionately to morbidity and mortality among young to middle-aged women (Jacobson et al. 1997; Walsh and Rau 2000). Despite high prevalence of these diseases, molecular mechanisms underlying systemic autoimmune response remain largely unknown. In recent years, increasing evidence suggests that free radical-mediated reactions could play a potential role in the pathogenesis of ADs (Khan et al. 2001; Hadjigogos 2003; Frostegard et al. 2005; Kurien and Scofield 2008; Wang et al. 2008, 2010a; Vasanthi et al. 2009; Iuchi et al. 2010). Indeed increased oxidative stress is reported in various ADs (Grune et al. 1997; Frostegard et al. 2005; Tam et al. 2005; Morgan et al. 2009; Vasanthi et al. 2009; Shah et al. 2010; Wang et al. 2010a; Al-Shobaili and Rasheed 2012; Al-Shobaili et al. 2013).

Reactive oxygen species (ROS) including superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$) and other reactive molecules containing oxygen formed during aerobic metabolism in cells as well as due to phagocyte and neutrophil activation during inflammation, have potential to initiate cellular damage to lipids, proteins and DNA (Biemond et al. 1984; Halliwell and Gutteridge 1984;

Finkel 2011). A variety of ROS-mediated modifications of proteins have been reported in ADs and aging (Stadtman and Berlett 1998; Oates et al. 1999; Beal 2002; Morgan et al. 2005; Sheikh et al. 2007; Wang et al. 2010a; Al-Shobaili and Rasheed 2012; Al-Shobaili et al. 2013). Sheikh et al. (2007) observed increased protein carbonyls and recognition of ROS-modified human serum albumin by circulating SLE autoantibodies in SLE patients. Recently, Al-Shobaili et al. (2013) reported higher level of anti-oxidized-catalase (CAT)-antibodies in SLE patients with varying levels of disease activity according to SLE Disease Activity Index (SLEDAI). These antibodies showed strong relation with the SLEDAI, disease induction and progression (Al-Shobaili and Rasheed 2012; Al-Shobaili et al. 2013), suggesting oxidized protein may be a useful biomarker in evaluating the progression of SLE and in elucidating the mechanisms of disease pathogenesis.

Reactive nitrogen species (RNS) are nitrogen-containing molecules, i.e., nitric oxide ($^{\cdot}NO$), peroxynitrite ($ONOO^{-}$) and nitroxyl anion (HNO^{-}) (Hill et al. 2010). Like ROS, RNS could also play a significant role in the pathogenesis of SLE and other ADs, and have drawn considerable attention in recent years. $^{\cdot}NO$, generated by the enzyme inducible nitric oxide synthase (iNOS), is one of the most important and widely studied RNS. The potential of $^{\cdot}NO$ in disease pathogenesis lies largely to the extent of its production and generation of $O_2^{\cdot-}$, leading to formation of peroxynitrite ($ONOO^{-}$). $ONOO^{-}$ is a potent nitrating and oxidizing agent which can react with tyrosine residues to form nitrotyrosine (NT; Weinberg et al. 1994; Xia and Zweier 1997; Khan et al. 2003). In addition, $ONOO^{-}$-mediated modifications of endogenous proteins and DNA may enhance their immunogenicity, leading to a break in immune tolerance (Khan et al. 2003; Ohmori and Kanayama 2005; Kurien et al. 2006). Accumulating evidence in murine lupus shows increasing iNOS activity with the development and progression of ADs, and studies using competitive inhibitors suggest that iNOS could play a pathogenic role in murine ADs (Weinberg et al. 1994; Xia and Zweier 1997; Karpuzoglu and Ahmed 2006; Wang et al. 2009). Also elevated presence of nitrated proteins, particular NT, a stable end product of increased RNS production, has been found in many diseases including ADs (Oates et al. 1999; Morgan et al. 2005; Khan et al. 2006; Ohmori and Kanayama 2005). Growing observational data in humans also suggest that overexpression of iNOS and increased production of $ONOO^{-}$ may contribute to glomerular and vascular pathology and in the pathogenesis of many other ADs (Wanchu et al. 1998; Nagy et al. 2007a; Morgan et al. 2009). There is appreciable evidence that NT and other markers of protein oxidation are enhanced in diabetes and many other ADs, and may contribute to the pathogenesis of these diseases (Stadtman and Berlett 1998; Oates et al. 1999; Martín-Gallán et al. 2003; Morgan et al. 2005; Ohmori and Kanayama 2005; Khan et al. 2006; Khan and Ali 2006; Renke et al. 2007; Wang et al. 2010a).

Reactive lipid species (RLS) are usually derived from unsaturated lipids, including lipid peroxidation-derived aldehydes (LPDAs) and reactive prostaglandins of A- and J-series (Higdon et al. 2012). LPDAs such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) are highly reactive and can bind covalently to proteins resulting in their structural modifications and may elicit an autoimmune response and contribute to disease pathogenesis (Khan et al. 1997, 1999; Januszewski et al. 2005; Reed et al. 2009; Wang et al. 2010a; Ben Mansour et al. 2010). Indeed higher levels of MDA-/HNE-modified proteins have been observed in AD patients (Grune et al. 1997; Kurien

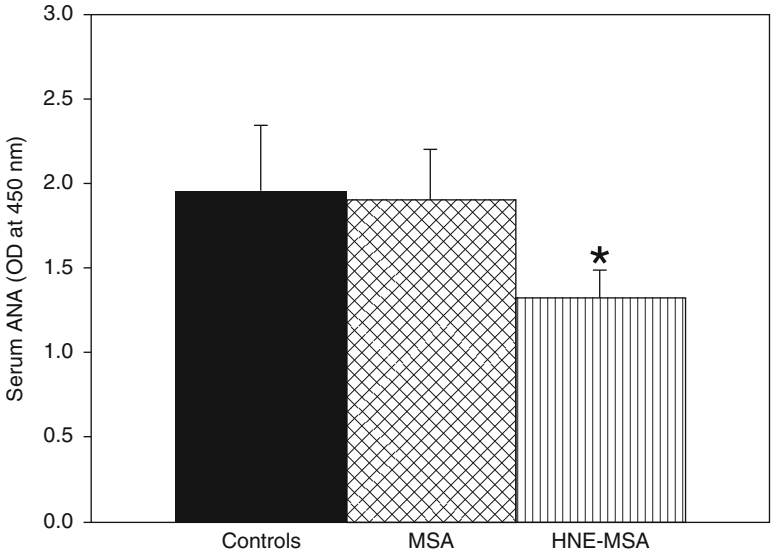

Fig. 4.1 Inhibitory effect of HNE-mouse serum albumin (*MSA*) on ANA binding to nuclear antigens. Serum from 18-week MRL/lpr mice was pre-incubated with HNE-MSA followed by ANA determination using ELISA kit. The values are means ± SD. *$p < 0.05$ vs. controls (serum only) (Adapted from Informa Healthcare)

and Scofield 2003; Frostegard et al. 2005; D'souza et al. 2008; Ben Mansour et al. 2010; Wang et al. 2010a), suggesting a potential role for these oxidatively modified proteins in ADs. Wang et al. (2010a) analyzed the sera from 72 SLE patients with varying levels of disease activity (according to the SLEDAI) and 36 age- and gender-matched healthy controls for oxidative stress markers and showed significantly higher levels of both MDA/HNE protein adducts and anti-MDA/anti-HNE protein adduct antibodies in SLE patients compared with healthy controls. Interestingly, not only was there an increased number of subjects positive for anti-MDA or anti-HNE antibodies, but also the levels of both of these antibodies were significantly higher among SLE patients whose SLEDAI scores were ≥6 as compared with SLE patients with lower SLEDAI scores (<6). In addition, a significant correlation was observed between the levels of anti-MDA or anti-HNE antibodies and the SLEDAI score, suggesting a possible causal relationship between these antibodies and SLE. The stronger response observed in serum samples from patients with higher SLEDAI scores suggests that markers of oxidative stress may be useful in evaluating the progression of SLE and in elucidating the mechanisms of disease pathogenesis. Recently, Wang et al. (2012b) observed age-related increases in the formation of MDA-/HNE-protein adducts, their corresponding antibodies and MDA-/HNE-specific immune complexes in MRL/lpr mice, the widely used model for SLE. Interestingly, HNE-MSA adducts mimic nuclear antigens and cause significant inhibition in ANA binding to nuclear antigens (Fig. 4.1; Wang et al. 2012b), suggesting that LPDA-modified proteins could be important sources of autoantibodies and CICs in these mice, and thus contribute to autoimmune disease pathogenesis.

4.5 TCE Exposure and Oxidative Stress

Free radical-mediated reactions have drawn increasing attention as the potential mechanism in the pathogenesis of ADs and other diseases (Khan et al. 2001; Hadjigogos 2003; Karpuzoglu and Ahmed 2006; Kurien et al. 2006; Cuzzocrea 2006; Nagy et al. 2007b). TCE has been shown to generate free radicals and induce oxidative stress both in vivo and in vitro (Ogino et al. 1991; Channel et al. 1998; Khan et al. 2001; Zhu et al. 2005; Wang et al. 2007a, b, 2008, 2012a). A list of studies leading to TCE-induced oxidative stress are summarized in Table 4.1. Several studies have reported an association between TCE exposure and increased oxidative stress, especially lipid peroxidation (Ogino et al. 1991; Channel et al. 1998; Khan et al. 2001; Zhu et al. 2005; Wang et al. 2007a, b, 2008, 2012a). Most of earlier studies (Cojocel et al. 1989; Ogino et al. 1991; Channel et al. 1998; Toraason et al. 1999) used high doses of TCE (125–2,000 mg/kg) to demonstrate TCE-induced lipid peroxidation which contributes to toxic response in the livers or kidneys. Wang et al. (2007b, 2008) reported that TCE exposure for 6 or 12 weeks led to significantly increased formation of MDA-/HNE-protein adducts in the livers of TCE-treated female MRL +/+ mice at both 6 and 12 weeks, but with greater response at 12 weeks. Further characterization of these adducts in liver microsomes showed increased formation of MDA-protein adducts with molecular masses of 86, 65, 56, 44, and 32 kD, and of HNE-protein adducts with molecular masses of 87, 79, 46, and 17 kD in TCE-treated mice (Wang et al. 2007b). In addition, significant induction of anti-MDA- and anti-HNE-protein adduct-specific antibodies was observed in the sera of TCE-treated mice, and showed a pattern similar to MDA- or HNE-protein adducts. TCE-induced formation of MDA-/HNE-protein adducts and their respective antibodies were also observed in mice exposed to a relatively lower dose of TCE (Wang et al. 2007a, 2012a)**.**

The potential of TCE in inducing nitrosative stress has also drawn attention recently (Wang et al. 2007a, 2009; Blossom et al. 2012). TCE exposure resulted in increased formation of NT and induction of iNOS in the serum of female MRL +/+ mice. TCE treatment also led to greater NT formation, and iNOS protein and mRNA expression in the livers and kidneys (Wang et al. 2009). TCE-induced formation of NT was also observed at relatively lower dosages of TCE (Wang et al. 2007a; Blossom et al. 2012), which could potentially contribute to TCE-induced autoimmune response.

The potential of TCE exposure leading to carbonylation of proteins has also been examined. TCE exposure (10 mmol/kg, i.p., every fourth day) in female MRL+/+ mice resulted in increased (~3 fold) serum protein carbonyls (a marker of protein oxidation) at both 6 and 12 weeks. Increased protein carbonyls were also observed in the livers and kidneys (2.1 and 1.3 fold, respectively) at 6 weeks, and to a greater extent at 12 weeks (3.5 and 2.1 fold, respectively) following TCE treatment (Wang et al. 2009). Increased protein carbonyls were also observed in ovaries, oocytes, sperms and kidneys if rats or mice exposed to TCE via drinking water (DuTeaux et al. 2004; Wu and Berger 2007; Fan et al. 2012). Fan et al. (2012) analyzed and

Table 4.1 Experimental studies of TCE exposure and oxidative stress

References	Studies	TCE exposure	Effect
Huang et al. (2012)	Humans	Occupational exposure	Oxidative stress
Blossom et al. (2012)	In vivo, female MRL+/+ mice	0.0 or 0.1 mg/ml in drinking water with 1 % of EL-620 for 6 weeks	Increased nitrotyrosine
Wang et al. (2012a)	In vivo, female MRL+/+ mice	0.5, 1.0 or 2.0 mg/ml in drinking water with 1 % of EL-620 for 12, 24, 36 weeks	Increased MDA-/HNE-protein adducts and antibodies; increased autoantibodies
Ali and Sultana (2012)	In vivo, male Swiss albino mice	200 µl of TCE (80 %, v/v, dissolved in acetone)	Depletion in GSH and SOD activity, induction of iNOS expression
Tabrez and Ahmad (2011)	In vivo, Swiss albino rats	1,000 mg/kg, i.p., in corn oil	Increased lipid peroxidation (MDA levels) and GST activity
Gharib (2009)	In vivo, male albino rats	1,000 mg/kg, oral, for 2 weeks	Increased MDA and NO, and decreased GSH
Khan et al. (2009)	In vivo, male Wistar rats	1,000 mg/kg/day, i.p., in corn oil for 25 days	Increased lipid peroxidation and declined SOD activity
Wang et al. (2009)	In vivo, female MRL+/+ mice	10 mmol/kg, i.p., every fourth day for 6 or 12 weeks	Increased MDA-/HNE-protein adducts & their antibodies; increased autoantibodies
Blossom et al. (2008)	In vivo, female MRL+/+ mice	0.1 mg/ml in drinking water with 1 % of EL-620 for 26 weeks	Increased ROS and decreased GSH
Wang et al. (2008)	In vivo, female MRL+/+ mice	10 mmol/kg, i.p., every fourth day for 4 weeks	Increased MDA-/HNE-protein adducts, their antibodies and autoantibodies
Shen et al. (2008)	Skin exposure, BALB/c hairless mice	50 µl of TCE dissolved in olive oil, skin exposure for 4 h, twice daily for 2 weeks	Increased MDA levels and inhibition of SOD activities
Hu et al. (2008)	In vitro, human HepG2 cells	0.5–4 mM	Increased 8-OhdG and increased lipid peroxidation (TBARS)
Shen et al. (2007)	In vitro, epidermal keratinocytes	0.125, 0.25, 0.50, 1.0 and 2.0 mM	Dose-dependent increases of iNOS activities and mRNA expression
Wang et al. (2007b)	In vivo, female MRL+/+ mice	10 mmol/kg, i.p., every fourth day for 6 or 12 weeks	Increased nitrityrosine, protein carbonyls with increased autoantibodies

Wang et al. (2007a)	In vivo, female MRL+/+ mice	0.5 mg/ml in drinking water with 1 % of EL-620for 48 weeks	Increased anti-MDA-/HNE-protein adduct antibodies, nitrotyrosine along with increased autoantibodies
Wu and Berger (2007)	In vivo, albino rats	0.45 % TCE (v/v) in 3 % Tween in drinking water for 4–5 days	Increased protein carbonyls
DuTeaux et al. (2004)	In vivo, Sprague-Dawley rats	0, 0.2 % or 0.4 % (v/v) in drinking water for 14 days	Increased protein carbonyls
Zhu et al. (2005)	In vitro, epidermal keratinocytes	0.01–31.6 mM for 1, 2, 3, 4 h	Time- and concentration-dependent increases of MDA and decreases in GSH
Chen et al. (2002)	In vitro, cell lines: H460, H1299	0.9–6.5 μl/cm^2 for 24 h	Increased TBARS and MDA; decreased GSH
Khan et al. (2001)	In vivo, female MRL+/+ mice	10 mmol/kg, i.p., every fourth day for 6 weeks	Increased anti-MDA specific antibodies
Toraason et al. (1999)	In vivo, Fisher rats	0, 100, 500, 1,000 mg/kg, i.p., in 1:4 (v/v) of Alkamuls/water	Increased TBARS and 8OHdG formation
Channel et al. (1998)	In vivo, B6C3F1 mice	0, 400, 800, and 1,200 mg/kg in corn oil, orally, once daily for 8 weeks	Increased TBARS and 8OHdG formation
Ogino et al. (1991)	In vivo, male Wistar rats	2,000 mg/kg, i.p., in olive oil	Increased MDA
Cojocel et al. (1989)	In vivo, Male NMRI mice	125–150 mg/kg, i.p., in sesame oil	Increased MDA

characterized the carbonylated proteins by using two-dimensional (2D) gel electrophoresis, Western blot along with matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI TOF/TOF MS/MS) in the kidneys following TCE exposure in female MRL+/+ mice (2 mg/ml via drinking water) for 36 weeks. TCE treatment led to significantly increased protein carbonyls in the kidney protein extracts. Interestingly, among 18 identified carbonylated proteins, 10 were found only in the kidneys of TCE-treated mice, whereas other eight were present in the kidneys of both control and TCE-treated mice. The identified carbonylated proteins represent skeletal proteins, chaperones, stress proteins, enzymes, plasma protein, and proteins involved in signaling pathways. Huang et al. (2012) examined the serum proteome in the TCE-induced hypersensitivity dermatitis patients via 2D gel coupled with MALDI-TOF-TOF/MAS and also found that inflammatory responses and oxidative stress might contribute to TCE-induced hypersensitivity dermatitis.

In vitro studies (Chen et al. 2002; Zhu et al. 2005; Shen et al. 2007; Hu et al. 2008) using a variety of human cell lines such as human epidermal keratinocytes, human lung cancer H460 and p54-null H1299 cells have shown that TCE can induce oxidative stress, particularly lipid peroxidation in a time- and concentration-dependent pattern, and GSH, an intracellular antioxidant, provided protection against TCE-induced oxidative damage.

4.6 TCE-Induced Oxidative Stress and Induction of Autoimmunity

The role of oxidative stress in the TCE-induced autoimmune response was first proposed by Khan and his colleagues (Khan et al. 2001). That led to a series of studies examining the contributions of oxidative stress, especially the LPDAs, in TCE-induced autoimmune response by his research group (Wang et al. 2007a, b, 2008, 2009, 2012a; Fan et al. 2012). Their observations led them to hypothesize (Fig. 4.2) that TCE-induced oxidative stress leads to a variety of RONS-mediated structural modifications of the endogenous proteins, such as increased formation of LPDA-protein adducts (e.g. MDA-/HNA-protein adducts), carbonylation and nitration of proteins, which could potentially lead to generation of neoantigens. After antigen processing, these neoantigens could elicit autoimmune response by stimulating T and B lymphocytes, especially Th1 and Th17 cells (Wang et al. 2008, 2012a).

Wang et al. (2007b) detected increased formation of MDA- and HNE-protein adducts in the livers of MRL+/+ mice treated with TCE (10 mmol/kg, i.p., every fourth day) for 6 and 12 weeks. Significant induction of anti-MDA- and anti-HNE-protein adduct specific antibodies was also observed in the sera of TCE-treated mice, which showed a pattern similar to MDA-/HNE-protein adducts. More importantly, the increases in anti-MDA- and anti-HNE-protein adduct antibodies were associated with significant elevation in serum anti-nuclear (ANA)-, anti-ssDNA- and anti-dsDNA-antibodies at 6 weeks and, to a greater extent at 12 weeks. Those studies, even though at a relatively high dose, served as the basis for further evaluation of TCE-induced oxidative

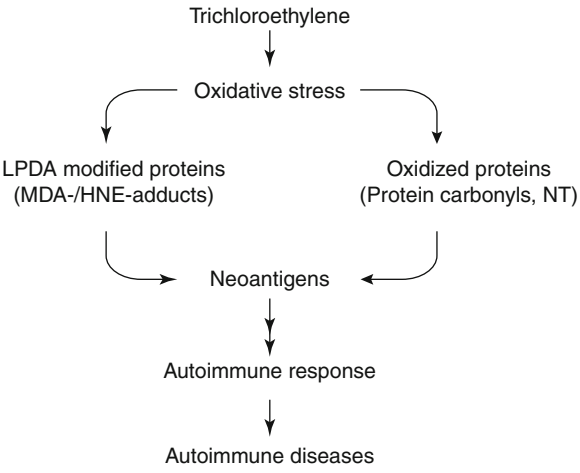

Fig. 4.2 Projected sequence of events leading to ADs following TCE exposure

modification of proteins at occupationally relevant doses. Wang et al. (2007a, 2012a) observed that occupationally-relevant doses of TCE (0.5, 1.0 or 2.0 mg/ml in drinking water for 12, 24, 36, 48 weeks) led to dose- and time-related increases in MDA-/HNE-protein adducts and their corresponding antibodies in the sera. Furthermore, strong relationship between the increases in MDA-/HNE-protein adducts and significant elevation in serum ANA and anti-ssDNA-antibodies, suggested an association between TCE-induced oxidative stress and autoimmune response. Interestingly, stimulation of cultured splenic lymphocytes from both control and TCE-treated female MRL +/+ mice (10 mmol/kg, i.p., every fourth day for 4 weeks) with MDA-adducted mouse serum albumin (MDA-MSA) or HNE-MSA for 72 h showed significant proliferation of $CD4^+$ T cells in TCE-treated mice as analyzed by flow cytometry. Also, splenic lymphocytes from TCE-treated mice released more IFN-γ and IL-2 into cultures when stimulated with MDA-MSA or HNE-MSA, suggesting a Th1 cell activation (Wang et al. 2008). Similarly, after female MRL +/+ mice were orally exposed to TCE (0.5, 1.0 or 2.0 mg/ml in drinking water) for 12, 24, 36 weeks, the splenocytes from mice treated with TCE for 24 weeks secreted significantly higher levels of IL-17 and IL-21 than did splenocytes from controls after stimulation with MDA-MSA or HNE-MSA adducts. The increased release of these cytokines was dose-dependent and more pronounced in mice treated with TCE for 36 weeks (Wang et al. 2012a; Fig. 4.3). These studies provide evidence that MDA- and or HNE-modified proteins contribute to TCE-mediated autoimmunity, which may be via activation of Th1, Th17 cells (Wang et al. 2008, 2012a). Recent studies in groups of female MRL +/+ mice treated with TCE, NAC or TCE plus NAC for 6 weeks (TCE, 10 mmol/kg, i.p., every fourth day; NAC, 250 mg/kg/day through drinking water), showed that NAC supplementation not only attenuated the TCE-induced formation of anti-MDA/-HNE-protein adduct antibodies and increased carbonylation of serum proteins, but also increases in serum levels of ANA, anti-Sm- and anti-dsDNA-antibodies, evidenced by their reduced levels in the sera of TCE plus NAC treated mice, further supporting a role of oxidatively modified proteins in TCE-induced autoimmune response (Wang et al. 2010b).

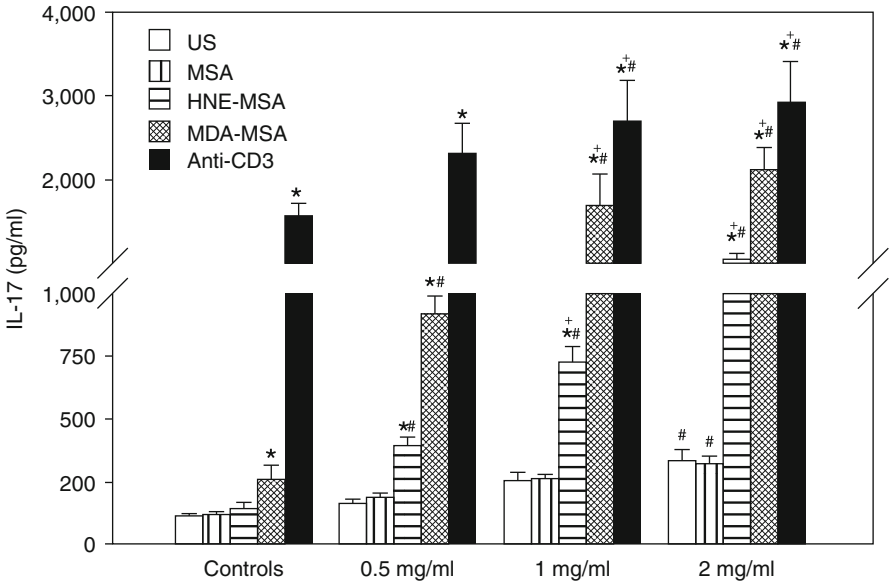

Fig. 4.3 IL-17 release in the culture supernatants of splenocytes from control and TCE-treated mice (0.5, 1.0, 2.0 mg/ml of TCE in drinking water for 36 weeks). Splenocytes were stimulated with MSA alone, HNE-MSA, MDA-MSA or anti-CD3 antibody for 72 h. *US* un-stimulated cells. *$p<0.05$ vs. US; $^{\#}p<0.05$ vs. stimulated control group; $^{+}p<0.05$ vs. stimulated lower dose groups (0.5 and 1 mg/ml) (Adapted from Elsevier)

Recent studies have also explored the contribution of protein oxidation (carbonylation and nitration) in the induction of TCE-induced autoimmune response (Wang et al. 2007a, 2009, 2010b, 2013). TCE exposure (10 mmol/kg, i.p., every fourth day) in female MRL +/+ mice for 6 or 12 weeks (10 mmol/kg, i.p., every fourth day), led to time-dependent increases in carbonylation and nitration of proteins with enhanced iNOS activity. More importantly, the increases in TCE-induced protein oxidation (carbonylation and nitration) were associated with significant increases in Th1-specific cytokine (IL-2, IFN-γ) release into splenocyte cultures (Wang et al. 2009). These data along with the evidence that TCE induces autoimmune response (Khan et al. 1995; Wang et al. 2007a, 2008), suggest an association between oxidative modification of proteins and autoimmunity. The modification of proteins, such as nitration or carbonylation, may alter immunogenicity of self-antigens (converting them to neoantigens), and may lead to an autoimmune response by stimulating T cells (especially activation of Th1 cells; Wang et al. 2009). Lower dose of TCE exposure (0.5 mg/ml via drinking water) also led to significant increases of serum NT along with elevation of ANA and anti-dsDNA antibodies (Wang et al. 2007a). Interestingly, TCE treatment in iNOS-null female MRL+/+ mice even though still led to increases in serum ANA and anti-dsDNA, but the increases in these autoantibodies induced by TCE were significantly less pronounced compared to that in MRL+/+ mice (Wang et al. 2013). These results suggest an association between

protein oxidation and induction/exacerbation of autoimmune response, and present a potential mechanism by which oxidatively modified proteins could contribute to TCE-induced autoimmune response (Wang et al. 2007a, 2009, 2010b, 2013).

4.7 Conclusions and Future Direction

Recent studies have contributed to the understanding of the role of oxidatively modified proteins, especially LPDAs modified proteins, in TCE-induced autoimmune response. These studies support that oxidative modification of proteins (e.g., MDA-/HNE-protein adducts, nitration and carbonylation of proteins) cause structural alterations to endogenous proteins, resulting in the formation of neoantigens which elicit autoimmune responses by stimulating T and/or B lymphocytes, particularly Th1 and Th17 lymphocytes (Khan et al. 2001; Wang et al. 2007a, b, 2008, 2009, 2012a). These studies not only demonstrated that TCE exposure leads to increased formation of MDA-/HNE-protein adducts, nitration and carbonylation of proteins, and formation of anti-MDA-/HNE-protein adduct antibodies, but more importantly, observed a significant association between formation of these modified proteins, corresponding antibodies and increased autoantibodies. Furthermore, MDA-/HNE-MSA stimulated greater release of IFN-γ, IL-2, IL-17 and IL-21, suggesting the contribution of oxidatively modified proteins in TCE-mediated autoimmune responses. Further detailed studies to unravel the distinct pathways by which oxidative stress contributes to autoimmunity, especially mapping of gene expression, analyzing proteome, blocking/inhibiting specific signal transduction pathways, knocking out/down target genes and exploring the epigenetic involvement will also provide critical mechanisms in TCE-induced autoimmunity.

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