are consistent with our conclusion that the present fluxes of tin through the atmosphere and surface ocean are dominated by anthropogenic effects.

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Bronchial Hyperreactivity Associated with Tracheal Gangliosides

Abstract. *Gangliosides, which are membrane constituents of animal cells, may be altered under various conditions that cause change in metabolism. In this study, gangliosides from tracheal and lung tissues were extracted and measured as a function of bronchial hyperreactivity in a guinea pig model of bronchial asthma. When plotted logarithmically, the data showed that tracheal gangliosides decreased with an increase in airway reactivity index, suggesting a linear relation between them. Differential analysis of tracheal gangliosides in the acute stage of bronchial hyperreactivity indicates accumulation of polysialogangliosides. These data support the hypothesis that tracheal gangliosides are intimately involved in the development of bronchial asthma.*

Bronchial asthma is characterized by increased responsiveness of the trachea and bronchi to various stimuli and is manifested by a narrowing of the airways. The severity of the disease may change either spontaneously or as a result of therapy (*1*). Nearly 7 percent of the population in the United States (*2*) and 1.5 percent in India have this disease. The etiology of asthma is unknown, but the lungs of asthmatic patients release histamine both in vitro (*3*) and in vivo (*4*) when challenged with an appropriate allergen, and the plasma histamine concentration is increased in spontaneously occurring asthmatic attacks (*5, 6*). Confirmation of the proposal by Ash and Schild (*7*) that at least two types of receptors (H1 and H2) are involved in the histamine response has caused a renewed interest in histamine in the pathogenesis of bronchial asthma (*8*). Because it is the airway muscle that is ultimately mobilized during anaphylactic bronchospasm, the present studies were designed to test the hypothesis that the defect in the guinea pig model of asthma may stem from a specific abnormality of a cell surface component of the airway smooth muscle.

The guinea pig model of allergic asthma was developed by Agrawal (*9*), who monitored airway reactivity to histamine. Agrawal recorded three parameters, that is, (i) airflow at nares, dV_2/dt; (ii) the difference between the change in chest cage volume and the air volume respired at the nares ($V_1 - V_2$); and (iii) the difference between the rate of change in chest cage volume and the airflow at the nares, $d(V_1 - V_2)/dt$ (Fig. 1). The airway reactivity index (ARI) is the ratio of the peak values of $d(V_1 - V_2)/dt$ after and before histamine inhalation. The peak values of $d(V_1 - V_2)/dV_2$ were calculated by dividing the magnitude of dV_2/dt waveform at the corresponding points.

Gangliosides, a subclass of glycosphingolipids, are constituents of the surface membrane of mammalian cells and may be altered under various conditions that cause changes in metabolism (*10, 11*). Moreover, gangliosides have receptors for tetanus toxin, interferon, Sendai virus, and cholera toxin (*12, 13*). They also appear to bind 5-hydroxytryptamine (*12, 14*).

In this study, total gangliosides from guinea pig tracheal tissue were extracted

Table 1. Glycosphingolipid composition (per gram of tissue, wet weight) of guinea pig trachea and lung.

Glycosphingolipids	Trachea		Lung	
	Normal	Hyperreactive	Normal	Hyperreactive
Gangliosides (nmole of sialic acid)	2422.62 ± 808.52 (8)	398.47 ± 38.41*(6)	71.91 ± 22.59 (6)	65.83 ± 17.15 (6)
Sphingomyelin (μg)	141.67 ± 29.15 (8)	115.03 ± 30.50 (6)	202.16 ± 17.84 (8)	210.17 ± 20.84 (7)
Cerebroside (μg)	268.40 ± 26.90 (8)	269.70 ± 40.10 (7)	21.46 ± 1.22 (7)	21.48 ± 4.33 (6)
Sulfatide (μg)	110.40 ± 25.50 (8)	132.40 ± 22.50 (7)	1.15 ± 0.09 (7)	1.42 ± 0.13 (6)

*$P < .001$. Animals with an ARI between 1.1 and 2.75 were grouped as normal and those between 3.0 and 5.38 were grouped as hyperreactive. Total lipids and then gangliosides were extracted from trachea and lung tissues as described in Fig. 1. After removal of the upper phase lipid for gangliosides, the lower phase was dried under a stream of N_2. Ten to fifteen milligrams of lower phase lipid was dissolved in 1 ml of 0.5*N* methanolic sodium hydroxide. The solution was allowed to stand for 4 hours at room temperature, then 1 ml of 1.5*N* methanolic hydrochloric acid was added and the solution was mixed thoroughly and left for 30 minutes. To this acidified solution, 3 ml of chloroform and 1 ml of distilled water were added and mixed. The upper aqueous phase was discarded, the lower phase was washed twice with methanol and water, 1:1 by volume (1 ml). Sphingomyelin was separated on silica gel H in chloroform, methanol, and water (60:25:4 by volume) and the lipid phosphorus was determined (*22*) [micrograms of phosphorous × 25 = micrograms of sphingomyelin (*23*)]. The cerebrosides and sulfatides were isolated by column chromatography with a combination of Florisil and DEAE-cellulose (acetate) (*24*). The results are expressed as means ± standard error. The significance of the results is analyzed by Student's *t*-test. The number of animals used in each experiment is given in parentheses.

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Table 2. Differential analysis of gangliosides.

Ganglioside	Trachea		Lung	
	Normal	Hyperreactive	Normal	Hyperreactive
GM_3	47.3 ± 1.8	9.7 ± 0.5	14.2 ± 1.4	10.6 ± 0.3
GM_2	20.8 ± 0.5	14.8 ± 0.7	68.2 ± 1.1	35.7 ± 1.2
GM_1	29.9 ± 1.0	13.1 ± 0.5	6.4 ± 0.4	21.2 ± 1.1
GD_{1a}	1.9 ± 0.2	2.3 ± 0.2	4.9 ± 0.2	14.4 ± 0.7
GD_{1b}	N.D.	6.4 ± 0.6	6.3 ± 0.2	8.3 ± 0.9
GT_1	N.D.	53.7 ± 1.0	N.D.	8.3 ± 0.5

A solution of gangliosides containing 60 to 80 nmole of NeuAc from animals with an ARI of 1.1 (normal) and 5.38 (hyperreactive) were applied to silica gel G plates (250 μm thick) and developed in a solvent system consisting of chloroform, methanol, and 2.5*N* ammonium hydroxide (60:40:9 by volume) (*25*). Bovine brain gangliosides were used as markers. After they were air-dried at room temperature the spots corresponding to the authentic samples were scraped off and the sialic acid was assayed after acid hydrolysis at 80°C for 1 hour. Each ganglioside fraction was further analyzed with respect to their carbohydrate residue. The results are expressed as percentages of total sialic acid (mean ± standard error) of three determinations. The nomenclature of the gangliosides is according to Svennerholm (*26*). N.D., not detected.

and quantitated. When measured as a function of the ARI, total gangliosides decreased with increasing ARI (Fig. 2). A theoretical curve for this decrease fits very well with the experimental data. Moreover, the slope (0.464 ± 0.081) calculated from the straight line in log-log scale presents a highly significant correlation ($P < .0001$). This suggests that there is a linear relation between the tracheal gangliosides and airway reactivity (Fig. 2, inset). Similar results were obtained from a double-blind study.

Since bronchial hyperreactivity might be related to the tracheal gangliosides alone or to other glycosphingolipids as well, the glycosphingolipids from tracheal and lung tissues were analyzed and compared. The lipid content of both tracheal and lung tissues was increased by 53 and 41 percent, respectively, in hyperreactive animals. But whereas there was an overall reduction in glycosphingolipids in the tracheal tissue of hyperreactive guinea pigs, lung tissue showed an insignificant increase in glycosphingolipids. Since total phospholipids in trachea are reduced in the hyperreactive state (*15*), the increase in total lipid in both tissues might be due to higher neutral lipids. As shown in Table 1, the concentration of gangliosides was reduced to one-sixth of the control in tracheal tissue whereas sphingomyelin was lowered by only 19 percent. The concentration of sulfatide was increased by 20 percent in hyperreactive trachea with no change in cerebroside. The glycosphingolipid content of the lung tissue was low, with the possible exception of sphingomyelin. The glycosphingolipids are generally high in membrane organelles or organs where transport activities are also high (*16*). Apart from gangliosides, sulfatides are believed to be involved in regulating the adenosenetriphosphatase activity in tissues (*17*). The 1.5- to 2-fold increase in Na^+- and Ca^{2+}-dependent adenosinetriphosphatase activities in hyperreactive trachea (*15*) is in agreement with the increased sulfatide level found here that supports the earlier observation (*17*).

The low level of gangliosides in the trachea of hyperreactive animals raised a question about its distribution among various mono-, di-, and trisialogangliosides. The gangliosides from normal and hyperreactive animals were therefore separated by thin-layer chromatography on silica gel G and subjected to differential analysis. The results (Table 2) indicated that the concentration of monosialogangliosides decreased with a concomitant increase in polysialogangliosides, that is, GD_{1b}, and GT_1, in the tracheal tissue of hyperreactive guinea pigs. The total polysialogangliosides were increased by 32-fold in the case of bronchial hyperreactivity. Among the monosialogangliosides, GM_3 was most reduced, leaving GM_1 and GM_2 in the range of 44 and 71 percent, respectively. The pattern of gangliosides in lung tissue was little different. The normal pattern of distribution in lung tissue is $GM_2 > GM_3 > GM_1 > GD_{1b} > GD_{1a} > GT_1$, which changed to $GM_2 > GM_1 > GD_{1a} > GM_3 > GD_{1b}$, and GT_1 in hyperreactive animals. The quantitative consideration of the data indicated that the concentration of GM_2 became one-half of the control, whereas that of GM_1, GD_{1a}, and GT_1 increased by 3.5- to 8-fold in the hyperreactive state.

There are four mechanisms by which gangliosides might participate in bronchoconstriction:

1) There might be an altered ganglioside pattern and a high level of polysialogangliosides in tracheal tissue under the hyperreactive state. The presence of *N*-acetylneuraminic acid (sialic acid, NeuAc) in gangliosides contributes to the negative charge carried by these molecules and to their ability to form a complex with histamine. The binding of histamine to gangliosides is primarily ionic and the amount of histamine bound per mole of gangliosides depends on the number of sialic acid residues in these molecules (*18*). But the role of gangliosides as natural receptors of amines is

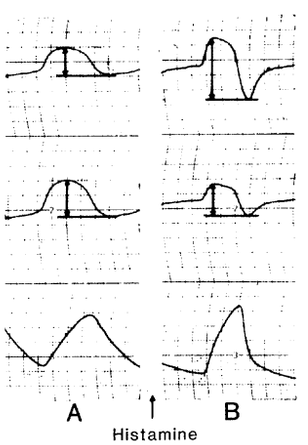

Fig. 1. Determination of the ARI. Male guinea pigs weighing 200 to 500 g were screened for their airway reactivity by a modified noninvasive technique with a whole-body plethysmograph (*9*). Histamine diphosphate (5 mg/ml, as base) was administered for 4 seconds per 100 g of body weight at a flow rate of 2 liters per minute. (A) Before and (B) after histamine inhalation. The calibration scale is omitted because it is not needed for calculating the ARI.

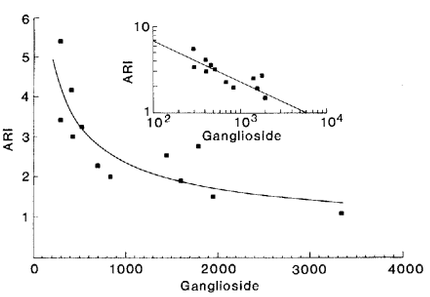

Fig. 2. The relation between tracheal gangliosides and ARI. Guinea pigs with a known ARI were anesthetized by an intraperitoneal injection of Nembutal. From each animal the trachea was quickly removed, freed of adhering tissues, and weighed. The total gangliosides were extracted according to the modified procedure of Suzuki (*27*). The gangliosides are assayed by measuring *N*-acetylneuraminic acid (NeuAc) after acid hydrolysis at 80°C according to the procedure of Warren (*28*) as modified by Saifer and Gerstenfeld (*29*). The ARI is plotted as a function of ganglioside content (g): ■, observed value; —, value predicted by using the expression $ARI = 58.2 \div g^{0.464}$. The inset shows the log of ARI plotted as a function of the log of g and the straight line fitted to the data by the method of least squares; the equation of the line is $\log(ARI) = 1.765 - 0.464 \log(g)$. The slope of the line [0.464 ± 0.081 (±standard error)] is significantly different from zero ($P < .0001$).

doubtful (*12*), particularly in view of their relatively low association constant (2.9×10 liter/mole) calculated from binding studies at equilibrium in vitro (*18*). When histamine is increased as a result of its being released from mast cells or inhaled, it binds to the cell surface and causes an increase in Ca^{2+} influx, which facilitates smooth muscle contraction. An increased capacity for exchange of cellular Ca^{2+} with La^{3+} was observed in cultured heart cells after the specific removal of sialic acid from the cell surfaces with purified neuraminidase (*19*). Moreover, from nuclear magnetic resonance studies, Jaques *et al.* (*20*) concluded that sialic acid forms a complex with Ca^{2+} in the proportion of 1:1 at neutral *p*H. It is reasonable, therefore, to assume that at least one molecule each of histamine and Ca^{2+} can bind per molecule of disialoganglioside, but the ratio may change in the case of trisialogangliosides. It is also possible that Ca^{2+} acts as bridge in the binding process.

2) The change in ganglioside sequence from $GM_3 > GM_1 > GM_2 > GD_{1a} > GD_{1b}$ and GT_1 to $GT_1 > GM_2 > GM_1 > GM_3 > GD_{1b} > GD_{1a}$ in the hyperreactive trachea might result from a defect in the degradation which could be due to decreased enzyme secretion. Earlier observations indicate that when either neutrophils or granulocytes from patients with active atopic eczema are incubated with histamine, the release of the lysosomal enzyme β-glucuronidase is inhibited (*21*).

3) Lung tissue might contribute to bronchoconstriction; however, analysis of the glycosphingolipids of lung tissue indicates that, other than releasing histamine from the mast cells, the contribution of the lung to bronchial hyperreactivity is almost negligeable. Further, Agrawal's data show that $d(V_1 - V_2)/dV_2$ is independent of lung volume because the larger the lung, the larger will be the value of $d(V_1 - V_2)$ for a given change in alveolar pressure and the larger will be the amount of air respired (dV_2) (*9*).

4) Although inhalation of histamine causes bronchoconstriction in susceptible animals, the altered ganglioside pattern is probably not due to the histamine because in each experiment the animals were killed after a 4-day recovery period. Moreover, 8 to 10 percent of guinea pigs have low concentrations of gangliosides in the tracheal tissue.

Clearly, this relation between the levels of gangliosides and bronchoconstriction offers a new approach for studying the mechanisms involved in the coupling of histamine release and bronchoconstriction and has important clinical implications.

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A New Subtype of Human T-Cell Leukemia Virus (HTLV-II) Associated with a T-Cell Variant of Hairy Cell Leukemia

Abstract. *Human T-cell leukemia virus (HTLV) is a human type-C RNA tumor virus (retrovirus) previously identified in and isolated from several patients with T-cell leukemias or lymphomas. The known virus isolates from the United States and Japan are closely related and are found in adults with an acute malignancy of mature T cells. A related retrovirus has been found in a patient (Mo) with a somewhat different disease (a T-cell variant of relatively benign hairy cell leukemia). Serum from Mo contains antibodies to the major internal core protein (p24) of HTLV. A T-cell line established from the spleen of Mo expresses HTLV antigens. However, HTLV from Mo is significantly different from all previous HTLV isolates in immunological cross-reactivity tests of p24. The usual prototype HTLV isolate is represented as HTLV-I, and the HTLV from Mo is represented as HTLV-II. Individual members of each subgroup may then be identified by subscript initials of the patient [for example, HTLV-$I_{(CR)}$, HTLV-$I_{(MB)}$, and HTLV-$II_{(Mo)}$].*

A novel human retrovirus (RNA tumor virus) designated human T-cell leukemia virus (HTLV) was first isolated from some cases in the United States of human leukemia and lymphoma involving relatively mature T cells (*1, 2*). Several additional isolates of HTLV were subsequently identified in other cases from the United States, England, and elsewhere (*3–6*), and recent evidence has shown that HTLV is endemic in certain geographical areas (*5*). HTLV is different from all the animal retroviruses isolated by comparisons of viral nucleic acids by nucleic acid hybridization (*7*), by immunological analyses of the structural proteins (*8, 9*) and of reverse transcriptase (*10*), and by amino acid sequencing of structural proteins (*11*). That HTLV is acquired by infection is suggested by the findings of high titer antibodies to HTLV structural proteins in serums of patients and in some normal people (*12–16*) and is conclusively shown by the finding of proviral sequences in the DNA of neoplastic T cells, but not in various normal human cells (*7, 17*). HTLV sequences were also absent in normal B cells from

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