bulk-isolated cell preparations (*12*). Our results now clearly demonstrate that in genetic galactosylceramidase deficiency, rapidly progressive accumulation of psychosine in white matter occurs from very early stages of the disease. This finding establishes step ii of the psychosine hypothesis.

Although the fundamental genetic defects of most of the lysosomal storage disorders have been clarified, the molecular mechanisms that lead to the clinical and pathological manifestations remain largely obscure. While the psychosine hypothesis has by no means been proven, evidence accumulated in the past dozen years since its proposal has made it a probable explanation for the biochemical pathogenesis of globoid cell leukodystrophy. Similar mechanisms may play important roles in the pathogenesis of other lysosomal storage diseases. In an analogous disease, Gaucher disease, which is caused by a genetic defect of glucosylceramidase, presence of glucosylsphingosine in the spleen has been shown (*13*), and more recent data suggested a correlation between the amount of glucosylsphingosine in the brain of patients and the degree of neurological involvement (*14*). In other diseases also, abnormal accumulation of "normal" constituents may not be sufficient to account for clinical and pathological manifestations. Attention to possible involvement of "abnormal" constituents may prove rewarding.

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Is α_1-Protease Inhibitor Inactivated by Smoking?

Stone *et al.* (*1*) report that functional α_1-protease inhibitor (α_1-PI) in the lower respiratory tract is not decreased by cigarette smoking, whereas Gadek *et al.* (*2*) and Carp *et al.* (*3*) found decreased functional α_1-PI in the lower respiratory tract of smokers. Carp also found 4 mole of methionine sulfoxide per mole of inactive α_1-PI in lung lavage fluids of smokers but oxidized methionine was not found in α_1-PI of nonsmokers (*3*). Oxidation of two to four methionine residues in α_1-PI has been associated with loss of its inhibitory activity toward human neutrophil elastase (*4, 5*). The apparent contradictions between Stone's observations and the above findings are perplexing and suggest that further studies are needed. In future experiments, it may be helpful if the following points are borne in mind.

Ideally, focal areas of centrilobular emphysema would be sampled, rather than whole lung. This being impractical, some effort should at least be made to lavage superior segments of the lung, since lesions of centrilobular emphysema in smokers are more common and more severe in the upper than in the lower zones of the lung (*6*). Also, subject selection may be critical; heavier smokers of unfiltered high-tar cigarettes should be preferred, especially those with signs of airflow limitation.

Furthermore, as mentioned by Stone *et al.* in their report, the time interval between smoking and lavage may be an important variable. One of us (S.K.C.) recently explored the time course of lung α_1-PI inactivation in mice after short-term and long-term exposure to smoke. Inactivation of α_1-PI occurred much sooner after a standard smoke exposure if the animals had been repeatedly exposed to smoke beforehand. Recovery of normal values of lung α_1-PI activity after the standard smoke exposure also occurred earlier in mice that inhaled smoke frequently than in mice never previously challenged with smoke. Despite this, the extent of α_1-PI inactivation was the same or greater in the group repeatedly exposed to smoke. Although these results were obtained in an animal model and their direct extrapolation to humans may be unwarranted, humans who are heavy smokers could also have significant decreases in lung α_1-PI activity immediately after smoking, but these decreases may be transient.

We agree with Stone *et al.* that "additional studies of the effects of cigarette smoke on α_1-PI in the lower respiratory tract are needed" and look forward to an eventual resolution of this controversy.

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Stone *et al.* (*1*) recently reported that functional α_1-protease inhibitor (α_1-PI) in the bronchoalveolar lavage fluid of smokers is not decreased. They cited our study (*2*) in which we assayed α_1-PI in lung fluid from young smokers who were subjected to bronchoalveolar lavage before smoking, and again 10 to 60 minutes after smoking. We later studied another 18 smokers and found no significant decrease in α_1-PI activity in lung fluids of smokers after smoking, except in smokers who were subjected to lavage 1 hour after smoking two cigarettes; these smokers showed a statistically significant mean decrease of 10 percent in α_1-PI activity compared with the values before smoking.

Stone *et al.* reported that α_1-PI in the lung fluids of nonsmokers was only 47 percent active, whereas the data of Carp *et al.* (*3*) indicated a mean activity of 118 percent; we found it about 90 percent active. We had initially reported (*2*) that

α_1-PI in nonsmokers was 54 percent active; however, when we repeated the immunological quantitation with a purified α_1-PI preparation (*4*), we found that the commercial α_1-PI plasma standard used had overestimated α_1-PI, resulting in an apparent decrease in activity expressed as percent of immunological α_1-PI. Jeppson *et al.* (*5*) pointed out that published values of normal plasma α_1-PI varied widely because of lack of uniform standards; their mean plasma concentration in healthy male subjects was 132 mg/100 ml, 40 percent below the value often quoted in the literature. The difference in α_1-PI activity in nonsmokers' lung lavage fluids may be related to the use of different standards for immunological quantitation; this issue could be resolved if standards used in different laboratories were checked against each other.

Gadek *et al.* (*6*) and Carp *et al.* (*3*) found that α_1-PI activity in smokers was decreased to about 60 percent of the activity in nonsmokers; Stone *et al.* (*1*) did not find that α_1-PI activity in smokers was decreased in comparison with values in nonsmokers; and we found α_1-PI activity was slightly decreased in some smokers.

Perhaps the differences between these results may arise in part from differences in the characteristics of the smokers studied (such as total cigarette consumption and the presence or absence of respiratory abnormalities). The differences could also be due to differences in the timing and intensity of smoking before lavage or may occur because variations in the techniques of bronchoalveolar lavage result in sampling differences. Further studies are needed to resolve this controversy.

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We agree with Janoff and Chan that the apparent contradictions between our study (*1*) and those of Gadek *et al.* (*2*) and Carp *et al.* (*3*) are perplexing. We are unable to pinpoint differences in the studies which might have accounted for the different findings. For example, in our study (*1*), Gadek's study (*2*) and, possibly, the study by Carp *et al.* (*3*) a middle lobe of the lung was lavaged. The time interval between cigarette smoking and lavaging was not explicitly stated in the two earlier studies (*2, 3*). In our study, in which lavage fluid from two cigarette smokers was obtained within 15 minutes after each had smoked one cigarette, we did not find evidence for functionally inactive alpha-1-protease inhibitor (α_1-PI).

Janoff and Chan propose that further studies be done using an experimental design that will maximize the chances of finding smoke inactivation of α_1-PI in the lungs. Such inactivation might be found only in microenvironments [as we suggested (*1*)] and be diluted beyond detectable limits in the lavage fluid. Inactivation might be more prevalent in upper lobes of the lung or in heavy smokers of unfiltered tar cigarettes, as suggested by Janoff and Chan. It is possible that the relative difficulty in detecting inactive α_1-PI may be related to the observation that most smokers apparently escape debilitating emphysema during their lifetimes (*4*).

With regard to the comments by Abboud and his co-workers on our immunological quantitation of α_1-PI, we double-checked the commercial α_1-PI serum standard against purified α_1-PI, the concentration of which had been independently confirmed by measuring its absorbancy at 280 nm (5.2 for a 1 percent solution). In a binding assay with ^{125}I-labeled human neutrophil elastase the purified α_1-PI showed 54 ± 4 percent (mean ± standard error, $N = 3$) functional activity (unpublished data). Addition of 2 mg of bovine serum albumin to the 0.00212 mg of α_1-PI used in each binding assay resulted in functional activity of 66 ± 6 percent ($N = 2$). We noted in the discussion of Table 2 of our report (*1*) that two other groups (*5, 6*) studied purified human α_1-PI and found 57 and 59 percent functional activity, respectively.

In summary, three questions addressed in these comments remain to be answered: (i) Do smokers generally have lower levels of functional α_1-PI in their lower respiratory tracts than nonsmokers? (ii) If not, does such inactivation of α_1-PI occur in vivo under narrowly defined circumstances, as outlined above? (iii) What is the functional activity of pure α_1-PI and α_1-PI in lavage fluid toward elastase?

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