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CELLULAR MORPHOMETRY OF THE BRONCHI OF HUMAN AND DOG LUNGS

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Abstract

One hundred and fourty-seven bronchial samples (generations 3-6) from 66 patients (62 useable; 36 female, 26 male; median age 61) have been dissected by generation from fixed surgical lung specimens obtained after the removal of pathological lesions.. In addition, one hundred and fifty-six mongol dog bronchi (generations 2-6) dissected from different lobes of 26 dog lungs have also been similarly prepared. One hundred and twenty-seven human samples have been completely processed for electron microscopy and have yielded 994 electron micrographs of which 655 have been entered into the Computerized Stereological Analysis System (COSAS) and been used for the measurement of the distances of basal and mucous cell nuclei to the epithelial free surface. Similarly 328 micrographs of dog epithelium from 33 bronchial samples have been used to measure the distances of basal and mucous cell nuclei to the epithelial free surface and have been entered into COSAS. Using the COSAS planimetry program, we continue to expand our established data bases which describe the volume density and nuclear numbers per electron micrograph for 5 cell types of the human bronchial epithelial lining of men and women, as well as smokers, non-smokers and ex-smokers and similar parameters for the same 5 epithelial cell types of dog bronchi. Our micrographs of human bronchial epithelium have allowed us to analyze the recent suggestion (Bridges, et al., 1991) that the DNA of lymphocytes may be subject to significant damage from Rn progeny while within the lung. Since the last progress report three papers have been submitted for publication.

Human and Dog Tissues

All 66 human lung specimens dissected to date (147 samples each with one to four different bronchi of the same generation; generations 3 to 6) and dog bronchi similarly dissected (156 samples from 26 dog lungs; generations 2 to 6 and branch points of 4 generations) have been processed through embedding in epoxy resin blocks. Approximately 90% of our human samples have been sectioned and stained for light microscopic examination. Of these approximately 85% have been judged to be adequately preserved and usable for electon microscopic morphometric analysis. One hundred and twenty seven human bronchial samples have been completely processed for electron microscopy. From the 994 electron micrographs taken of the epithelial lining of these human bronchi 655 have been entered into the Computerized Stereological Analysis Program (COSAS, Cornacchia and Black , 1988) and have also been used for the meaurement of the distances of basal and mucous cell nuclei to the apical free surface.

Table 1 summarizes the patients who were the sources of our tissues, the diagnoses of their lesions and the lung lobes from which we obtained bronchi. Processed dog bronchi continue to be sectioned and electron micrographs are being taken. Table 2 summarizes the generations represented in our dog samples. As seen in this Table we have attempted to determine if the epithelia at areas of airway bifurcation differ from those of adjacent airways and so we have also been preparing branch point samples as well as those from individual bronchial generations.

Using our expanding electron micrograph collection and COSAS, we continue to add to our established data bases which describe the nuclear volume density as well as other nuclear parameters such as maximum and minimum diameters and

nuclear numbers per electron micrograph for five cell types (basal, mucous, intermediate/indeterminate, ciliated, and intraepithelial white blood cells) of the bronchial epithelium of humans and dogs. As sufficient human samples are processed, we are comparing these data for men and women, as well as smokers, non-smokers and ex-smokers.

As we accumulate additional electron micrographs of bronchial gland duct epithelium we will analyze these in the same manner, since we intend to extend our analysis to include the basal cells of these ducts. Both the ciliated and the collecting ducts of human bronchial glands clearly have basal cells (Meyrick et al. 1969). Although these cells have been recently ignored as possible stem cells for carcinogenesis (McDowell and Trump, 1983), duct hyperplasia similar to that seen in the lining epithelium of smokers has been reported and suggested as a possible source of bronchogenic carcinoma (Auerbach, 1967); and bronchial gland volume is increased in smokers compared to non-smokers (Thurlbeck et al., 1963; Ryder et al., 1971). In view of the general phenomenon from other glandular systems in the human body such as salivary and mammary glands where duct cells are the stem cells (Alberts et al., 1989), duct basal cells should be considered in radon/carcinogenesis risk modelling. We have collected 20 micrographs of the epithelium of bronchial gland ducts but have not analyzed them as yet.

Of the 450 electron micrographs from our dog bronchial epithelium samples 328 have been entered into COSAS and also used for the measurement of the distances of basal and mucous cell nuclei to the epithelial free surface. The branch points of dog bronchi are also being examined to determine if their epithelia differ from that of the adjacent airways.

Results

Since we have access to patient records and are able to interview most of the patients from whom we get bronchial samples, we are comparing the airways of ex-smokers with smokers and non-smokers. For this initial analysis we have considered individuals who stopped smoking more than 10 years ago as ex-smokers and included those who stopped more recently as smokers. Table 3 summarizes the volume density data to date for the 5 cell types of the human bronchial epithelial nuclei and intraepithelial white blood cells of smokers, non-smokers, and ex-smokers. Overall there are trends but no statistically significant differences among these groups. It seems, if one examines the V_D data for basal cell and mucous cell nuclei, that ex-smokers may be different from both non-smokers and smokers. Although mucous metaplasia is known to accompany smoking (Chang 1957; Hayashi et al. 1978) its apparent persistance after years of non-smoking is somewhat unexpected. These initial data thus may suggest that smoking may cause changes in the basal and mucous cells such that they do not revert to the non-smoking values even 10 years after cessation of smoking when cancer risk has reverted to the level of the general population (Fishman, 1988). We continue to record the sex of our patients and will analyze this parameter when the data base is larger since we intend to include age as well as sex in our analyses.

The figures for the distances of basal and mucous cell nuclei to the epithelial free surface we generated have been used to update a model of the ^{222}Rn decay product dose to these nuclei in the human bronchial epithelium (Harley, et al, 1991a)

We have found intraepithelial leucocytes and occasional mast cells and are quantifying these as one of our cell populations (Tables 3, 4). Such cells have been often present in the bronchial epithelium although not always noted (for example

see Fig. 1 of McDowell et al. 1978). In addition, Langerhans cells have been added to the types observed (Richard et al., 1987) and the intraepithelial T-lyphocytes subsets in human bronchi have been characterized (Fournier et al. 1989). A recent paper by Henshaw and colleagues (Bridges et al, 1991) suggests a possible association between mutations of the hypoxanthine-guanine phosphoribosyl transferase gene in human blood T-lymphocytes and the radon concentrations in the homes of the individuals whose cells were examined. These authors clearly indicate that their findings do not prove causality but they speculate that Rn could affect the lymphocytes while they are "resident in various organs including bone marrow and the lung" (Bridges et al.,1991, p.1187). We (Harley and Robbins, 1991) have examined the possibilities of significant lymphocyte exposure to Rn decay products while they are in various lung loci (intraepithelial, BALT [bronchus associated lymphoid tissue],within the connective tissue space or capillaries of the interalveolar septa, within lymphatic vessels accompanying major airways or in the pleura). A provocative suggestion such as the one made by Bridges and colleagues must however, explain the apparent lack of increased risk of primary lung lymphoma in miners exposed to high Rn levels. It is apparent that the populations of lymphocytes which may be subjected to the highest alpha particle dose while in the lung are within the epithelium or just below it within BALT (Harley and Robbins, 1991). At present we are examining our electron micrograph set to determine the percentages of the different intraepithelial white blood cell types.

Table 4 summarizes the volume density data for the five types of epithelial cell nuclei of dog bronchi from generations 2 to 6 and includes as separate categories the epithelia of bronchi at two branch points (generation 4 to 5 and generation 5 to 6). In view of the possible non-uniformity of basal and mucous cell populations at these regions of greater stresses and particle deposition we extended our analysis to

examine these areas in comparison to the airways just proximal and just distal to the branch points. Micrcographs of additional branch points will be added as we complete the processing of our tissues. In comparison with the V_D data for human bronchial lining cells (Table 3) dog basal cell nuclei and mucous cell nuclei occupy (Table 4) a somewhat larger percentage of the epithelial volume.

Measurements of the distances on the electron micrographs of basal and mucous cell nuclei to the epithelial free surface analagous to those of human bronchial epithelium are also being made of dog bronchial epithelium. Some of our measurements of these distances have been utilized to update the ^{222}Rn dosimetry for the dog lung (Harley et al., 1991b).

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TABLE 1

DIAGNOSES AND LUNG SITES

DIAGNOSIS	FEMALE	MALE
SQUAMOUS CELL CARCINOMA	4	8
LARGE CELL CARCINOMA	2	4
EPIDERMOID CARCINOMA	3	0
ADENOCARCINOMA	15	7
CARCINOID CARCINOMA	3	0
UNKNOWN AT THIS TIME	8	4
OTHER	1	3

TISSUE SOURCE	FEMALE	MALE
RIGHT UPPER LOBE	6	12
RIGHT MIDDLE LOBE	6	2
RIGHT LOWER LOBE	4	1
LEFT UPPER LOBE	14	8
LEFT LOWER LOBE	4	2
RIGHT LUNG	2	1

TOTAL FEMALES= 36
TOTAL MALES= 26

Table 1. Lung samples are classified by location and pathology. Compared to the expected incidence of lung cancer in the U.S. our group shows more adenocarcinoma in woman and less in men as might have been expected (Churg,1988; Yesner,1988). It has been reported that the upper lobes are a predominant site of carcinoma (Churg,1988) and our cohort shows that both the left and right upper lobes as very frequent sites of tumor occurence.

TABLE 2

DOG BRONCHI

GENERATION	NUMBER OF SAMPLES
GENERATION 1	20
GENERATION 2	28
GENERATION 2 BRANCH	2
GENERATION 3	29
GENERATION 3 BRANCH	4
GENERATION 4	33
GENERATION 4 BRANCH	2
GENERATION 5	35
GENERATION 5 BRANCH	2
GENERATION 6	21
GENERATION 6 BRANCH	2
GENERATION 7	1
TOTAL	177 (156, generations 2-6)

Table 2. Twenty-six male dogs of various ages and sizes have been the source of these bronchi which were taken from all lobes. Initial fixation procedures were developed using dog tissues and of those thirty samples were found to be inadequately preserved and were discarded. Of the 177 samples 31 have been analysed to date. The total number of micrographs of dog bronchial epithelium in COSAS at present is 338, of these 328 micrographs have been analyzed.

TABLE 3

Number and Volume Density of Human Bronchial Epithelial Cell Nuclei

A. Smokers

		Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
Gen	# µgrphs	V_D	#	V_D	#	V_D	#	V_D	#	V_D	#
3	66	0.025±0.015	4.6±2.8	0.032±0.013	7.0±3.0	0.059±0.032	10.3±5.2	0.074±0.035	8.9±3.8	0.004±0.005	1.6±2.4
4	85	0.019±0.014	3.3±2.4	0.036±0.013	7.4±2.8	0.056±0.034	9.6±6.6	0.084±0.032	10.1±3.8	0.006±0.006	1.9±1.8
5	121	0.018±0.014	2.8±2.3	0.033±0.016	6.0±2.8	0.040±0.024	7.2±5.2	0.101±0.035	10.1±3.9	0.006±0.007	1.6±2.2
6	77	0.023±0.018	4.4±3.3	0.031±0.011	7.5±3.3	0.045±0.027	10.6±10.2	0.082±0.031	11.1±4.6	0.006±0.006	1.9±1.8

B. Non-Smokers

		Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
Gen	# µgrphs	V_D	#	V_D	#	V_D	#	V_D	#	V_D	#
3	47	0.018±0.015	2.7±2.5	0.038±0.012	7.2±2.2	0.056±0.032	9.1±4.7	0.087±0.029	9.1±2.7	0.004±0.004	1.1±1.2
4	81	0.017±0.016	2.8±2.8	0.041±0.016	7.1±2.4	0.046±0.028	7.7±4.9	0.091±0.033	9.8±3.5	0.002±0.005	0.8±1.1
5	55	0.018±0.014	3.5±2.4	0.037±0.014	7.9±3.5	0.051±0.024	11.3±6.4	0.097±0.035	12.8±4.8	0.004±0.005	1.1±1.0
6	42	0.019±0.015	3.6±2.9	0.034±0.013	8.1±3.2	0.050±0.027	10.9±5.7	0.088±0.032	12.4±3.6	0.005±0.005	1.7±1.6

C. Ex-Smokers

		Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
Gen	# µgrphs	V_D	#	V_D	#	V_D	#	V_D	#	V_D	#
3											
4	25	0.029±0.016	5.6±3.1	0.036±0.013	7.3±2.3	0.045±0.020	7.8±3.1	0.062±0.027	7.2±3.5	0.003±0.004	1.1±1.6
5	38	0.018±0.015	3.4±2.9	0.036±0.019	8.6±3.9	0.042±0.023	8.4±5.3	0.086±0.029	12.3±4.2	0.003±0.003	0.9±1.1
6	18	0.015±0.019	2.7±3.2	0.034±0.015	7.3±2.6	0.044±0.028	8.5±5.1	0.081±0.033	8.1±3.4	0.001±0.003	0.6±0.8

Table 3. This summarizes the data from COSAS on the volume density (V_D) of the nuclei of airway cell types (mucous, basal, intermediate/indeterminate, ciliated, and intraepithelial white blood cells) and the average numbers of nuclei of each type per micrograph. The data are presented for four bronchial generations and smokers, non-smokers, and ex-smokers are compared.

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TABLE 4

Number and Volume Density of Dog Bronchial Epithelial Cell Nuclei by Airway Generation

		Mucous Cells		Basal Cells		Indet/Intermed Cells		Ciliated Cells		White Blood Cells	
Gen	# ugrphs	V_D	#	V_D	#	V_D	#	V_D	#	V_D	#
2	28	0.020±0.014	2.43±1.14	0.041±0.013	8.89±2.95	0.044±0.028	9.00±7.11	0.089±0.031	9.85±3.50	0.005±0.006	1.50±1.71
3	66	0.021±0.016	2.77±1.92	0.039±0.013	7.86±2.25	0.042±0.029	6.29±3.56	0.120±0.041	12.29±3.59	0.003±0.004	0.86±1.64
4	87	0.035±0.017	4.41±2.25	0.038±0.012	7.55±2.78	0.035±0.021	6.17±4.36	0.125±0.042	13.16±4.05	0.004±0.007	0.93±1.50
4,5	18	0.034±0.011	4.66±2.11	0.032±0.013	6.16±2.17	0.028±0.016	4.38±3.18	0.124±0.028	13.33±4.86	0.004±0.006	1.05±1.89
5	74	0.039±0.018	5.54±2.63	0.034±0.010	7.92±2.75	0.033±0.022	6.11±4.08	0.117±0.031	14.81±5.16	0.002±0.003	0.43±0.79
5,6	7	0.030±0.014	5.86±2.67	0.036±0.016	7.43±3.05	0.033±0.019	6.57±4.12	0.141±0.019	17.43±2.64	0.003±0.003	0.57±0.53
6	48	0.032±0.015	5.06±2.71	0.036±0.012	7.79±2.85	0.027±0.016	5.31±2.82	0.119±0.033	15.27±4.46	0.002±0.003	0.56±0.97

Table 4. Summary of the data from COSAS on the volume density (V_D) of the nuclei of airway cell types (mucous, basal, intermediate/indeterminate, ciliated, and intraepithelial white blood cells) and the average numbers of nuclei of each type per micrograph. The data are presented for 5 generations as well as 2 branch points (generations 4 to 5 and 5 to 6).

Total of 328 dog micrographs entered and analyzed.

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