Structure and immunological function of oxidised albumin in lung cancer: its potential role as a biomarker of elevated oxidative stress

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ABSTRACT

The role of reactive oxygen species (ROS)-damaged human serum albumin (HSA) in lung cancer (LC) patients is investigated. The binding characteristics of LC antibodies with native and ROS-damaged HSA are assessed. Smoking and non-smoking LC patients (n=40) are examined by a direct binding enzyme-linked immunosorbent assay (ELISA) and the results are compared with healthy age- and gender-matched smoking and non-smoking subjects (n=38). A high degree of specific binding in approximately 70% of cancer immunoglobulin G (IgG) towards ROS-damaged HSA was observed, compared to results with its native analogue ($P<0.05$). Affinity purified IgG from those LC patients with a history of smoking showed substantially stronger binding to damaged HSA over native HSA. Competitive inhibition ELISA substantiated the enhanced recognition of ROS-HSA by circulating antibodies in LC patients. The increase in total serum protein carbonyl levels in the LC patients was largely due to an increase in oxidised albumin. Purified HSA from LC patients (LC-HSA) contained higher levels of carbonyls than did HSA from healthy subjects (normal-HSA; $P<0.01$). LC-HSA was conformationally altered and showed greater exposure of its hydrophobic regions. Collectively, the oxidation of plasma proteins, especially HSA, might enhance oxidative stress in LC patients.

KEY WORDS: Human serum albumin.
Lung neoplasms.
Reactive oxygen species.

Introduction

Lung cancer is the leading cause of cancer deaths worldwide.[1] The number of lung cancer (LC) patients has been increased by exposure to environmental risk factors including cigarette smoking and asbestos.[2] The prevention of lung cancer is a serious worldwide challenge, but it is clear that the successful prevention of lung cancer will depend on the reduction of risk factors along with better methods of screening and early detection.

Reactive oxygen species (ROS) play a key role in both normal biological function and in the pathogenesis of certain human diseases. They are generated continuously in cells by cellular metabolism and by exogenous agents, but increases in their steady states are thought to be responsible for a variety of pathological conditions including carcinogenesis, and lung cancer in particular.[3–6]

Excess generation of ROS has the ability, either directly or indirectly, to damage proteins, DNA and other biomolecules.[7] In fact, proteins are major targets for free-radical attack, and especially damaging in this respect is the hydroxyl radical. Protein oxidation, which results in functional disruption, is not random but appears to be associated with increased oxidation in specific proteins.[8,9] Recent studies indicate a strong role for increase in protein oxidation as a primary cause of cellular dysfunction observed in many diseases[10,11] including carcinogenesis.[12] Furthermore, ROS can modulate the activity of the proteins that respond to stress and which regulate genes involved in cell proliferation, differentiation and apoptosis.[13]

Increased levels of circulating antibodies and autoantibodies directed against smooth muscle, nuclei[14] and phospholipids[15] have been reported in the serum of patients with malignancies and it is now well accepted that ROS play an important role in carcinogenesis and that hydroxyl radicals contribute to the structural changes that characterise the cancer-like phenotype.[16]

Human serum albumin (HSA) is the most abundant protein in the circulatory system. It is documented that autoantibodies to normal human albumin are observed in cancer. Many reported cases of monoclonal protein produced by myelomas have anti-HSA immune specificity,[17,18] but it is not clear why this prevalent extracellular protein becomes antigenic. It is well documented that HSA is quite vulnerable to ROS.[19,20]

The authors and others have previously demonstrated that ROS cause extensive damaged to HSA.[21,22] For example, ROS-damaged HSA is highly immunogenic in experimental animals, with the induced antibodies exhibiting variable binding to nucleic acid conformers,[23] and ROS-damaged HSA is recognised by antibodies of patients with various diseases[22,24] including cancer.[25] Therefore, HSA is exposed

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continually to oxidative stress so alterations to the conformation and function of HSA can occur, resulting in modification of its biological properties.

The aim of the study is to test the hypothesis that ROS-damaged HSA is involved in lung cancer pathogenesis. It examines the presence of circulating antibodies directed against native and ROS-modified HSA in lung cancer in cigarette smokers and non-smokers. It also attempts to evaluate the effects of oxidative stress on the structural and biological properties of isolated HSA from smoking and non-smoking lung cancer patients.

Materials and methods

Materials

Human serum albumin (essentially fatty acid free), anti-human IgG-alkaline phosphatase conjugate, p-nitrophenyl phosphate, Tween-20, Freund's complete and incomplete adjuvants and bovine serum albumin were obtained from the Sigma Chemical Company. Protein-A sepharose CL-4B was obtained from Genei, India. Sephacryl S-200 HR was obtained from Pharmacia Fine Chemicals, Sweden. Polystyrene microtitre flat-bottomed enzyme-linked immunosorbent assay (ELISA) plates and modules were obtained from Nunc, Denmark. All other reagents/chemicals were of the highest analytical grade available. Protein was estimated by the method of Lowry *et al*.[26] and HSA concentration was determined spectrophotometrically at 280 nm.[27]

Subjects

Sera and blood were collected from lung cancer patients attending the J.N. Medical College Hospital, AMU, Aligarh, and the All India Institute of Medical Sciences, New Delhi. Samples were taken from patients with a history of cigarette smoking (n=24; all male; age range: 63±8.2 years) and non-smokers (n=16; male=7; female=9; age range: 56± 4.1 years) with proven histopathological diagnosis after informed consent had been obtained. Sera and blood from normal, healthy individuals with a history of smoking (n=20; male=20; age range: 62±9.3 years) and not smoking (n=18; male=10; female=8; age range: 55±9.4 years) served as negative controls. The details of the study subjects are summarised in Table 1. All sera were decomplemented by heating at 56°C for 30 min and stored in aliquots at –20°C.

Table 1. Characteristics of the study subjects.

	Lung cancer patients		Normal human subjects	
	Smoking	Non-smoking	Smoking	Non-smoking
Number of cases	24	16	20	18
Age (years)	63±8.2	56±4.1	62±9.3	55±9.4
Gender (M/F)	24M	7M/9F	20M	10M/8F
Smoking duration (years)	31±7.2	–	29±10.7	–
Smoking intensity (cigarettes/day)	29±12.1	–	27±14.0	–

Results represent mean±SD values.

Modification of human serum albumin

Human serum albumin was modified in PBS (10 mmol/L sodium phosphate buffer containing 150 mmol/L NaCl [pH 7.4]) by a published procedure.[22,23] An aqueous solution of native HSA (3.75 µmol/L) was modified by hydroxyl radicals generated by the ultraviolet (UV)-irradiation (30 min) of hydrogen peroxide (15.1 mmol/L) at 254 nm. Excess hydrogen peroxide was removed by extensive dialysis against PBS.

Enzyme-linked immunosorbent assay

An ELISA was carried out on polystryrene plates.[23] Polystyrene polysorp immunoplates were coated with 100 µL native or modified HSA (10 µg/mL) in carbonate-bicarbonate buffer (0.05 mol/L, pH 9.6). The plates were coated for 2 h at 37°C and overnight at 4°C. Each sample was coated in duplicate and half of the plates served as controls without antigen coating. Unbound antigen was washed (x3) with TBS-T (20 mmol/L Tris, 150 mmol/L NaCl [pH 7.4] containing 0.05% Tween-20) and unoccupied sites were blocked with 2% fat-free milk in TBS (10 mmol/L Tris, 150 mmol/L NaCl [pH 7.4]) for 4–6 h at 37°C.

After incubation the plates were washed (x4) with TBS-T. The protein A-sepharose CL-4B-purified LC-IgG in TBS (100 µL/well) was adsorbed for 2 h at 37°C and overnight at 4°C. Bound IgG was assayed with anti-human alkaline phosphatase conjugate using p-nitrophenyl phosphate as substrate. The absorbance (A) of each well was monitored at 410 nm on an automatic microplate reader. Each sample was run in duplicate. The control wells were treated similarly. Results were expressed as a mean of A_{test}–$A_{control}$.

Competition ELISA

Antigenic specificity of the antibodies was determined by competition ELISA.[24] Varying amounts of inhibitors (0–20 µg/mL) were mixed with a constant amount of IgG.

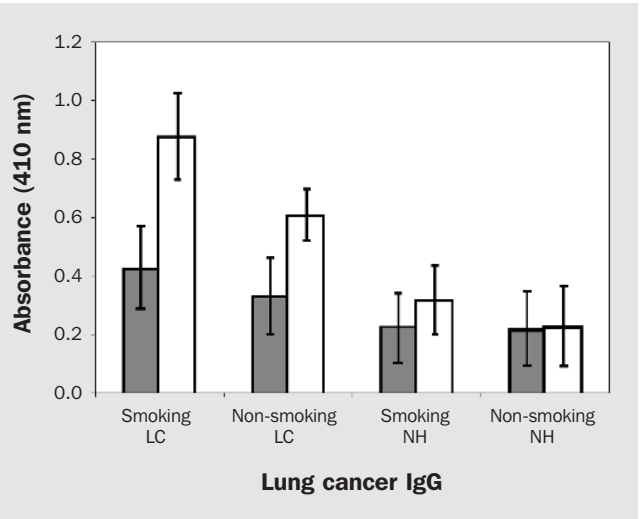

Fig. 1. Direct binding ELISA of affinity purified IgG from smoking lung cancer patients (smoking LC), non-smoking lung cancer patients (non-smoking LC), healthy smoking subjects (smoking NH) and healthy non-smoking subjects (non-smoking NH) to native (■) and ROS-modified (□) HSA. The number of smoking LC, non-smoking LC, smoking NH and non-smoking NH samples were 18, 10, 20 and 18, respectively.

Table 2. Competitive inhibition of affinity purified IgG from smoking and non-smoking LC patients by native and ROS-modified HSA.

IgG samples	Number of patients	Maximum inhibition (%)	
		Native HSA	ROS-HSA
Smoking LC	18	25.3±4.7	61.7±2.5*
Non-smoking LC	10	24.1±3.4	43.4±2.5*
Smoking NH	20	20.5±3.2	25.1±5.2
Non-smoking NH	18	19.3±6.1	19.4±4.1

Results represent mean±SD values.
ELISA plates were coated with ROS-HSA (10 µg/mL).
*$P<0.001$ compared with native HSA.

The mixture was incubated at room temperature for 2 h and overnight at 4°C. The immune complex formed was coated in the wells instead of IgG. The remaining steps were as for the direct binding ELISA. Inhibitors used were native and ROS-modified HSA. Percentage inhibition was calculated using the formula:

$$\% \text{ inhibition} = (1-[A_{\text{inhibited}}/A_{\text{uninhibited}}]) \times 100.$$

Purification of IgG

IgG from cancer patients and from normal human sera was isolated by affinity chromatography on a protein A-sepharose CL-4B column. Serum (0.3 mL) diluted with an equal volume of PBS (pH 7.4) was applied to the column (12 mm × 45 mm) equilibrated with the same buffer. The flow through was reloaded on the column (x2/3). Unbound proteins were removed by extensive washing with PBS (pH 7.4). The bound IgG was eluted with 0.58% acetic acid in 0.85% sodium chloride and neutralised with 1 mL Tris-HCl (1 mol/L; pH 8.5), 3-mL fractions were collected and read at 251 and 278 nm. The IgG concentration was determined considering 1.38 A_{278} = 1.0 mg IgG/mL. The isolated IgG was dialysed against PBS (pH 7.4) and stored at –20°C.[10] The homogeneity of isolated IgG was checked by polyacrylamide gel electrophoresis.

Purified albumin from healthy control and LC patients

Human serum albumin samples were isolated by ammonium sulphate fractionation followed by gel exclusion chromatography on Sephacryl S-200 HR.[28] The purity of the HSA samples was checked by SDS-PAGE. Fatty acid was depleted from purified HSA samples by a published procedure.[29]

Determination of serum protein and purified albumin oxidation

Carbonyl content in serum and in purified HSA was analysed using the method of Levine *et al.*,[30] with slight modification. Briefly, the reaction mixture containing an appropriate amount of serum/purified HSA, 0.5 mL 10 mmol/L 2, 4-dinitrophenylhydrazine (DNPH) in 2.5 mol/L HCl was added and mixed thoroughly. After addition of 250 µmol/L TCA (20%) and centrifugation, the pellet was collected and washed (x3) with 1 mL ethanol:ethylacetate (1:1) mixture. The pellet was then dissolved in 1 mL guanidine solution (6 mol/L) and incubated at 30°C for 15 min. After centrifugation, the supernatant was collected and carbonyl content was estimated from A at 370 nm. Samples were analysed against a blank of 1 mL guanidine solution (6 mol/L). Protein concentration was determined by the method of Lowry *et al.*[26]

Circular dichroism measurements

Far UV-CD measurements were carried out using a Jasco spectropolarimeter (model J-720) equipped with a microcomputer. The instrument was calibrated with D-10-camphorsulphonic acid. The CD measurements were made at 25°C with a thermostatically controlled cell holder attached to a Neslab RTE 110 water bath (temperature accuracy: ±0.1°C). Spectra were taken with a scan speed of 20 nm/min at a response time of 1 sec. Each spectrum was the average of three scans. The CD spectra were taken at a protein concentration of 3 µmol/L (cell path length: 1 mm). The mean residue ellipicity (MRE) was calculated as follows:

$$\text{MRE} = \text{CD} / (10 \times n \times l \times \text{Cp})$$

CD is millidegrees, n is the number of amino acid residues (585), l is the cell path length, and Cp is the mole fraction.

Helical content was calculated from the MRE values at 222 nm using the following equation:[31]

$$\% \text{ alpha helix} = (\text{MRE222 nm} - 2340/30300) \times 100.$$

Effective protein hydrophobicity

The effective hyrophobicity of all albumin samples (1.5 µmol/L) in 67 mmol/L sodium phosphate buffer (pH 7.4) was probe with 4,4`-dianilino-1,1`-binaphthyl-5,5`-disulphonic acid (bis-ANS) (10 µmol/L) at 25°C. The compound was excited at 394 nm and fluorescence spectra were recorded on an Hitachi F-200 spectrofluorimeter using 1-cm quartz 10 cells. Increase in fluorescence intensity (FI) was calculated using the following equation:

$$\% \text{ Increase in FI} = ([\text{FI modified HSA} - \text{FI native HSA}] / \text{FI modified HSA}) \times 100$$

Statistical analysis

Data were presented as mean±SD. Significance of difference from control values was determined using Student's t-test (Statgraphics, Origin 6.1). $P<0.05$ was considered to indicate statistical significance.

Results

Earlier reports[22–25] showed alterations in HSA following exposure to the hydroxyl radical generated from UV-irradiation of hydrogen peroxide. Loss of secondary and tertiary structures, hyperchromicity at 280 nm, loss of tryptophan fluorescence intensity, exposure of the hydrophobic areas and an increase in protein carbonyl content were observed in HSA following hydroxyl treatment. Previously, the authors demonstrated that a direct-binding ELISA showed ROS-modified HSA as a potent immunogen in rabbits that induced high-titre (>1:12,800) antibodies, whereas native HSA showed a titre <1:6400. Preimmune serum serving as a negative control did not show appreciable binding with the respective immunogens under identical experimental conditions. Protein A-sepharose CL-4B-isolated anti-ROS-HSA IgG and anti-HSA IgG showed strong binding with their respective immunogens.[23]

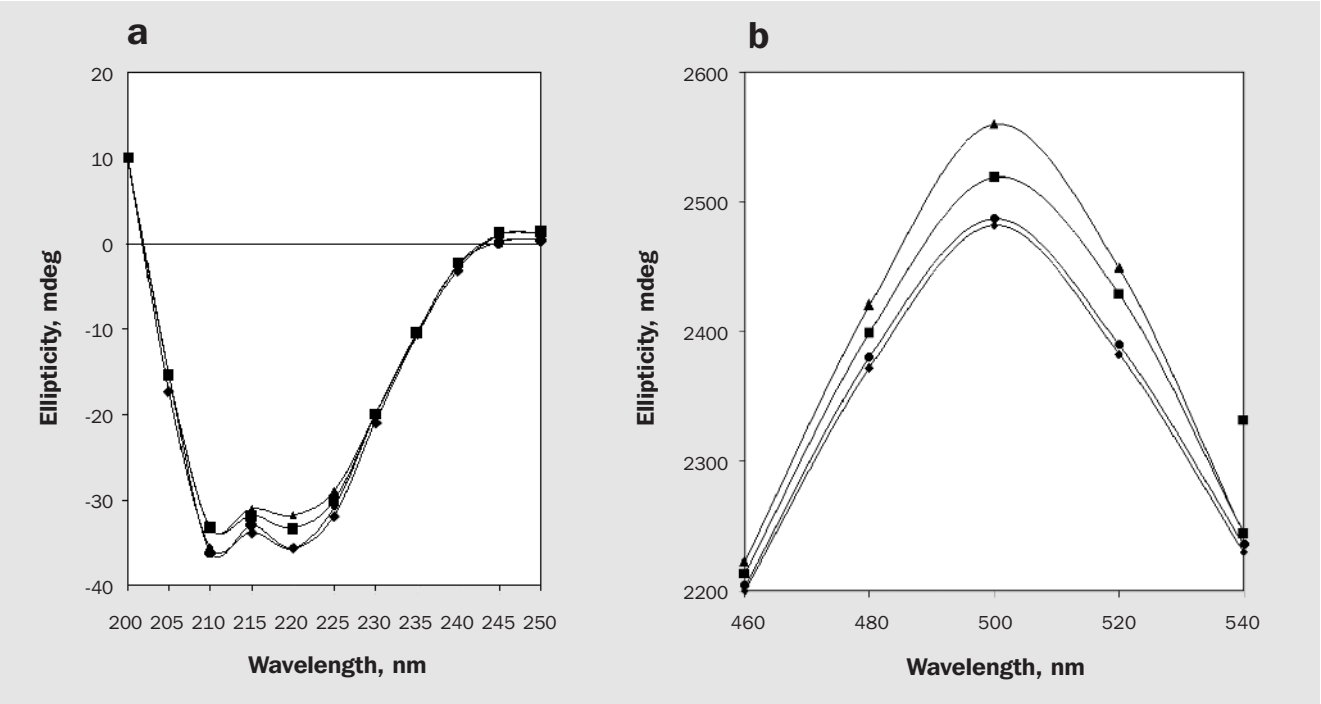

Fig. 2. a) CD spectra of purified HSA from smoking LC patients (▲), non-smoking LC patients (■), healthy smoking subjects (●) and healthy non-smoking subjects (⧫). Spectra are the average of eight determinations. b) Florescence spectra of purified HSA-bound bis-ANS. Purified HSA from smoking LC patients (▲), non-smoking LC patients (■), healthy smoking subjects (●) and healthy non-smoking subjects (⧫). Spectra are the average of eight determinations.

In the present study, the purified IgGs were found to elute in a single symmetrical peak. SDS-PAGE of purified IgG under non-reducing conditions showed a single homogeneous band (data not shown). The majority of smoking (18/24) and non-smoking (10/16) LC IgG showed strong binding to ROS-HSA over native HSA ($P<0.05$). No appreciable binding was observed in the healthy smoking and non-smoking subjects. The average A at 410 nm (±SD) of 18 smoking LC IgG binding to native and ROS-damaged HSA was 0.43±0.14 and 0.88±0.15, respectively, whereas the results for 10 non-smoking LC IgG binding to native and ROS-damaged HSA was 0.33±0.13 and 0.61±0.09, respectively. Average A at 410 nm (±SD) of 20 smoking NH IgG binding to native and ROS-damaged HSA was 0.23±0.12 and 0.31±0.13, respectively, whereas the results for 18 non-smoking NH IgG binding to native and ROS-damaged HSA was 0.23±0.14 and 0.22±0.13, respectively.

The binding specificity of antibodies from smoking and non-smoking sera from LC patients was further evaluated by competitive inhibition ELISA using native and ROS-HSA as inhibitors. Average percentage inhibition (±SD) in the binding of 18 smoking LC IgG to native and ROS-HSA was 25.3±4.7 and 61.7±2.5, respectively, whereas the results for 10 non-smoking LC IgG to native and ROS-HSA was 24.1±3.4 and 43.4±2.5, respectively (Table 2). IgG from the negative controls did not show significant inhibition to either inhibitor. The data revealed striking differences in the recognition of native and ROS-damaged HSA by LC antibodies ($P<0.001$).

Protein carbonyl content is a general indicator and the most commonly used biomarker for protein oxidation. The data showed significant increase in total serum protein carbonyl content in 10 cigarette smoking and 10 non-smoking LC patients, compared with their respective control subjects ($P<0.05$). The average carbonyl content (±SD) of 10 independent assays of smoking LC serum proteins and smoking NH serum proteins were 3.62±0.28 and 2.21±0.15 nmol/mg protein, respectively, whereas in healthy non-smoking LC serum proteins and non-smoking NH serum proteins the average results were 2.74±0.15 and 2.00±0.16 nmol/mg protein, respectively (Table 3).

To investigate the extent of alterations in the biological properties of HSA in lung cancer patients, HSA was isolated from 10 smoking LC patients (S-LC-HSA), 10 non-smoking LC patients (NS-LC-HSA), 10 healthy smoking subjects (S-NH-HSA) and 10 healthy non-smoking subjects (NS-NH-HSA). Purified HSA was found to elute in a single symmetrical peak on a Sephacryl S-200 HR column. Polyacrylamide gel electrophoresis of purified HSA showed a single homogenous band (data not shown).

Average carbonyl contents (±SD) of 10 independent assays of S-LC-HSA and S-NH-HSA were 2.84±0.24 and 1.84±0.13 nmol/mg protein, respectively ($P<0.01$), whereas in NS-LC-HSA and NS-NH-HSA the results were 1.91±0.15 and 1.62±0.12 nmol/mg protein, respectively ($P<0.01$). $P<0.05$ indicated a significant difference in carbonyl content in the S-LC-HSA and NS-LC-HSA groups (Table 3).

The structural properties of NH-HSA and LC-HSA were examined by circular dichroism and ANS fluorescence. Fatty acid-free S-NH-HSA, NS-NH-HSA, S-LC-HSA and NS-LC-HSA were used. The far-UV CD spectra of S-LC-HSA and NS-LC-HSA showed 52.1% and 54.8% alpha-helix, respectively, whereas the results for S-NH-HSA and NS-NH-HAS were 58.1% and 58.0%, respectively (Fig. 2a). The effect of oxidation on the exposure of the hydrophobic areas was examined using the fluorescence probe bis-ANS. Fluorescence intensity of S-LC-HSA, NS-LC-HSA, S-NH-HSA and NS-NH-HSA was 2560, 2519, 2487 and 2482

arbitrary unit, respectively, at 500 nm (Fig. 2b). These results support the view that binding of ANS to the hydrophobic regions of proteins results in an increase in fluorescence intensity.[32]

Discussion

The lung is directly exposed to higher levels of oxygen than are most other tissues. The level of ROS in the lung is further increased by cigarette smoke, inflammation, pollutants, chemicals and carcinogens, all of which lead to DNA and protein damage.[33,34] Oxidants and an imbalance between the cellular redox state and the pulmonary defence systems play a role in the pathogenesis and progression of malignant lung disease.[34]

Lung cancer is the most common malignancy worldwide[1] and its incidence continues to increase. Reactive oxygen species such as singlet oxygen, superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) are generated by various stimuli and it is suggested that they function as intracellular messengers in redox signalling pathways.

Excess generation of ROS has the ability, either directly or indirectly, to damage proteins, DNA and other cell biomolecules. In fact, proteins are major targets of free-radical attack, and especially damaging in this respect is the hydroxyl radical. Protein oxidation results in cellular dysfunctions, functional disruption and structural changes,[7,35] and contributes to the aetiology of many human diseases including cancer.[9–12]

Serum albumin is the most abundant protein in the circulatory system and its redox modification modulates its physiological function as well as serving as a biomarker of oxidative stress. It is well documented that HSA is vulnerable to ROS.[19] Therefore, HSA is exposed continually to oxidative stress such that alterations of the conformation and function of HSA could occur, resulting in modification of its biological properties.

The authors and others have reported the presence of elevated levels of oxidised protein products (advanced oxidation protein products) such as oxidised albumin in patients with various diseases.[20–24] Furthermore, ROS-modified HSA is highly immunogenic in rabbits, and the antigenic specificity of affinity purified anti-ROS-HSA IgG and anti-HSA IgG suggest that induced antibodies are immunogen-specific. The substantially enhanced immunogenecity of ROS-HSA in comparison to native HSA may be due to the generation of potential neo-epitopes against which antibodies are raised.[23]

It is documented that autoantibodies to normal human albumin are found in cancer. Many reported cases of monoclonal protein produced by myelomas have anti-HSA immune specificity,[17,18] but it is not clear why this prevalent extracellular protein becomes antigenic. In the present study, LC serum antibodies were screened for the presence of autoantibodies reactive to native and ROS-modified HSA using a direct-binding ELISA. Results showed that approximately 70% of affinity purified cancer IgG samples showed preferentially high binding to ROS-HSA, compared to its native analogue, whereas control samples did not show appreciable binding. Competitive inhibition ELISA was also performed and the results indicated that the ROS-modified HSA was an effective inhibitor showing substantial difference in the recognition of damaged HSA over native HSA ($P<0.001$).

Table 3. Carbonyl content in serum proteins and in purified albumin from smoking and non-smoking lung cancer patients.

Study subjects	Number of samples	Carbonyl content (nmol/mg protein)	
		Serum proteins	Purified HSA
Smoking LCS	10	3.62±0.28	2.84±0.24
Non-smoking LCS	10	2.74±0.15	1.91±0.15
Smoking NHS	10	2.21±0.15	1.84±0.13
Non-smoking NHS	10	2.00±0.16	1.62±0.12

Results represent mean±SD values.
LCS: lung cancer sera; NHS: normal human sera.

As the most abundant plasma protein, albumin could play a major role as an antioxidant in plasma, at least by thiol oxidation and carbonyl formation.[36] In this context, the characterisation of the oxidation status of serum albumin would provide not only useful information about the redox state of the human body but also alterations in the conformation and function of HSA that may result in modification of its biological properties. The oxidation of a protein typically results in an increase in carbonyl content. This increase is due to the oxidation of lys, arg, pro and other amino acid residues. In short, protein carbonyl groups are the biomarkers of oxidative stress.[37]

In human plasma, all amino acids in protein are susceptible to oxidative modification by oxidants such as hydroxyl radicals and hypochlorous acid.[38] In the present study, total serum protein carbonyl content was significantly ($P<0.05$) increased in cigarette smoking and non-smoking LC patients compared with controls. Thus, to investigate the extent of alterations in the biological properties of HSA in LC patients, carbonyl content was compared and $P<0.01$ indicated a significant difference in carbonyl content between smoking LC patients and non-smoking healthy controls. Similar results were obtained in non-smoking LC patients and normal subjects. Higher carbonyl content was also found in smoking LC patents compared with non-smoking LC patients ($P<0.05$).

These results suggest that HSA is exposed continually to oxidative stress such that alterations in its biological properties could result in conformational change, and the significance of this conformational change to purified HSA was examined. A slight decrease in alpha helical content was observed in LC-HSA. The effect of oxidation on the exposure of hydrophobic areas was also examined using bis-ANS, and results indicated that the conformation of HSA in LC patients involves an increase in accessible hydrophobic regions.

The present data support the view that cigarette smoking increases oxidative stress in lung cancer patients, and the formation of reactive oxygen/nitrogen species results in oxidation/nitration of plasma proteins.[34] Patients with lung cancer show higher production of oxygen free radicals than do the normal population, and HSA from normal subjects offers significant protection against free radicals.[39] This suggest that LC patients with increased oxidative stress show greatly enhanced oxidative modification of plasma

proteins. Thus, as the most abundant plasma protein, albumin is likely to be extensively damaged.

Despite the power of modern molecular approaches and persistent investigative efforts, lung cancer remains an enigmatic disorder and the agents that trigger it remain to be identified. The present report is the first to demonstrate structural perturbation in albumin due to oxidative stress, rendering it immunogenic. The neo-epitopes generated may be one factor responsible for the induction of autoantibodies in patients with lung cancer.

The link between autoimmunity and cancer may result from a common aetiological origin (i.e., genetic, hormonal or environmental) or from paraneoplastic syndromes. Many autoantibodies have been identified in cancer patients[6,25] and these represent potentially valuable tools for identifying new biomarkers and therapeutic targets. Recently, it has been shown that the amplification of signals provided by the host's immune system to low levels of tumour-associated antigens in early disease provides a potential route to the early diagnosis of cancer.[40,41]

Further studies are required to assess the diagnostic and/or prognostic value of autoantibodies reacting with plasma proteins, polynucleotides, modified DNA, DNA-protein adducts, oncoproteins, tumour suppression genes and proliferation-associated antigens. Work over the past 25 years suggests that it is feasible to use specific autoantibody markers to improve diagnostic accuracy in systemic autoimmune disease.

Circulating autoantibodies to self-antigens over-expressed by cancer cells are common in cancer patients; thus, distinct binding of circulating antibodies to ROS-modified HSA in cancer patients might play a significant role in the aetiology of this disease. □

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