Human SP-A genetic variants and bleomycin-induced cytokine production by THP-1 cells: effect of ozone-induced SP-A oxidation

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Submitted 31 July 2003; accepted in final form 12 November 2003

Huang, Weixiong, Guirong Wang, David S. Phelps, Hamid Al-Mondhiry, and Joanna Floros. Human SP-A genetic variants and bleomycin-induced cytokine production by THP-1 cells: effect of ozone-induced SP-A oxidation. Am J Physiol Lung Cell Mol Physiol 286: L546–L553, 2004. First published November 14, 2003; 10.1152/ajplung.00267.2003.—Surfactant protein A (SP-A) plays a role in innate host defense. Human SP-A is encoded by two functional genes (SP-A1 and SP-A2), and several alleles have been characterized for each gene. We assessed the effect of in vitro expressed human SP-A genetic variants, on TNF-α and IL-8 production by THP-1 cells in the presence of bleomycin, either before or after ozone-induced oxidation of the variants. The oligomerization of SP-A variants was also examined. We found 1) cytokine levels induced by SP-A2 (1A, 1A3) were significantly higher than those by SP-A1 (6A2, 6A4) in the presence of bleomycin. 2) In the presence of bleomycin, ozone-induced oxidation significantly decreased the ability of 1A and 1A/6A2, but not of 6A4, to stimulate TNF-α production. 3) The synergistic effect of bleomycin/SP-A, either before or after oxidation, can be inhibited to the level of bleomycin alone by surfactant lipids. 4) Differences in oligomerization were also observed between SP-A1 and SP-A2. The results indicate that differences among SP-A variants may partly explain the individual variability of pulmonary complications observed during bleomycin chemotherapy and/or in an environment that may promote protein oxidation.

inflammation; enzyme-linked immunosorbent assay; oligomerization; ozone

PULMONARY SURFACTANT, which is produced by alveolar type II cells and consists of surfactant lipids and several associated proteins, is essential for normal lung function because it prevents alveolar collapse during end expiration (7). Pulmonary surfactant-associated protein A (SP-A), in addition to surfactant-related function (14), plays a role in innate host defense and regulation of inflammatory processes in the lung (8). It has been previously reported that natural human SP-A stimulates cytokine production (17, 28), nitric oxide production (2), alveolar macrophage phagocytosis (52), immune cell proliferation (27), NF-κB activation (25), and matrix metalloproteinase-9 production (53). In vivo studies suggest a role for SP-A in neutrophil recruitment in the lung of preterm lambs (26). However, in other systems, an anti-inflammatory role has been attributed to SP-A (1, 3, 4, 32). Some in vivo and in vitro studies show SP-A inhibition of LPS-induced cytokine production, nitric oxide, and lymphocyte proliferation. The reasons for these apparent discrepancies are not clear, but differences in the experimental systems are likely to contribute. Because surfactant lipids can moderate the proinflammatory effect of SP-A in vitro (25, 27, 28), it has been proposed that surfactant lipids and SP-A are counterregulatory. Therefore, changes in the relative amounts of surfactant lipids to SP-A may be important in determining the immune status of the lung. It is also possible that the role of SP-A changes at different stages of the inflammatory response and is modulated by different activating molecules (15, 47), as has been proposed recently for NF-κB (30).

Human SP-A is encoded by two functional genes (SP-A1 and SP-A2) and several alleles have been characterized for each gene based on nucleotide differences within the coding region. The most commonly observed alleles are 6A, 6A2, 6A3, and 6A4 for SP-A1 and 1A, 1A0, 1A1, 1A2, 1A3, and 1A5 for SP-A2 (10, 24). Several studies have shown differences among human SP-A genetic variants expressed in vitro in their abilities to stimulate cytokine production (55, 56) to bind carbohydrate (35) and lipids (16) and to enhance phagocytosis by alveolar macrophages (our unpublished observations). Moreover, a number of studies have revealed quantitative associations between SP-A alleles or genotype and mRNA levels, and different ratios of SP-A1 to SP-A2 mRNA have been observed among individuals (23). Associations between certain SP-A alleles and patient groups indicate that functional and/or structural differences of SP-A may play a role in the pathogenesis of certain diseases (13, 42).

Bleomycin is an effective chemotherapeutic agent and is used in the treatment of squamous cell carcinoma, lymphomas testicular carcinoma, etc. However, bleomycin-induced pulmonary fibrosis is a serious problem that limits the usefulness of the drug in some patients (49). Bleomycin induces inflammatory cells to secrete multifunctional cytokines, such as tumor necrosis factor (TNF-α), interleukin (IL)-1β, IL-8, and transforming growth factor-β. These cytokines stimulate fibroblast proliferation and are probably involved in the development of pulmonary fibrosis (44, 57). It has been observed that bleomycin specifically stimulates macrophage-like THP-1 cells to produce TNF-α, IL-8, and IL-1β in a time- and dose-dependent pattern (22). In a clinical study, the TNF-α level was shown to be significantly increased after bleomycin infusion (46). TNF-α is considered to be a central mediator in bleomycin-induced pulmonary fibrosis, and TNF-α receptor knockout mice are protected from lung injury after exposure to bleomycin (38). Although the mechanism of bleomycin-induced toxicity has not been fully elucidated, it has been demonstrated that bleomycin-induced DNA damage is associated with reactive oxygen species. It is postulated that bleomycin exerts its
toxic effects in the lung through the generation of oxygen free radicals that initiate pulmonary fibrosis (20). Clinical data have implicated oxygen supplementation during bleomycin chemotherapy with increased bleomycin toxicity (31).

Lung surfactant lines the surface of lung alveoli and is likely to be the first target of reactive oxygen species generated by oxygen and ozone (40). The effects of oxidants on the pulmonary surfactant system include the oxidation of surfactant proteins (including SP-A) and the peroxidation of surfactant phospholipids (50). Moreover, a number of studies have shown that oxidation of SP-A in vitro by ozone (36, 37, 56), nitrogen dioxide (34), and reactive oxygen and nitrogen species (9, 19) impairs many aspects of SP-A function, including lipid aggregation, mannose binding capacity, stimulation of cytokine production, and phagocytosis. In vivo oxidation of SP-A in different systems (34, 37, 48) has also resulted in functional differences in SP-A.

Our previous studies showed that SP-A enhances bleomycin-induced inflammatory cytokine production and that this synergistic effect can be attenuated by surfactant lipid (22). In the present study, we used the surfactant protein genetic variants as a model to gain insight into the basis of individual variability to bleomycin-induced pulmonary toxicity and specifically in microenvironments that may promote protein oxidation. In this regard, we assessed 1) differences among human SP-A genetic variants, expressed in vitro by insect cells either before or after ozone-induced oxidation, in their ability to stimulate cytokine production in the presence of bleomycin; and 2) whether functional differences correlate with differences in the oligomeric patterns of normal and ozone-oxidized SP-A variants under various electrophoretic conditions. The rationale for these studies is twofold. First, the functional and biochemical properties of SP-A change following ozone exposure (36, 37). In this regard, we hypothesized that the functional ability of SP-A variants is differentially affected by ozone-induced damage/oxidation. This differential alteration is due to amino acid differences among alleles. The second reason is that differences in SP-A oligomer size has already been associated with disease (21). Therefore, it is possible that ozone-induced alterations, as assessed by changes in the electroforetic mobility, correlate with functional differences.

MATERIALS AND METHODS

Cell lines and cell culture conditions. The insect cell line Sf9 (GIBCO-BRL, Life Technologies) from Spodoptera frugiperda was used for protein expression. The cells were cultured in SF-900 II SFM medium (GIBCO-BRL) in an incubator at 27°C. Protein expression proceeded in suspension culture of cells in a 250-ml Erlenmeyer flask (50 ml culture medium/flask) with shaking at 110 rpm.

The THP-1 cell line was obtained from the American Type Culture Collection (Manassas, VA). Cells were grown in RPMI 1640 (Sigma, St. Louis, MO) with 0.05 mM 2-mercaptoethanol and 10% heat-inactivated fetal bovine serum (BioWhittaker, Walkersville, MD) at 37°C in an atmosphere of 5% CO₂. The cells were split periodically inactivated fetal bovine serum (BioWhittaker, Walkersville, MD) at 90% confluence. After detachment, the cells were collected with 0.01 pg LPS/μg SP-A. SP-A was stored at −80°C until use.

Expression of human SP-A genetic variants by insect cells. The SP-A genetic variants were expressed by the baculovirus-mediated insect cell system as described in our previous study (55). Briefly, PCR-amplified cDNA fragment was cloned into donor plasmid pFastBac DUAL (GIBCO-BRL). The recombinant plasmid was then transformed into Escherichia coli DH10Bac in which the foreign gene was transferred into the baculovirus genome (bacmid). After cloning, selection, and purification, the recombinant bacmid DNA was transfected into insect cell line Sf9. Three days after transfection, SP-A expression was examined by Western blot analysis, and recombinant baculovirus particles in the supernatant of the cell culture were collected. We achieved the expression of single SP-A gene products by infecting insect cells with the virus particles containing a single SP-A allele. A 50-ml culture at 2 × 10⁶ cells/ml was cultured with 1 ml of a viral stock at 1 × 10⁶ pfu/ml. For coexpression of SP-A1/SP-A2 products, the virus particles containing SP-A1 and SP-A2 genes were mixed at a 1:1 ratio of virus titers and used for infecting the insect cells. To confirm expression of the specific gene and/or allele, we determined the SP-A genotype of the insect cell mRNA by the PCR-converted restriction fragment length polymorphism (CRFLP) method (10). The culture supernatants were harvested at 72 h after infection and purified with mannose-affinity chromatography. The purified SP-A variants were then dialyzed against 5 mM Tris (pH 7.5) and examined by Western blotting and silver staining. Protein concentration and LPS content were determined by the methods described above. The LPS content of SP-A variants used in this study was <0.2 pg/μg SP-A.

Bleomycin. Bleomycin (Blenoxane; Bristol-Myers Squibb, Princeton, NJ) solutions were prepared immediately before use with endotoxin-free saline (American Pharmaceutical Partners, Los Angeles, CA). LPS was not detected in the stock solution of bleomycin at a bleomycin concentration of 5 U/ml (1 U = 1 mg) using the method described above.

Electrophoretic analysis of in vitro expressed SP-A variants and native human SP-A under various conditions. Human SP-A genetic variants and human SP-A from BAL fluid were subjected to electrophoresis under reducing, nonreducing, and native conditions. Reducing PAGE analysis was done following the procedure described by Laemmli (29). Electrophoresis (90 V, 1.5 h) of each protein sample was performed in a 10% polyacrylamide gel in the presence of SDS after reduction of protein by dithiothreitol and heating at 95°C for 10 min. The nonreducing PAGE (75 V, 5 h) was done in a 4–15% gradient polyacrylamide gel under the same conditions as the reducing PAGE, except there was no reducing agent in the loading buffer. Gel electrophoresis under native conditions was performed in a 4–15% gradient polyacrylamide gel with an electrophoretic running buffer (90 mM Tris, 80 mM boric acid, and Na₂ EDTA, pH 8.4) and a loading buffer containing sodium. In addition, electrophoresis was carried out at 4°C at 50 V for 1 h followed by 95 V for 13 h. Stimulation of THP-1 cells with SP-A and bleomycin. After differentiation with 10⁻⁸ M vitamin D₃ for 72 h, THP-1 cells were pelleted by centrifugation at 1000 g for 5 min. The pellet was then washed with cold PBS. THP-1 cells were suspended in RPMI 1640 at a concentration of 2 × 10⁶ cells/ml and incubated in 24-well culture plates (0.5 ml/well). Cells were stimulated with nonozone or ozone-exposed SP-A (20 μg/ml), bleomycin (5 μU/ml), or SP-A plus bleomycin. In
some experiments, Infasurf (Forest Pharmaceuti
cals, St. Louis, MO), an extract of natural surfa
tant from calf lung, was used to assess the
inhibitory effect of surfactant lipid on SP-A, either with or without
ozone-induced oxidation, and bleomycin on induced cytokine pro
duction. Cells were incubated for 4 h after treatment. TNF-α and IL-8
levels in culture medium were then quantified by ELISA.

ELISA assay. The ELISA assays for TNF-α and IL-8 (OptEIA
Human ELISA Sets; Pharmingen, San Diego, CA) were performed
according to the instructions recommended by the manufacturer. The
ELISA kits were capable of measuring levels of 7.8–500 pg/ml for
TNF-α and 6.2–400 pg/ml for IL-8. We obtained a reference curve for
each of these cytokines by plotting the concentration of several
dilutions of standard protein versus the corresponding absorbance.

Exposure of SP-A to ozone and detection of protein oxidation.
SP-A protein at a concentration of ~0.5 mg/ml was exposed to ozone
to 24-well culture tissue plates as described previously (51). Briefly,
each well contained 100 μl of the protein solution and was exposed to
ozone (1 ppm for 4 h) for protein oxidation. Protein oxidation was
detected with the OxyBlot oxidized protein detection kit (Intergen,
Purchase, NY). The ozone-exposed proteins were derivatized with
2,4-dinitrophenylhydrazine (DNPH). 200 ng of DNPH-derivatized
protein were blotted onto nitrocellulose, and immunodetection of
oxidized proteins was done with anti-DNPH and goat anti-rabbit IgG
(horseradish peroxidase-conjugated) antibodies. Blots were exposed
to XAR film following enhanced chemiluminescent
detection.

Statistics. Values are presented as means ± SE. Data were ana-
lyzed using SigmaStat statistical software (SPSS, Chicago, IL). For
comparison among SP-A variants, statistical treatment included a
one-way analysis of variance followed by a Student-Newman-Keuls
test for pairwise comparison. A paired t-test was used for comparing
the effect of SP-A before and after ozone-induced oxidation exposure.
A value at P < 0.05 was judged to be significantly different.

RESULTS

Differentiated THP-1 cells were stimulated with SP-A vari-
ant (20 μg/ml), bleomycin (5 mM/ml), or SP-A variant plus
bleomycin. In this study, in vitro expressed genetic variants of
two SP-A1 alleles (6A2, 6A4), two SP-A2 alleles (1A, 1A0)
that are commonly observed in the general population (12), as
well as two combinations of SP-A1 and SP-A2 alleles (1A/6A2,
1A/6A4) were used. For the SP-A1/SP-A2 combinations
we used a ratio of 1:1 viral SP-A1 and SP-A2 titers as noted in
MATERIALS AND METHODS. Although it has been proposed that
SP-A consists of a 2:1 ratio of SP-A1 to SP-A2 (54), the ratio
of SP-A1 to SP-A2 at the mRNA level among unrelated
individuals varies considerably (23). Therefore, it remains to
be determined whether there is a unique ratio of SP-A1 to
SP-A2 that applies to all individuals. A dose of 20 μg/ml
of SP-A was chosen rather than the dose of 50 μg/ml we have
used in previous experiments (55, 56), because the lower dose
more effectively shows the combined effects of bleomycin and
SP-A before or after ozone-induced oxidation. The doses of
bleomycin and ozone used in this study were chosen based on
findings of our previous studies (22, 56) and on other published
reports (36, 44).

Combined effect of in vitro expressed SP-A variants and
bleomycin on cytokine production by THP-1 cells. As depicted
in Fig. 1A, TNF-α levels induced by SP-A1 variants (6A2, 6A4)
are significantly lower than those by SP-A2 variants (1A, 1A0)
and by coexpressed variants (1A/6A2, 1A/6A4). When these
variants were combined with bleomycin, a synergistic effect on
TNF-α level was observed for all SP-A variants. In the pres-
ence of bleomycin, 6A2 and 6A4 stimulated production of
TNF-α by THP-1 cells to levels of 441 ± 34.9 pg/ml and
432 ± 64.9 pg/ml, respectively, which are significantly lower
than those induced by 1A (635 ± 91.5 pg/ml) and 1A0
(603.8 ± 49.5 pg/ml). When IL-8 production was studied after
the cells were treated with SP-A variants and bleomycin, the
response pattern of IL-8 was very similar to that observed in
TNF-α assay (Fig. 1B). No significant difference was found
between SP-A2 and coexpressed variants in their ability to
stimulate cytokine production in the presence of bleomycin.

Effect of ozone-induced oxidation on SP-A/bleomycin-in-
duced TNF-α production. Functional changes in SP-A follow-
ing ozone-induced oxidation are shown in Fig. 2. TNF-α
production was significantly reduced after ozone-induced oxidation of SP-A (99.0 ± 16.2 vs. 48.5 ± 5.8 pg/ml for 1A, 45.8 ± 2.8 vs. 33.0 ± 2.1 pg/ml for 6A2, 93.3 ± 7.1 vs. 47.1 ± 2.7 pg/ml for 1A/6A4). The synergistic effect of SP-A plus bleomycin on TNF-α production was also significantly decreased with oxidized 1A and 1A/6A4 variants but not with the 6A2 variant. When differences among SP-A variants in their ability to stimulate cytokine production before and after SP-A oxidation were compared, it was found that the TNF-α levels induced by 6A2 were significantly lower than those induced by 1A or 1A/6A4 in all circumstances except in the presence of bleomycin. Enhancement of TNF-α production by native human SP-A was also reduced after in vitro oxidation both in the presence and absence of bleomycin.

Effect of surfactant lipids (Infasurf) on oxidized SP-A-induced TNF-α production by THP-1 cells in the presence of bleomycin. The inhibitory effect of surfactant lipids (Infasurf) on native human SP-A plus bleomycin-induced TNF-α production was examined, both before and after ozone-induced oxidation of SP-A. Significant findings were observed in both the presence and absence of bleomycin (Fig. 3) either before or after SP-A oxidation.

Patterns of oligomerization of in vitro expressed SP-A variants and human SP-A under various electrophoretic conditions. To study whether the functional differences observed above were related to structural differences among SP-A variants, we analyzed in vitro expressed SP-A variants by PAGE under reducing, nonreducing, and native conditions. Under reducing conditions, the SP-A variants showed a monomeric form with a lower molecular mass than native human SP-A by both Western blot analysis (Fig. 4A) and silver staining (Fig. 4B) due to the absence of certain posttranslational modifications in the baculovirus-mediated expression system. A low-intensity dimeric band (~60 kDa) of SP-A variants was observed sometimes, especially by Western blot. The SP-A1 (6A2, 6A4) presented with a larger molecular mass than the SP-A2 (1A, 1A2) variants. The native human SP-A presented with both monomeric and dimeric forms under the reducing condition.

Under nonreducing conditions, the inter- and intramolecular disulfide bonds remain intact because no reducing agents, such as dithiothreitol and 2-mercaptoethanol, are used. The majority of oligomers of the in vitro expressed SP-A variants under this condition (Fig. 5A) are resolved into dimers (2X) and trimers.
In contrast, the oligomers of native human SP-A are highly oligomerized and most of them are >6X. Differences between SP-A1 and SP-A2 variants in the pattern of oligomerization under the nonreducing condition were observed. These include the presence of bands of higher intensity corresponding to the trimer (3X) and an additional band between the dimer (2X) and the trimer (3X) for SP-A1. A band corresponding to a higher-order oligomer (>6X) is present in SP-A2 and absent in SP-A1.

Differences among SP-A variants were also observed under the native condition (Fig. 5B), where proteins are not denatured by chemicals and heating and both covalent and noncovalent interactions are maintained. Differences between SP-A1 and SP-A2 variants include the presence in SP-A1 (and absence in SP-A2) of a high-intensity band, approximately a 9X oligomer. Conversely, a high-intensity band at ~12X is present in SP-A2 and absent in SP-A1. Subtle differences between alleles of a given gene and/or of coexpressed SP-A1/SP-A2 gene products are also observed.

**Electrophoretic characteristics of oxidized SP-A variants and native human SP-A.** No significant oxidation was detected for each of the SP-A variants (1A, 6A4, 1A/6A4) before ozone exposure, but all of the SP-A variants were oxidized after ozone exposure at a concentration of 1 ppm for 4 h (Fig. 6A). Although oxidation of native human SP-A was detected before ozone exposure (Fig. 6A), a higher level of oxidation was observed after ozone exposure as assessed by a shorter film exposure (Fig. 6B). Densitometric analysis of two independent experiments performed in duplicate indicates that SP-A oxidation before ozone exposure is approximately (42 ± 4%) that following ozone exposure. Examination of native SP-A indicated that SP-A from the BAL fluid of patients with alveolar proteinosis is partially oxidized in vivo in the human body, and this has been shown previously (51).

Under reducing conditions, SP-A variants, but not native human SP-A, demonstrated bands of higher apparent molecular weight after ozone exposure (Fig. 7A). Although no significant loss of SP-A was detected by protein quantification after oxidation, bands of lower intensity in oxidized SP-A variants were observed in gel electrophoresis under nonreducing conditions (Fig. 7B). A major electrophoretic change in 6A4 was seen, compared with other SP-A variants after oxidation (Fig. 7, A and B). Increased smearing in the spaces between the oligomeric bands was observed in ozone-oxidized SP-A variants under nonreducing conditions (Fig. 7B). These observations point to the possibility that higher-size oligomers are formed and some of these may or may not enter the gel, explaining perhaps both the lighter band intensity and the smearing.

**DISCUSSION**

SP-A, a major surfactant-associated protein, plays important roles in surfactant-related activities and in the innate host defense of the lung. Due to its high level of heterogeneity and polymorphism, SP-A may contribute to the individual variability of susceptibility to pulmonary disease under certain conditions. The effect of bleomycin therapy on pulmonary toxicity may present an opportunity where the impact of SP-A variants could be studied. In the present study, we assessed the functional and structural differences among human SP-A genetic variants either before or after ozone-induced oxidation on
cytokine production by THP-1 cells in the presence of bleomycin, as well as the electrophoretic pattern of SP-A variants. The rationale for the latter is based on the high level of polymorphism of the SP-A genes, which may lead to quantitative and/or qualitative differences of the SP-A protein, as we observed previously (16, 35). This is probably due to the oxidation state of SP-A.

The high level of polymorphism of the SP-A genes may mean that functional and structural differences exist among individuals, and this may contribute to the individual variability of susceptibility to pulmonary disease and bleomycin-induced pulmonary fibrosis. In the present study, we found that the ability of SP-A variants to enhance bleomycin-induced cytokine production is similar to that of native SP-A. However, differences with regards to the level of cytokine enhancement and the electrophoretic pattern between SP-A1 and SP-A2 variants were observed. Published data have shown the level of SP-A mRNA, as well as the ratio of SP-A1 to SP-A2 mRNA, to vary among individuals, indicating that, in some individuals, single SP-A gene products are in excess (23).

An overabundance of one SP-A gene product over the other (23) may be reflected in functional differences among individuals, and such differences under certain compromised conditions may contribute to disease pathogenesis (11, 13, 42). Support for such a possibility is provided by two groups of experiments. First, functional differences between SP-A1 and SP-A2 have been observed not only by the findings in the present study but also by several in vitro studies. These include differences between the two SP-A gene products in cytokine production (55, 56), carbohydrate binding (16, 35), lipid aggregation (16, 35), structural stability (16), and phagocytosis (our unpublished observation). Second, association between certain SP-A alleles and patient groups has been observed (13, 42), suggesting that SP-A functional differences play a role in the pathogenesis of certain diseases. The present data, along with previously published reports, suggest that the SP-A1 and SP-A2 single gene products are not entirely equivalent in their functional capabilities at the protein concentrations studied.

A remarkable difference in the oligomeric pattern between SP-A1 (6A2, 6A4) and SP-A2 (1A, 1A0) was observed under various electrophoretic conditions. This may be of potential clinical interest in view of previous findings where patients with birch pollen allergy were identified with a larger fraction of smaller-size oligomers of SP-A (21). The difference in oligomeric patterns between SP-A1 and SP-A2 may relate to differences, among SP-A, in posttranslational modification (39), assembly (18), and thermal stability (16). For example, biochemical studies have shown: 1) the SP-A2 structure to be more stable than the SP-A1 structure and 2) differences in the ability of SP-A to undergo self-aggregation and to induce lipid LPS aggregation, in order of native SP-A > SP-A2 > SP-A1/SP-A2 > SP-A1 (16). The higher structural stability of SP-A2 and/or of other structural differences between SP-A1 and SP-A2 may explain its higher capacity in cytokine production. In the present study, we did not observe a difference between the coexpressed variants and the single SP-A2 gene product, either in the function or in the oligomerization pattern, as we observed previously (16, 55). This is probably due to the variable ratio of SP-A1 to SP-A2 in the baculovirus-mediated expression system. In this system, the SP-A1/SP-A2 gene product was coexpressed by isolating baculovirus containing both genes at a ratio of 1:1 (55). Although mRNA expression of both genes was confirmed by genotyping using PCR-RFLP analysis (10), the ratio of SP-A1 to SP-A2 expression in each preparation may vary, and this variation may result in subtle differences in function and/or structure.

Bleomycin, which is used in several protocols of chemotherapy, is an exogenous lung oxidant and produces reactive oxygen species (20). In addition, patients who receive bleomycin therapy may need oxygen supplementation, and this hyper-

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Fig. 7. Electrophoretic analysis of in vitro expressed SP-A variants and native hSP-A after ozone exposure under reducing and nonreducing conditions. In vitro expressed hSP-A genetic variants (1A, 6A4, 1A/6A4) and native hSP-A purified from BAL of an alveolar proteinosis patient were exposed to ozone at a concentration of 1 ppm for 4 h. A: 1 µg protein was subjected to 10% polyacrylamide gel electrophoresis in the presence of SDS after reduction of protein in 50 mM dithiothreitol at 95°C for 10 min. B: 5 µg of each protein sample were subjected to a 4–15% gradient polyacrylamide gel electrophoresis in the presence of SDS after 95°C for 10 min without dithiothreitol where inter- and intramolecular disulfide bonds remain intact (nonreducing condition). Protein bands were detected by silver staining. Notation on the right (2X, 3X, etc) indicates oligomers.
In summary, with regard to cytokine production by macrophage-like cells in response to SP-A, we found 1) differences between the SP-A1 and SP-A2 single gene products in the presence of bleomycin, 2) a difference between the SP-A 6A4 variant and the other SP-A variants after ozone-induced oxidation in the presence of bleomycin, and 3) electrophoretic differences in the oligomeric band pattern between SP-A variants either before or after ozone-induced oxidation. We speculate that differences observed by gel analysis have an impact on the function of SP-A variants under unperturbed conditions and conditions that promote protein oxidation. We further speculate that a better understanding of the mechanisms that underlie the differences among SP-A variants may contribute to our better understanding of individual variability of pulmonary complications observed during bleomycin chemotherapy and/or in an environment with increased oxidative burden.

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