Toxicology 338 (2015) 86–94

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Enhancement of regulatory T cell-like suppressive function in MT-2 by long-term and low-dose exposure to asbestos

Chen Ying[a,c,1], Megumi Maeda[b,c,1], Yasumitsu Nishimura[c], Naoko Kumagai-Takei[c], Hiroaki Hayashi[a,d,1], Hidenori Matsuzaki[c], Suni Lee[c], Kei Yoshitome[c], Shoko Yamamoto[c], Tamayo Hatayama[c], Takemi Otsuki[c,*]

[a] Division of Pneumoconiosis, School of Public Health, China Medical University, Shenyang, PR China
[b] Department of Biofunctional Chemistry, Division of Bioscience, Okayama University Graduate School of Natural Science and Technology, Okayama, Japan
[c] Department of Hygiene, 4: Department of Dermatology, Kawasaki Medical School, Kurashiki, Okayama, Japan
[d] Department of Dermatology, Kawasaki Medical School, Kurashiki, Japan

ARTICLE INFO

Article history:
Received 7 September 2015
Received in revised form 8 October 2015
Accepted 13 October 2015
Available online 23 October 2015

Keywords:
Asbestos
Regulatory T cell
IL-10
TGF-β1
CTLA-4

ABSTRACT

Asbestos exposure causes lung fibrosis and various malignant tumors such as lung cancer and malignant mesothelioma. The effects of asbestos on immune cells have not been thoroughly investigated, although our previous reports showed that asbestos exposure reduced anti-tumor immunity. The effects of continuous exposure of regulatory T cells (Treg) to asbestos were examined using the HTLV-1 immortalized human T cell line MT-2, which possesses a suppressive function and expresses the Treg marker protein, Foxp3. Sublines were generated by the continuous exposure to low doses of asbestos fibers for more than one year. The sublines exposed to asbestos showed enhanced suppressive Treg function via cell–cell contact, and increased production of soluble factors such as IL-10 and transforming growth factor (TGF)-β1. These results also indicated that asbestos exposure induced the reduction of anti-tumor immunity, and efforts to develop substances to reverse this reduction may be helpful in preventing the occurrence of asbestos-induced tumors.

1. Introduction

Asbestos exposure causes not only lung fibrosis, which is a typical form of pneumoconiosis known as asbestosis, but also malignant tumors such as lung cancer and malignant mesothelioma (MM) (Brody, 2010; Jamrozik et al., 2011; Liu et al., 2013; Stayner et al., 2013). In addition, cancers of the larynx, gastrointestinal tracts and bladder have frequently been found in asbestos-exposed individuals (Friedman, 2011). The causes of asbestos-induced cancer are thought to include (i) DNA damage induced by reactive oxygen species (ROS) and produced mainly by the Fenton reaction due to the iron present in asbestos fibers, (ii) direct chromosomal and genomic injury caused by the firm and rigid physical characteristics of asbestos fibers, and (iii) adsorption of various carcinogenic substances around asbestos fibers inhaled into the respiratory system (Barrett et al., 1989; Kamp et al., 1992; Toyokuni 2014).

The core chemical component of asbestos fibers is silica (SiO_2). It is well known that exposure to silica particles also causes a type of lung fibrosis known as silicosis, another typical form of pneumoconiosis, as well as various disorders of autoimmunity such as rheumatoid arthritis (known as Caplan's syndrome), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and ailments detailed in many recent reports involving complications of anti-neutrophil cytoplasmic antibody-induced vasculitis/nephritis in silicosis patients (Steenland and Goldsmith, 1995; Wichmann et al., 1996; D'Cruz, 2000; Mulloy, 2003; Gómez-Puerta et al., 2013). Although silica-induced dysregulation of autoimmunity has been considered an adjuvant disease, we have

Abbreviations: MM, malignant mesothelioma; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; Teff, effector T cells; Treg, regulatory T cells; DcR3, decoy receptor 3; HTLV, human T-lymphotropic virus; PP, pleural plaque; IL, interleukin; TGF, transforming growth factor; STAT3, signal transducer and activator of transcription 3; CXCR3, C-X-C chemokine receptor type 3; IFN, interferon; NK, natural killer; CTL, cytotoxic T lymphocytes; MLR, mixed lymphocyte reaction; FCS, fetal calf serum; PBMCs, peripheral blood mononuclear cells; FITC, fluorescein isothiocyanate; Tresp, responder T cells; iDCs, immature dendritic cells; GM-CSF, granulocyte macrophage-colony stimulating factor; CBA, cytometric bead array; ELISA, enzyme-linked immunosorbent assay; DC, dendritic cells; PLSD, parametric least significant difference; TCR, T cell receptor; SMRP, soluble mesothelin-related peptide.

* Corresponding author at: Department of Hygiene, Kawasaki Medical School, 577 Matsushima, Kurashiki 701-0192, Japan. Fax: +81 86 464 1125.
E-mail address: takemi@med.kawasaki-m.ac.jp (T. Otsuki).

[1] These two authors contributed equally to this study.

http://dx.doi.org/10.1016/j.tox.2015.10.005
0300-483X/

been investigating the direct effects of silica particles on immunocompetent cells and found unbalanced levels of effector T cells (Teff) and regulatory T cells (Treg). Silica particles induce chronic activation of both Teff and Treg. Activated Teff showed escape from Fas/CD95-mediated apoptosis by producing inhibitory molecules such as soluble Fas and decoy receptor 3 (DcR3) that resulted in its long survival. As a consequence of the chronic activation of Treg, these cells enhanced their Fas/CD95 expression and experienced early death. Finally, the unbalance of Teff/Treg leads to subsequent dysregulation of autoimmunity (Hayashi et al., 2010; Lee et al., 2012, 2014).

Given that silica affects immunocompetent cells, it is supposed that asbestos fibers, which possess SiO_2 as the core chemical, also affect immune cells. Considering the above-mentioned complications exhibited by asbestos-exposed patients, exposure to asbestos is likely to decrease anti-tumor immunity. It is on this basis that we have been investigating the immunological effects of asbestos fibers on human T cells using the human T-lymphotropic virus (HTLV)-1 immortalized polyclonal T cell line MT-2 as Treg-like cells (Chen et al., 2006), and freshly isolated human T cells from healthy volunteers, as well as cells from asbestos-exposed patients such as those with pleural plaque (PP), who only show the plaque without any clinical evidence of cancers, and patients with MM (Otsuki et al., 2007; Maeda et al., 2010).

The results of our investigations revealed apoptosis of MT-2 cells through production of ROS, activation of the mitochondrial apoptotic pathway by transient high-dose exposure to asbestos (Hyodoh et al., 2005), acquisition of asbestos-induced apoptosis by continuous and low-dose exposure to asbestos in MT-2 cells through Src-activation, enhanced production of interleukin (IL)-10, activation of signal transducer and activator of transcription 3 (STAT3), and upregulation of bcl-2 located downstream of STAT3 activation (Miura et al., 2006). Furthermore, asbestos-resistant strains of MT-2 cells produced transforming growth factor TGF-β1 at high levels (Maeda et al., 2014). Continuously exposed MT-2 cells showed reduction of C-X-C chemokine receptor type 3 (CXCR3) expression with reduced production of interferon (IFN)-γ (Maeda et al., 2011a,b). These reductions of CXCR3 and IFN-γ were also found in patients with PP and MM (Maeda et al., 2011a,b). Furthermore, the natural killer (NK) cell line, freshly isolated peripheral blood NK cells, and NK cells isolated from asbestos-exposed patients showed decreased expression of NK cell activating receptors. In particular, reduction of NKp46 was the significant effect of asbestos exposure and caused a decrease in the tumor-killing function of experimental asbestos-exposed NK cells and of patient NK cells (Nishimura et al., 2009a,b, 2013). Moreover, asbestos exposure caused inhibition of differentiation and proliferation of CD8+ cytotoxic T lymphocytes (CTL) in vitro when CTL from healthy volunteers were examined in a mixed lymphocyte reaction (MLR) with or without chrysotile asbestos (Kumagai-Takei et al., 2013, 2014). The overall findings indicate that asbestos affects human immune cells and causes a reduction of anti-tumor immunity.

In this study, we assessed whether exposure to asbestos affects Treg function. We have independently established six asbestos-resistant sublines as a model of continuous (more than one year) and low-dose (10 μg/mL) exposure to asbestos in the MT-2 cell line (Org), which has Treg functionality and has never been exposed to asbestos fibers. The asbestos-resistant sublines were designated CA 1, 2 and 3, which were exposed to chrysotile A, and CB 1, 2 and 3, which were exposed to chrysotile B. The difference between chrysotile A and B concerns the amounts of mineralogical minor components such as TiO_2, Al_2O_3 and Fe_2O_3 (Kohyama et al., 1996). Finally, we compared the Treg suppressive function regarding the cell-to-cell contact manner, as well as soluble molecules such as IL-10 and TGF-β1 as typical soluble factors that reflect Treg functionality (Horwitz et al., 2003; Chattopadhyay et al., 2005; Linehan and Goedegebuure, 2005; Taylor et al., 2006).

2. Materials and methods

2.1. Cell lines and asbestos

MT-2Org and the asbestos-resistant sublines were cultured as reported previously (Miura et al., 2006; Maeda et al., 2011a,b, 2014). These cells were maintained in a humidified atmosphere of 5% CO_2 at 37 °C in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), streptomycin and penicillin. The International Union Against Cancer standard chrysotile-A and -B were kindly provided by the Department of Occupational Health at the National Institute for Occupational Health of South Africa. The mineralogical features of these fibers were reported previously (Kohyama et al., 1996). Six asbestos-resistant sublines (CA 1–3 and CB 1–3) were generated by continuous exposure to CA or CB (10 μg/ml, for more than eight months) of MT-2Org, as described previously. 293FT cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% FCS.

2.2. Lentiviral transduction

IL-10 and TGF-β1-targeting double-stranded oligonucleotides 5′- GATCCCCGCCTACATGACAATGAAGATTCAAGAGATCTTCATTGTCATGTAGGCTTTTTGGAAA -3′ (IL-10) and 5′- GATCCCCGGAGGTCACCCGCGTGCTATTCAAGAGATAGCACGCGGGTGACCTCCTTTTTGGAAA-3′ (TGF-β1) were subcloned into pSUPER digested by BglII-HindIII (Brummelkamp et al., 2002). Resulting constructs were digested with BamHI/SalI, and short hairpin RNA (shRNA) containing human H1 RNA polymerase III promoter subcloned into the BamHI-SalI site of the lentiviral vector pRDI292 that expresses shRNA and a protein associated with puromycin resistance as described previously (Naldini et al., 1996). The vesicular stomatitis virus G protein (VSV-G)-pseudotyped HIV-1-based vector system was generated as described previously (Bridge et al., 2003). The replication-defective lentiviral vector particles were produced by transient cotransfection of the second-generation packaging construct pCMV-DR8.91 (Zufferey et al., 1997), the VSV-G envelope plasmid pMDG2 and the lentiviral vector into 293FT cells with FuGene6 (Roche Diagnostics, Mannheim, Germany). The supernatant containing the virus was collected 48 h and 72 h after transfection. The lentivirus-containing supernatants were added to MT-2Org or the subline CB 1 (0.5×10^5 in 2 mL of medium) in a six-well plate. After 3 days, cells were treated with 1 μg/mL of puromycin to select stable clones expressing shRNA.

2.3. Purification of monocytes and primary T cells

Human peripheral blood was obtained from healthy donors after informed consent was received in accordance with procedures approved by the human ethics committee of Kawasaki Medical School, Kurashiki, Japan (#883). Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (Separate-L®, Muto Pure Chemical Co., Ltd., Tokyo, Japan). $CD14^+$ monocytes were isolated by positive selection using anti-CD14-coated beads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) in accordance with manufacturer's instructions. The remaining cells were labeled with anti-CD4-phycoerythrin (PE) (clone RPA-T4) and anti-CD25-fluorescein isothiocyanate (FITC) (clone M-A251) for 30 min at room temperature. After washing, $CD4^+CD25^-$ responder T cells (Tresp) and $CD4^+CD25^{high}$ Treg were sorted using a FACS Aria cell sorter (BD Biosciences, San Jose, CA) (Fig. 1A).