

Correspondence



The DNA-Repair Gene *MGMT* and the Clinical Response of Gliomas to Alkylating Agents

To the Editor: Esteller and colleagues (Nov. 9 issue)¹ claim that methylation of the O⁶-methylguanine-DNA methyltransferase (*MGMT*) promoter is associated with improved responsiveness to carmustine and prolonged survival in patients with high-grade gliomas. We believe there are methodologic flaws in the authors' study that call these results into question. First, the patients in the study were treated with a combination of surgery, radiation therapy, and chemotherapy with cisplatin and carmustine. We do not believe the response to carmustine is a discernible end point in this trial, because multiple treatments were administered along with carmustine.

Second, although the difference in age between the group of patients with unmethylated tumors and the group with methylated tumors was not statistically different, there was a trend toward an older age in the group with unmethylated tumors. We are not given the distribution of ages above or below 50 years. Since the prognosis worsens with each additional decade of age, such information would be useful in assessing the balance in age between the two groups.^{2,3}

Third, the median period of survival was approximately 20 months for the patients with unmethylated tumors and approximately 30 months for those with methylated tumors, as compared with an average of about 12 months in other series.^{2,3} Moreover, since no deaths occurred before 12 months, it is unlikely that the patients chosen for this study were representative of most patients with the disease. Finally, the small numbers of deaths in the two groups of patients make the results of a statistical comparison questionable.

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1. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene *MGMT* and the clinical response of gliomas to alkylating agents. *N Engl J Med* 2000;343:1350-4.
2. Nelson DF, Diener-West M, Horton J, Chang CH, Shoenfeld D, Nelson JS. Combined modality approach to treatment of malignant gliomas — re-evaluation of RTOG 7401/ECOG 1374 with long-term follow-up. In: National Cancer Institute monographs. No. 6. Bethesda, Md.: Department of Health and Human Services, 1988:279-84.
3. Dinapoli RP, Brown LD, Arusell RM, et al. Phase III comparative evaluation of PCNU and carmustine combined with radiation therapy for high-grade glioma. *J Clin Oncol* 1993;11:1316-21.

To the Editor: Esteller et al. describe differences in outcome based on the methylation status of the *MGMT* promoter in adults with high-grade gliomas who were treated with multimodal therapy incorporating the alkylating agent carmustine. Methylation of the *MGMT* promoter predicted an improved outcome with such therapy. This finding is potentially very important. However, neither the authors of the report nor Weinstein, in the accompanying editorial,¹ raise the question of whether methylation of the *MGMT* promoter is predictive of the outcome for patients who are not given chemotherapy as part of their primary treatment. Data from a study involving a sizable cohort suggest that the status of the *MGMT* promoter is not prognostic in patients treated with regimens that do not incorporate carmustine.² For the findings of Esteller et al. to be placed in context, not only will their study have to be replicated, but the absence of a prognostic effect in patients not given alkylating agents will also have to be confirmed.

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To the Editor: In a study of 47 patients with malignant gliomas, Esteller and colleagues report a positive correlation between inactivation of *MGMT* — by virtue of gene-promoter methylation — and survival. All the patients had undergone surgery before they received radiotherapy and chemotherapy, including carmustine. However, magnetic

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resonance imaging (MRI) or computed tomography was not performed immediately after surgery to determine the residual tumor volume, despite the well-known effect of the residual tumor volume on the prognosis.¹ Therefore, we are left with some uncertainty, because the results may have been biased by differences in the tumor volumes before the initiation of adjuvant chemotherapy.

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1. Albert FK, Forsting M, Sartor K, Adams HP, Kunse S. Early postoperative magnetic resonance imaging after resection of malignant glioma: objective evaluation of residual tumor and its influence on regrowth and prognosis. *Neurosurgery* 1994;34:45-60.

To the Editor: The methyl-excision-repair (MER)-negative phenotype, manifested by absent or decreased tumor MGMT, has been associated with a longer period of disease-free survival among patients with gliomas who are treated with carmustine. However, Esteller et al. provide definitive evidence of a correlation between survival after carmustine therapy and MGMT methylation. Their findings are thus important and provide the basis for an assay with potentially clinical value. We would, however, like to make the following comments about their report.

First, contrary to the statement by Esteller et al., the most frequent site of DNA base alkylation by monofunctional and bifunctional nitrosoureas and related alkylating agents, such as temozolomide and procarbazine, is not the O⁶-position of guanine but rather the N⁷ position of guanine and the N³ position of adenine. Second, MGMT does not repair the DNA interstrand cross-links resulting from the O⁶-chloroethylguanine adducts of nitrosoureas, as suggested in Figure 1 of the article by Esteller et al. Furthermore, in that figure, the DNA interstrand cross-link produced by carmustine is incorrectly depicted as a diguanyl cross-link. It is actually an N¹-deoxyguanosinyl-N³-deoxycytidyl cross-link.¹ Its correct structure is shown in Figure 1 here.

Finally, in the absence of methylation of the MGMT gene, translational² and post-translational³ processes can alter MGMT levels and MGMT functional activity in tumors and may confound the interpretation of the methylation status of the MGMT gene. We suggest that any clinical assay based on the findings reported by Esteller et al. be validated before it is used widely in selecting patients for chemotherapy.

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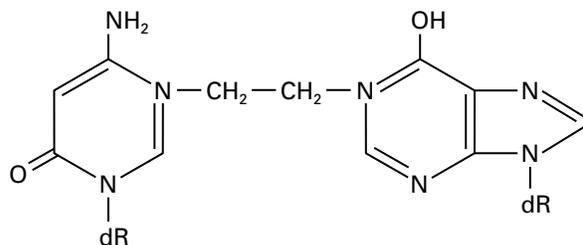


Figure 1. DNA Interstrand Cross-Link Formed by Carmustine, Lomustine, and Other Bifunctional Nitrosoureas. The abbreviation dR denotes deoxyribose.

in human and murine tumor cells following inactivation with O⁶-benzylguanine or 1,3-bis(2chloroethyl)-1-nitrosourea. *Biochemistry* 1996;35:1328-34.

The authors reply:

To the Editor: Dr. Quinn correctly points out that improvements in the response to treatment and survival cannot be definitively attributed to carmustine without a comparison with no treatment. The study Quinn cites showed no relation between MGMT enzyme activity and survival among patients not given chemotherapy, but it also failed to show such a relation among patients receiving chemotherapy.¹ Previous studies demonstrated a direct relation between MGMT expression in glioma cell lines and a response to the alkylating agent nimustine (ACNU), but not other chemotherapeutic agents.² Other work we have done suggests that MGMT inactivation predicts prolonged survival in patients with lymphoma who are treated with an alkylating agent (unpublished data) but not in patients with colorectal cancer who do not receive an alkylating agent (unpublished data).

Drs. Buckner and Moynihan raise several questions. Although there was a slight imbalance between the two groups in the number of patients who were over 50 years old, the age distribution did not differ statistically. In a univariate analysis, age was minimally associated with progression-free survival (hazard ratio for the risk of progression, 0.99) and overall survival (hazard ratio for the risk of death, 0.92); the associations were not statistically significant. Most important, the association of MGMT methylation with overall and progression-free survival was independent of age, as indicated in the legend to Figure 3 of our article. Differences in survival between our study and others may be due to differences in treatment regimens, performance status, and tumor grade (with a higher prevalence of grade 3 tumors in our study); however, these differences do not change the conclusions of our study. Our statistical analysis took into account the size of the sample.

We regret that in our article we did not clearly state that we obtained MRI scans for all patients after surgery in order to provide a base line for evaluating the response to treatment, and we thank Dr. Schlegel for allowing us to make this clarification. We thank Ali-Osman et al. for clarifying issues related to the chemistry of alkylating agents. Although other sites of DNA base alkylation may be more frequent, the O⁶ position appears to be most important for sensitivity to alkylating agents and the adduct most closely related

to *MGMT* expression.³ Although *MGMT* does not repair cross-links, it prevents their formation by the removal of alkyl groups. Finally, translational and post-translational changes in *MGMT* that were not determined by examination of promoter-region methylation would be relevant only in the tumors with unmethylated *MGMT* promoters, which transcribe the gene. Such changes, if they had been present, would not have led to the observed association.

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1. Silber JR, Blank A, Bobola MS, Ghatan S, Kolstoe DD, Berger MS. O6-methylguanine-DNA methyltransferase-deficient phenotype in human gliomas: frequency and time to tumor progression after alkylating agent-based chemotherapy. *Clin Cancer Res* 1999;5:807-14.
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3. Bignami M, O'Driscoll M, Aquilina G, Karran P. Unmasking a killer: DNA O(6)-methylguanine and the cytotoxicity of methylating agents. *Mutat Res* 2000;462:71-82.

The editorialist replies:

To the Editor: Dr. Quinn is correct when he points out that the value of the findings reported by Esteller et al. would be enhanced by comparison with data from a group of patients with gliomas who were not treated with carmustine (or other alkylating agents). If methylation of the *MGMT* promoter region in tumor samples from such patients were not correlated with improved overall and disease-free survival, that finding would strongly support the hypothesis of a causal relation between the activity of carmustine and the methylation. Going one step further, a survey of the methylation status of other promoter regions in the glioma samples analyzed by Esteller et al. would indicate whether the putative relation with methylation was specific to the *MGMT* promoter. Nonetheless, the authors' principal conclusion that "methylation of the *MGMT* promoter in gliomas is a useful predictor of the responsiveness of the tumors to alkylating agents" stands without such additional studies. One could argue that the term "alkylating agents" is too broad, since the data are only for the nitrosourea carmustine, but the data do identify a "useful predictor" if one assumes (on the basis of the clinical course and timing) that the observed responses were actually due to the treatment with carmustine. Dr. Quinn's critique illustrates the difficulty of establishing pharmacogenomic causality, especially in the clinical setting.

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Markers of Myocardial Damage and Inflammation in Unstable Coronary Artery Disease

To the Editor: Lindahl and colleagues (Oct. 19 issue)¹ report that elevated levels of troponin T and C-reactive pro-

tein are predictors of the long-term risk of death from cardiac causes in patients with unstable coronary artery disease. They specify the use of cardiac medications at admission in Table 1 but do not mention any concurrent treatment with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins). The statins do more than just lower cholesterol levels.² Recent evidence demonstrates that they appear to be potent and effective cardioprotective agents that inhibit leukocyte-endothelial cell interactions, possibly through enhanced endothelial release of nitric oxide,³ which itself has been shown to have a cardioprotective role in ischemia-reperfusion injury.⁴

We would appreciate it if the authors could provide the details of such treatment, in view of the potential impact of their findings on future preventive strategies to reduce long-term mortality in patients with coronary artery disease.

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4. Jones SP, Girod WG, Palazzo AJ, et al. Myocardial ischemia-reperfusion injury is exacerbated in absence of endothelial cell nitric oxide synthase. *Am J Physiol* 1999;276:H1567-H1573.

The authors reply:

To the Editor: Drs. Engelhardt and Cuthbertson point out that statins might have other cardioprotective effects besides lowering cholesterol levels — for example, anti-inflammatory effects — and request information about the use of statins in our study. We agree that this information would have been valuable, especially since it has been shown that long-term treatment with statins decreases inflammatory activity as measured by C-reactive protein levels¹ and that statin treatment might decrease the risk associated with elevation of C-reactive protein.² However, the use of statins at admission or during follow-up was not included in the case-report form in our study.

The study was begun in April 1992, before the results of the first large-scale trial of statins were presented.³ At that time, treatment with statins was very uncommon in clinical practice in Sweden. Even in 1995 and 1996, only 9 percent and 17 percent, respectively, of patients with myocardial infarction in Sweden were receiving statins at discharge (data from the Swedish register of cardiac intensive care). Therefore, one can assume that the influence of treatment with statins on the results of the present study was quite limited. Nevertheless, since the use of statins is now part of the standard treatment for coronary artery disease, the question of whether treatment with statins will influence the predictive value of markers of myocardial damage and inflammation is important. We hope to be able to answer that question as soon as we have the results of the Fragmin during Instability in Coronary Artery Disease inflammation

substudy (FRISC II), which will include detailed information about statin use, inflammatory markers, markers of myocardial damage, and long-term outcome.

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- Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. *Circulation* 1999;100:230-5.
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Adjuvant Chemotherapy for Completely Resected Non-Small-Cell Lung Cancer

To the Editor: In a randomized trial, three quantities related to sample size must be defined so as to ensure small enough error rates to make the conclusions credible: the significance level (α), the power of a test for a particular alternative hypothesis ($1-\beta$), and the difference one wishes to detect (δ). Since only the P value (α) is specified in the study by Keller et al. on adjuvant chemotherapy for completely resected non-small-cell lung cancer (Oct. 26 issue),¹ the negative results could be due to the use of a sample that was insufficiently large to show benefit.

Keller et al. state that newer agents with substantial activity against non-small-cell lung cancer appear to offer no survival advantage, but we disagree. There is evidence of improved survival or increased time to progression among patients with advanced non-small-cell lung cancer who are treated with a combination of paclitaxel and cisplatin,² a combination of cisplatin and gemcitabine,³ or with a regimen of three drugs (cisplatin and mitomycin with either vindesine or ifosfamide),⁴ as compared with those treated with the older regimen of cisplatin and etoposide. Furthermore, the conference abstract⁵ cited by Keller et al. does not show a comparison of survival according to study group and has not yet been published in a peer-reviewed medical journal.

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- Keller SM, Adak S, Wagner H, et al. A randomized trial of postoperative adjuvant therapy in patients with completely resected stage II or IIIa non-small-cell lung cancer. *N Engl J Med* 2000;343:1217-22.
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- Belani CP, Natale RB, Lee JS, et al. Randomized phase III trial comparing cisplatin/etoposide versus carboplatin/paclitaxel in advanced and metastatic non-small cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol* 1998;17:455a. abstract.

To the Editor: Keller et al. were unable to identify any survival advantage associated with postoperative chemotherapy in patients with completely resected stage II or III non-small-cell lung cancer. Although this was a well-designed phase 3 trial, the fact that a number of patients in the experimental group (those given chemotherapy and radiotherapy) did not receive adequate chemotherapy is an important limitation. Fourteen patients received no chemotherapy at all, and 160 patients "received all or part of the four cycles of chemotherapy." How many received only part, and how much of each cycle did they receive? We need to know more precisely the amount of chemotherapy delivered to the 246 patients assigned to chemotherapy plus radiotherapy, including the percentage of the doses planned that were actually delivered to the patients who received "part" of one, two, three, or four cycles.

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To the Editor: Keller et al. provide no information about how they established recurrence of disease, an important outcome of interest in this prospective investigation. Were participants screened for recurrence of disease in a systematic manner, or were evaluations symptom-driven and carried out at the discretion of the treating clinicians? Was a new radiographic abnormality sufficient to establish a diagnosis of recurrent disease, or was histopathological proof required?

The investigators' data show a trend toward a lower incidence of distant recurrence of disease, excluding the central nervous system, among participants treated with a combination of chemotherapy and radiotherapy (19 percent), as compared with participants treated with radiotherapy alone (23 percent) ($P=0.09$). Systematic prospective surveillance for distant recurrence of disease might have reduced noise in the measurement of this outcome and might have yielded a more accurate assessment of potential differences in the efficacy of the treatments. A rigorously defined strategy for detecting recurrence of disease, which is typically used in prospective trials of cancer treatments,¹⁻³ should have been delineated by the investigators.

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- Mayer R, Smolle-Juettner FM, Szolar D, et al. Postoperative radiotherapy in radically resected non-small cell lung cancer. *Chest* 1997;112:954-9. [Erratum, *Chest* 1998;113:564.]
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The authors reply:

To the Editor: Cortes et al. inquire about the trial's statistical power. The trial was designed to have 85 percent power to detect a 40 percent improvement in median survival with the use of a one-sided hypothesis test with a type I error of 5 percent. At a November 1998 meeting of the data-monitoring committee, when 71 percent of the information was available, the 90 percent repeated confidence interval for the hazard ratio, determined according to the method of Jennison and Turnbull,¹ was 0.76 to 1.29, which did not include the target alternative of 1.4. A 90 percent confidence interval was used because of the one-sided type I error of 5 percent in the design. Although early stopping in favor of the null hypothesis was not part of the original design of the trial, the data monitoring committee elected to release the results because there was strong evidence that any improvement in median survival due to adjuvant chemotherapy would be less than 40 percent. Cortes et al. also suggest that the results might have been different if a different drug regimen had been used, and they cite studies that demonstrate a survival advantage with newer drug regimens in advanced disease. However, substantial improvements in the survival of patients with completely resected stage II or IIIa non-small-cell lung cancer are not consistently achieved with newer regimens.^{2,3} Furthermore, the limited prolongation of survival in patients with advanced disease may not apply to patients receiving adjuvant chemotherapy.^{4,5} A randomized trial is required to demonstrate such a survival benefit.

Greco raises an important point pertaining to the adequacy of chemotherapy. Of the 246 patients assigned to receive chemotherapy, 69 percent received all four planned cycles. Common reasons for receiving less than the planned treatment included refusal by the patient (16 percent), toxic effects (8 percent), and progression of disease (3 percent). These figures are consistent with those in similarly designed trials. Survival advantages have been reported in randomized trials using fewer cycles of chemotherapy only when treatment was given before surgery.⁶ This suggests that differences in the effectiveness of treatment depend on the timing of the administration of chemotherapy relative to surgical intervention, rather than on the number of cycles delivered.

Shigemitsu and Kuschner inquire about follow-up procedures. All patients were followed systematically with physical examinations, screening chemistry, chest radiography, and computed tomographic scanning (including the upper abdomen) every three months for two years. Patients then had follow-up visits every six months for two to five years and annually thereafter. Between scheduled visits, patients returned as needed if they had symptoms, and the assessment was performed by the treating physician. Histologic proof of recurrence was requested whenever it was feasible

and safe. In other cases, the treating physician used clinical judgment.

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Mucosal Shedding of Human Herpesvirus 8

To the Editor: Transmission of human herpesvirus 8 (HHV-8) remains puzzling, despite the additional insights provided by Pauk et al. (Nov. 9 issue).¹ Whereas sexual transmission may occur, we endorse the authors' reasoning that oral transmission may be a more common route, although with a 15 percent frequency of salivary shedding, transmission must be somewhat difficult, since open-mouthed kissing between homosexual men is common. Open-mouthed kissing also is common in the heterosexual community, but the prevalence of HHV-8 in the general population is only 1 to 2 percent in the United States.^{2,3} Why?

Transmission is probably related not only to the presence of HHV-8 but also to the amount of infectious virus shed. HHV-8 levels could be low in HHV-8-infected persons who are not infected with the human immunodeficiency virus (HIV) and who are immunocompetent. This group would include most heterosexual persons. In the current report, the frequency of shedding in HIV-seropositive homosexual men appeared to be similar to that in HIV-seronegative homosexual men, but the study did not provide data about HHV-8 viral levels in these groups.

A simple rendering of the data in Figure 1 of the article to display viral levels in various body fluids according to HIV status would be of interest. Is HHV-8 prevalence among homosexual men higher because HIV-related immunosuppression has increased the amount of HHV-8 shed and thereby increased its transmission within this community? Such a finding might explain reports^{4,5} that the prevalence of HHV-8 in the homosexual community has increased in parallel with the rising AIDS epidemic.

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1. Pauk J, Huang ML, Brodie SJ, et al. Mucosal shedding of human herpesvirus 8 in men. *N Engl J Med* 2000;343:1369-77.
2. Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D. The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. *Nat Med* 1996;2:918-24.
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To the Editor: The frequent detection of HHV-8 in the oropharyngeal samples and saliva of men who have sex with men, as reported by Pauk and colleagues, is an important contribution. However, their study of risk factors has limited value because of its cross-sectional design, which may yield spurious associations.¹ The time of infection among their subjects is not known; infection may even have occurred during a period other than that in which sexual practices were investigated. Thus, although deep kissing with HIV-positive partners may appear to be a major risk factor for HHV-8 infection, it may well confound (or be confounded by) many other sex practices or simply the number of HIV-positive sex partners. Even in a study with a prospective design in which the time of infection is known, it is difficult to disentangle the relative contributions of various practices, as the authors noted. With such closely related factors, careful modeling in a prospective setting is required, but this requirement was not met in the study by Pauk et al. Furthermore, the authors give the reader no information about what sexual practices were evaluated and over what period.

Although Pauk et al. clearly demonstrate the presence of HHV-8 in oropharyngeal samples and saliva, the epidemiologic aspect of their study is difficult to interpret and is insufficient to identify deep kissing as the principal mode of HHV-8 transmission. Other modes, such as orogenital sex, may well have an important role.¹

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1. Dukers NH, Renwick N, Prins M, et al. Risk factors for human herpesvirus 8 seropositivity and seroconversion in a cohort of homosexual men. *Am J Epidemiol* 2000;151:213-24.

To the Editor: Our studies in a different epidemiologic setting support the conclusions of Pauk et al. about HHV-8 infection. Of 788 patients seen from 1998 through 2000 at our unit for sexually transmitted diseases, 394 consecutive patients (320 of whom were prostitutes) underwent serologic testing for HHV-8. Anti-HHV-8 antibodies were found in 56 (53 from sub-Saharan Africa) of the 320 prostitutes and in 8 (6 from sub-Saharan Africa) of the 74 other patients (Table 1). Mantel-Haenszel analysis, with adjust-

TABLE 1. HUMAN HERPESVIRUS 8 (HHV-8) SEROLOGIC STATUS AMONG PROSTITUTES AND OTHER PATIENTS, ACCORDING TO THEIR GEOGRAPHIC ORIGIN.

SEROLOGIC STATUS	PROSTITUTES		OTHER PATIENTS		TOTAL
	FROM SUB-SAHARAN AFRICA	FROM OTHER AREAS	FROM SUB-SAHARAN AFRICA	FROM OTHER AREAS	
	number of patients				
HHV-8–positive	53	3	6	2	64
HHV-8–negative	171	93	6	60	330
Total	224	96	12	62	394

ment for geographic origin, and multivariate analysis disclosed no association between prostitution or other indicators of sexual activity and HHV-8 infection, whereas being born in sub-Saharan Africa carried a relative risk of HHV-8 infection of 7.90 (95 percent confidence interval, 3.24 to 19.25; $P < 0.001$).

Molecular analysis with the polymerase chain reaction in both salivary and cervical specimens from 34 HHV-8–infected women led to the detection of HHV-8 viral DNA in 32.2 percent of the salivary samples and in none of the cervical specimens.

Our findings from areas where HHV-8 infection is endemic support those of Pauk et al. and further confirm the minor role of sexual contact in HHV-8 transmission. The role of deep kissing cannot be easily extrapolated from that of sexual intercourse; however, the most likely route of HHV-8 spread can be ascertained more clearly when infection with HHV-8 in a population consisting largely of prostitutes is associated only with birth in areas where the infection is highly endemic.¹ In such areas, HHV-8 infection is thought to be acquired during infancy, probably through mechanisms similar to those responsible for transmission of the Epstein-Barr virus.²

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The authors reply:

To the Editor: Biggar and Goedert request additional data on the amounts of HHV-8 DNA detected. In our

cross-sectional cohort, herpes simplex virus was present in the oral swabs of 5 of 11 HIV-seropositive men and 3 of 16 HIV-seronegative men. The mean amount of HHV-8 DNA present in these samples was $10^{3.1}$ and $10^{3.8}$, respectively. Among the participants from whom swabs of the oral mucosa were obtained daily, herpes simplex virus was present in the samples from 9 of 14 HIV-seropositive and 4 of 9 HIV-seronegative men. The mean amount of HHV-8 DNA detected was $10^{4.0}$ and $10^{3.6}$, respectively, on the days that HHV-8 was detected ($P=0.003$ by the Mann-Whitney test). We did not see a correlation between the amount of HHV-8 and the CD4 count; however, the number of people studied was small. There were too few genitourinary samples containing HHV-8 DNA to allow meaningful comparisons.

We agree with Dukers and colleagues that, as stated in our report, deep kissing is a potential but not proven mode of transmission. We hope that our findings will lead to prospective studies designed to obtain data about oral contact as a risk factor for HHV-8 acquisition. In our study, sexual practices that were not related to HHV-8 seropositivity among HIV-seronegative men who had sex with men were the consistent use of condoms, unprotected receptive and insertive anal sex, receptive and insertive oral-anal "rimming," and receptive and insertive orogenital sex. Because of space constraints, we presented only the final multivariate models in the article. Moreover, our study was designed to evaluate sites of shedding and to estimate shedding rates and patterns; the epidemiologic data reinforce the data on mucosal shedding.

We are gratified to see the data from Italy on the high detection rate of HHV-8 in the saliva of HHV-8-seropositive women. It would be of interest to compare the frequency of detection in HIV-seropositive women with that in HIV-seronegative women. The key goal now is to identify the factors critical to the increased transmission and acquisition of this herpesvirus.

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Narcolepsy and the HLA System

To the Editor: In the second part of their review of the HLA system (Sept. 14 issue),¹ Klein and Sato state that the HLA class II association in human narcolepsy is due to linkage disequilibrium with mutations in the gene coding for the hypocretin type 2 receptor (*HCRTR2*). *HCRTR2* is located on human chromosome 6, but at a very large genetic distance from the HLA loci; there is no linkage disequilibrium between the HLA alleles and *HCRTR2* over a distance of more than 30 million base pairs and 33.4 centimorgans. However, there is a different association between narcolepsy and the HLA system. Microsatellite-marker and sequencing studies of the HLA class II region have shown that HLA-DQ is the primary susceptibility locus for human narcolepsy in the HLA region.²⁻⁴

We have recently shown that most cases of narcolepsy in humans do not involve mutations in the hypocretin-system

genes but, rather, involve a loss of hypocretin-containing neurons in the perifornical hypothalamus.⁵ On the basis of current data, the most likely hypothesis is that human narcolepsy is an autoimmune disorder targeting hypocretin-containing cells.

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Treatment of Ciguatera Poisoning with Gabapentin

To the Editor: Ciguatera poisoning from fish is caused by a neurotoxin (ciguatoxin) present in the dinoflagellate *Gambierdiscus toxicus*. The toxin is transferred through herbivorous reef fish to carnivorous tropical reef fish, which are consumed by humans. The toxin is lipid-soluble and is not inactivated by cooking, cold, or gastric juice. More than 200 species of fish have been implicated in causing ciguatera poisoning, the most common being grouper, red snapper, and barracuda. The primary endemic areas include the Caribbean and South Pacific islands, where the incidence is between 50 and 500 cases per 10,000 population.^{1,2}

The symptoms of ciguatera poisoning, which develop 1 to 30 hours after the ingestion of poisoned fish, are nausea, vomiting, abdominal cramps, and watery diarrhea, followed by such neurologic symptoms as numbness and paresthesia of lips, tongue, and throat; pruritus, myalgia, or sharp, shooting pains in the legs; dysesthesia involving a reversal of the sensations of cold and heat; and in severe cases, hypotension, bradycardia, and respiratory paralysis. These symptoms can last for months and recur intermittently. There is no effective treatment.^{1,2}

We evaluated two patients who had ciguatera poisoning after the ingestion of dusky grouper in the Dominican Republic; both were treated successfully with gabapentin. Patient 1 was a 30-year-old woman who had an episode of diarrhea during a vacation in Punta Cana. Several hours later, dysesthesia developed, along with intense pruritus of the legs, hands, and breasts, which increased with exposure to cold. The physical examination and results of laboratory studies were normal. Patient 2, a 37-year-old

woman, had a similar history, except that she had generalized pruritus and sharp, shooting pains in her legs. In both patients, the symptoms were disabling and persisted for weeks.

One month after the onset of symptoms, the patients were treated with gabapentin (400 mg orally three times daily), with rapid improvement. Twenty days later, we stopped the drug; the symptoms returned in a few hours in both patients. Gabapentin therapy was resumed, and the patients had immediate relief of symptoms. The drug was then administered for three weeks. Subsequently, Patient 1 had only minor dysesthesia, and Patient 2 had some leg pain but chose not to resume treatment.

Gabapentin is an antiepileptic drug structurally related to γ -aminobutyric acid that has been used successfully to

treat neuropathic pain.^{3,4} We believe this drug is an effective treatment for ciguatera poisoning.

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