

## Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted by e-mail (science\_letters@aaas.org), the Web (www.letter2science.org), or regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

## Retraction of an Interpretation

**WE WRITE TO RETRACT AN INTERPRETATION** in our Report, "Contribution of human  $\alpha$ -defensin 1, 2, and 3 to the anti-HIV-1 activity of CD8 antiviral factor" (1), wherein we demonstrated that human  $\alpha$ -defensin 1, 2, and 3 account for much of the anti-HIV-1 activity of the CD8 antiviral factor (CAF) that is not attributable to  $\beta$ -chemokines. Although the antiviral activity of human  $\alpha$ -defensin has not been called into question, the cellular source of these  $\alpha$ -defensins has been reinterpreted in light of more recent experiments. Our experiments were done using purified CD8 T cells from long-term nonprogressors or normal persons that were stimulated with anti-CD3 and anti-CD28 antibodies, recombinant interleukin-2, phytohemagglutinin, and irradiated allogeneic peripheral blood mononuclear cells (PBMC). This method of stimulating CD8 T cells had been commonly used by many groups working on CAF (2-7). However, in a follow-up attempt to define the specific subpopulation of CD8 T cells that produce  $\alpha$ -defensins, we have found that in the absence of allogeneic irradiated PBMC (feeders), stimulated CD8 T cell supernatants do not contain  $\alpha$ -defensins. Although it could be argued that an allogeneic stimulus is a prerequisite for  $\alpha$ -defensin production by CD8 T cells, it is more likely that they are derived from a cell population residing within allogeneic feeders.

To pursue the exact source of  $\alpha$ -defensins in allogeneic feeders, we positively selected individual cell populations from irradiated PBMC and subjected them to the aforementioned stimulation conditions in the absence of allogeneic exposure.  $\alpha$ -Defensins were detected in the supernatants of CD4 and CD8 T cells and CD19 B cells. However, if the allogeneic PBMC feeders were first treated with an anti-CD15 monoclonal antibody to eliminate residual neutrophils before being subjected to irradiation, then  $\alpha$ -defensins were no longer detectable in the supernatants of stimulated T or B cells. These findings

suggest that under our experimental conditions, even minor degrees of neutrophil contamination could result in the detection of  $\alpha$ -defensins in the culture supernatant of other cell populations.

In a different set of experiments using antibody staining in flow cytometry or immunofluorescence, we also detected  $\alpha$ -defensins within several freshly isolated mononuclear cell populations, including CD8 T cells, as we have reported (1). But, in stark contrast, no  $\alpha$ -defensin mRNA could be detected in CD8 T cells using a sensitive RT-PCR assay. This discrepancy prompted a series of in vitro cell-mixing experiments, which led us to the conclusion that during the steps of cell processing, fixation, and permeabilization,  $\alpha$ -defensins readily leaked from neutrophils into other cells that are not natively producing these proteins. In preliminary experiments, we also find that the release of  $\alpha$ -defensins into the supernatant becomes more striking when activated CD8 T cells are present, perhaps because they release cytokines that facilitate degranulation of  $\alpha$ -defensins from contaminating neutrophils. Taken together, these new findings convinced us that  $\alpha$ -defensins cannot account for the CAF activity in experimental systems that do not use allogeneic feeders (8-11).

We wish to emphasize that the experimental findings in our Report (1) are repeatable. In particular, we have solidified the results shown in figs. 3 and 4 of our paper (1). The removal or neutralization of  $\alpha$ -defensins by specific antibodies again resulted in the loss of anti-HIV-1 activity in the supernatant of CD8 T cells stimulated by allogeneic feeders. It should be pointed out that there is, in fact, very little residual anti-HIV-1 activity remaining once  $\alpha$ -defensins and  $\beta$ -chemokines are eliminated. More importantly, we have also tested human neutrophil-derived  $\alpha$ -defensins from independent sources and found their antiviral potency to be equivalent to those previously described (1), irrespective of the viral strain or target cell used in the experiment. Other investigators have confirmed this observation (12, 13). Thus, the anti-HIV-1 activity of  $\alpha$ -defensins we have described (1) is not in doubt, and the mechanism of their antiviral effect should be pursued.

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## Brightening Depression

**CONSTANCE HOLDEN'S OVERVIEW "FUTURE brightening for depression treatments"** (Special Issue on Brain Disease, News, 31 Oct., p. 810) explored the current exciting approaches for creating novel antidepressants. Absent from this discussion were two major nonpharmacological, biological antidepressant treatments that have been clearly demonstrated to be highly efficacious and fast.



**Given the psychological suffering that depression inflicts..., it is surprising how little notice is taken of these remarkable chronobiological interventions [sleep deprivation and light therapy]."**

—WIRZ-JUSTICE ET AL.

A single night of total or partial sleep deprivation—"wake therapy"—induces rapid and dramatic, albeit usually short-lasting, improvement of mood in about 60% of all depressed patients, independent of diagnostic subgroup (1). A positive response to sleep deprivation predicts and hastens the response to antidepressant medication (1). Sleep deprivation can be combined with a variety of drugs to maintain the response attained within hours (2-4)—theoretically, a perfect combination (5).

Light therapy is the only treatment in psychiatry that evolved directly out of neurobiological models of behavior (6, 7). It is the treatment of choice for seasonal affective disorder, or winter depression (6), but is

also efficacious in nonseasonal depression (8–10). Light therapy is characterized by a fast onset of antidepressant action—within days—and it can prevent the depressive relapse after recovery sleep following sleep deprivation (4, 11). Furthermore, light and medication can be combined (8–12).

Sleep deprivation and light therapy cannot be patented, and they will not bring profits to the conventional psychopharmacology industry, but they can help the patient in a shorter time and with fewer side effects than drugs—and can be easily and successfully combined with medication (3, 4, 11, 12). Given the psychological suffering that depression inflicts—including the danger of suicide—and the financial pressures to minimize the duration of hospitalization, it is surprising how little notice is taken of these remarkable chronobiological interventions. We must include them in the therapeutic armamentarium. For light therapy, an American Psychiatric Association task force recently has concluded the same (13).

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## The Difficulties of Testing for SARS

WE READ WITH INTEREST THE ARTICLE "SEARCH for SARS origins stalls" (M. Enserink, D. Normile, *News Focus*, 31 Oct., p. 766). The experience of the Canadian National Microbiology Laboratory, stemming from a positive SARS test that was later found to be a false alarm (Sidebar, "Unexplained false alarm may hold lessons," M. Enserink, p. 767), struck a particularly resonant chord, as it mirrored almost exactly our own experience in Hong Kong, one of the places most affected by SARS.

As the provider of the only private test service for the SARS coronavirus (CoV) in Hong Kong, we had been contracted by a local private hospital to test patient samples from cases of atypical pneumonia. The majority of tests were conducted after Hong Kong had been declared free from SARS. After testing many samples with our enhanced real-time PCR method, which we developed in-house, one sample gave a preliminary positive result with our

## CORRECTIONS AND CLARIFICATIONS

standard set of primers against the viral polymerase gene. Before we were able to confirm the positive test by retesting the sample using primers directed at another part of the SARS-CoV genome, the patient was transferred to another designated hospital. Regrettably, the results of the initial positive test were passed to the Department of Health and Hospital Authority, which then leaked the information to the media, causing considerable distress. This premature disclosure resulted in the temporary reintroduction of SARS preventive measures in hospitals, a plunge in the stock market, and the return of fear and anxiety to the general population. The patient later provided not to have the virus.



**Random Samples:** "Eyes on FDA" (9 Jan., p. 167) The item on Food and Drug Administration Commissioner Mark McClellan was accompanied by an incorrect photograph. The correct photo appears at the left. The picture that ran was actually of Mark McLellan, a professor at the Institute of Food Science and Engineering at Texas A&M University in College Station.

**News Focus:** "Butler's samples spelled trouble for U.S. agencies" by D. Malakoff (19 Dec., p. 2058) is accompanied by a text box that incorrectly quotes from a 16 May 2002 e-mail from David Dennis, then a CDC official responsible for the agency's plague program, to Thomas Butler, the Texas Tech University researcher recently tried for mishandling samples from suspected Tanzanian plague patients. The excerpt should read, "I know this sounds terrible to someone who just wants to get the job done, but things are not what they used to be."; not "...as someone who just wants to get the job done, but things are changing and not for the better." The excerpt refers to regulations governing Butler's planned visit to CDC's laboratory in Fort Collins, Colorado, and to human subject research rules that barred CDC researchers from co-authoring research papers with Butler, not to Butler's plans for transporting his samples to the laboratory in his car. This excerpt, and a second 9 May 2002 e-mail in which Dennis commends Butler's proposed algorithm for testing specimens from suspected plague patients, should have been accompanied by an explanation that CDC treated Butler's samples as clinical specimens "intended for diagnostic, reference, or verification purposes," which, in accordance with then-existing federal regulations, exempted them from certain permit requirements for interstate shipment. As noted during Butler's trial, the method of transport of specimens from Tanzania to Texas Tech was unknown to CDC. Butler was acquitted of government charges that he illegally transported the samples from Tanzania to Texas Tech, and subsequently from Texas Tech to CDC.

We share the scientific community's concern at the lack of a standardized, sensitive, and specific test for SARS-CoV. Test sensitivity is critical to SARS control. Any positive results can be confirmed by other laboratories. But false negative results may go unchallenged, leading to a false sense of security, with the potential for the disease to spread silently in the community. Only when standardization is available will more direct questions concerning effective SARS control be addressed clearly. In light of the recently confirmed SARS case in Guangdong, China, the origins of the disease in China, the involvement of one or more reservoir species, and the dynamics of interspecies transmission are all vital in understanding the potential for future outbreaks.

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## TECHNICAL COMMENT ABSTRACTS

## COMMENT ON "Impact Ejecta Layer from the Mid-Devonian: Possible Connection to Global Mass Extinctions"

Grzegorz Racki, Christian Koeberl

Evidence presented by Ellwood *et al.* (Reports, 13 June 2003, p. 1734) for a mid-Devonian bolide impact requires comprehensive confirmation. Even if the impact occurred, a cause-and-effect relation between this event and global biocrisis remains doubtful. The late Eifelian "mass extinction" cited by Ellwood *et al.* is at the sixth position among the Devonian intervals in a relative ranking by Sepkoski. The search for a causal link between extra-terrestrial impact and an obligatorily major extinction represents a circular argument.

Full text at [www.sciencemag.org/cgi/content/full/303/5657/471b](http://www.sciencemag.org/cgi/content/full/303/5657/471b)

## RESPONSE TO COMMENT ON "Impact Ejecta Layer from the Mid-Devonian: Possible Connection to Global Mass Extinctions"

Brooks B. Ellwood, Stephen L. Benoist, Ahmed El Hassani, Christopher Wheeler, Rex E. Crick

We present three diagrams with new data supporting our previous work from the Eifelian-Givetian global boundary stratotype. These data show what appear to be two impact ejecta levels, within a rapidly accumulating, thin, multiphase marl bed containing the Kacák/*otomari* extinctions. These results are supported by our other work in Morocco and elsewhere.

Full text at [www.sciencemag.org/cgi/content/full/303/5657/471c](http://www.sciencemag.org/cgi/content/full/303/5657/471c)