

Correspondence

Interferon- γ -Mediated Antiviral Immunity against Orthopoxvirus Infection Is Provided by $\gamma\delta$ T Cells

To the Editor—Abate et al. [1] reported increases in the numbers of vaccinia virus (VV)-responding CD4⁺ and CD8⁺ T cells in recipients of smallpox vaccine, compared with those in nonvaccinated control subjects. The population of interferon (IFN)- γ -producing $\gamma\delta$ T cells was also expanded in vaccinated persons, suggesting that, in addition to CD4⁺ and CD8⁺ $\alpha\beta$ T cells, $\gamma\delta$ T cells might be involved in orthopoxvirus-induced immune responses.

These data confirm our previous observation that VV-specific CD4⁺ and CD8⁺ T cell recall responses are detectable by flow cytometry after recent smallpox vaccination [2]. Recently, we have observed that VV is also able to induce a substantial in vitro expansion of the $\gamma\delta$ T cell population in peripheral-blood mononuclear cell samples obtained from nonvaccinated volunteers (2.5–10-fold increases in $\gamma\delta$ T cell numbers were observed after 7 days of culture in the presence of VV in 4 of 5 nonvaccinated donors). Previously, substantial numbers of VV-specific $\gamma\delta$ T cells have been observed in uninfected mice [3], and $\gamma\delta$ T cells from a high proportion of apparently naive cattle could be activated by modified VV Ankara [4]. Cumulatively, these findings suggest that VV may induce $\gamma\delta$ T cell activation through innate pathways of immune recognition.

In our study, the VV-expanded human $\gamma\delta$ T cell population displayed the V γ 9V δ 2 T cell receptor (TCR) (the vast majority of V δ 2 chains form the V γ 9JpC1 γ /V δ 2DJ1C δ combination—i.e., the V γ 9V δ 2 TCR) [5]. Similar expansions of the V γ 9V δ 2 T cell subset have been noted in persons receiving live recombinant cana-

rypox virus vaccination [6]. Proliferative, cytotoxic, and cytokine responses of the V γ 9V δ 2 T cell subset are constitutively induced by both nonpeptidic antigens, such as pyrophosphomonoesters and alkylamines, and nitrogen-containing bisphosphonates (N-BPs), such as zoledronic acid (ZA) [7, 8]. Moreover, V γ 9V δ 2 T cells are known to exert broad antiviral activity against different viruses through cytolytic and noncytolytic mechanisms that include the release of soluble $\gamma\delta$ factors (GDFs) [9, 10]. Therefore, we tested the possible VV inhibitory activity of GDFs in vitro. Specifically, GDFs released by V γ 9V δ 2 T cells were collected after 24 h of stimulation with either isopentenyl-pyrophos-

phate (IPP) (one of the well-studied “non-peptidic antigens”) or ZA. The antiviral activity of the GDFs was assessed using the A549 cell line infected with VV. Because the supernatants of IPP-stimulated $\gamma\delta$ T cell cultures usually contain relevant amounts of IFN- γ (range, 50–500 IU/mL), neutralization experiments were performed using an IFN- γ -specific polyclonal rabbit antiserum (final concentration, 10,000 neutralization units/mL). Our data indicate that (1) the GDFs from the IPP- or ZA-stimulated $\gamma\delta$ T cells, but not from the control $\gamma\delta$ T cells, inhibited VV replication and (2) the neutralizing antibodies against IFN- γ virtually abrogated the virus inhibitory activity of the GDFs present

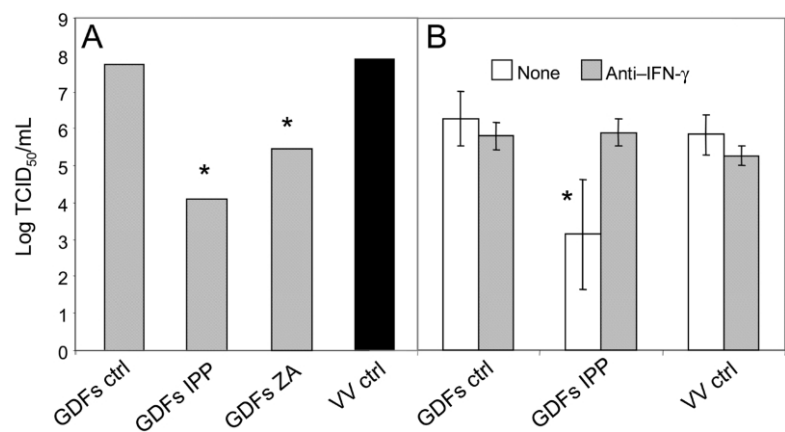


Figure 1. Vaccinia virus (VV) inhibitory activity of $\gamma\delta$ factors (GDFs). For the experiments shown in panel A, V δ 2 T cell lines (>80% V δ 2⁺ cells) were obtained by stimulating peripheral-blood mononuclear cells from healthy donors with isopentenyl-pyrophosphate (IPP; 10 μ mol/L) and interleukin-2 (100 IU/mL) for 12 days. The V δ 2 T cell lines were then restimulated with either IPP (10 μ mol/L) or zoledronic acid (ZA; 2 μ mol/L) for 24 h, and the culture supernatants that contained GDFs (i.e., factors released by activated $\gamma\delta$ T cells) were diluted 1:2 and added to A549 cell cultures for an additional 24 h. The A549 cell cultures were then washed and infected with VV at an MOI of 0.01. Seventy-two hours after infection, the A549 cell culture supernatants were collected, and the numbers of infectious VV particles were titrated by limiting dilution assay on uninfected target cells and expressed as the TCID₅₀ per milliliter. Panel B shows the results of the cytokine neutralization assay, which was performed using polyclonal rabbit anti-interferon (IFN)- γ antiserum, as described elsewhere [11]. ctrl, control. * P < .05 (statistically significant difference by Student's t test), for the comparison of GDFs IPP or GDFs ZA with GDFs ctrl (factors released without activating $\gamma\delta$ cells) or with VV ctrl (VV-infected control cultures) in panel A, and for the comparison of GDFs IPP with or without anti-IFN- γ antiserum in panel B.

in the supernatants from the stimulated V γ 9V δ 2 T cell cultures (figure 1). These findings suggest that the VV inhibitory activity of GDFs from in vitro-stimulated $\gamma\delta$ T cells is largely mediated through IFN- γ , confirming a main role for this cytokine in the $\gamma\delta$ T cell response to VV [1]. These data are also in agreement with those from a study showing that augmented $\gamma\delta$ T cell responses in recipients of canarypox vaccine are mediated primarily by IFN- γ -producing V γ 9V δ 2 T cells [6]. It is noteworthy that, despite substantial differences between mouse and human $\gamma\delta$ T cells, VV-infected mice deficient in $\gamma\delta$ T cells have significantly higher VV titers and mortality rates than do normal mice [3] and that rapid and substantial increases in IFN- γ -producing $\gamma\delta$ T cells occur in $\alpha\beta$ -TCR-deficient mice that survive VV infection [3].

These observations emphasize the importance of IFN- γ -producing $\gamma\delta$ T cells in innate resistance to VV infection. Moreover, it is likely that the increased IFN- γ production by $\gamma\delta$ T cells boosts the induction of protective type 1 memory immunity and augments the effectiveness of live vaccines. The human V γ 9V δ 2 T cell subset can be activated in vivo by the administration of nonpeptidic antigens or N-BPs [9, 10]. N-BPs are widely used to treat bone-demineralization disorders and certain cancers [8]. The low toxicity of N-BPs, combined with the current lack of effective smallpox therapies, suggests that the use of N-BP-induced activation of V γ 9V δ 2 T cells for possible postexposure prophylaxis and/or treatment of orthopoxvirus infections should be explored.

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Reply to Agrati et al.

To the Editor—We appreciate that Agrati et al. [1] have independently documented the induction of CD4⁺, CD8⁺, and $\gamma\delta$ T cells by vaccinia virus (VV). The work of Ennis et al. [2], Kennedy et al. [3], and our group [4] have demonstrated the induction of VV-specific T cell responses by VV vaccination in larger numbers of individuals. Agrati et al. report that they have induced potent expansions of the $\gamma\delta$ T cell population by in vitro stimulation of peripheral-blood mononuclear cells (PBMCs) from VV-naive individuals with VV lysates but do not present the details on how these experiments were conducted. In contrast, we have previously tried to induce $\gamma\delta$ T cells by live VV stimulation of PBMCs derived from VV-naive individuals without success. We found that if interleukin (IL)-2 was included in these attempts, similar expansions of the $\gamma\delta$ T cell population could be seen whether or not VV stimulation was included. We wonder whether IL-2 was included in the protocol Agrati et al. used to stimulate $\gamma\delta$ T cells; if so, it could have resulted in an expansion of the $\gamma\delta$ T cell population that was not necessarily VV specific.

We have generated protocols that induce expansions of $\gamma\delta$ T cell populations only when they are from persons who had been previously vaccinated with VV and, thus, had memory immunity. Shown in figure 1A are results demonstrating the dependence of enhanced VV-specific memory immune $\gamma\delta$ T cell responses on priming of these cells by vaccination with VV in vivo. Pairs of pre- and postvaccination samples of PBMCs harvested from volunteers recruited into a vaccinia vaccination trial were studied in a mix-and-match fashion. $\gamma\delta$ and $\alpha\beta$ T cells were separated by immunomagnetic sorting, with only minimal residual contamination of these purified preparations by the other T cell subset. Naive ($\alpha\beta$ and $\gamma\delta$ T cells purified from prevaccination PBMC samples) and memory immune ($\alpha\beta$ and $\gamma\delta$ T cells purified from postvaccination PBMC samples) populations were obtained. Different

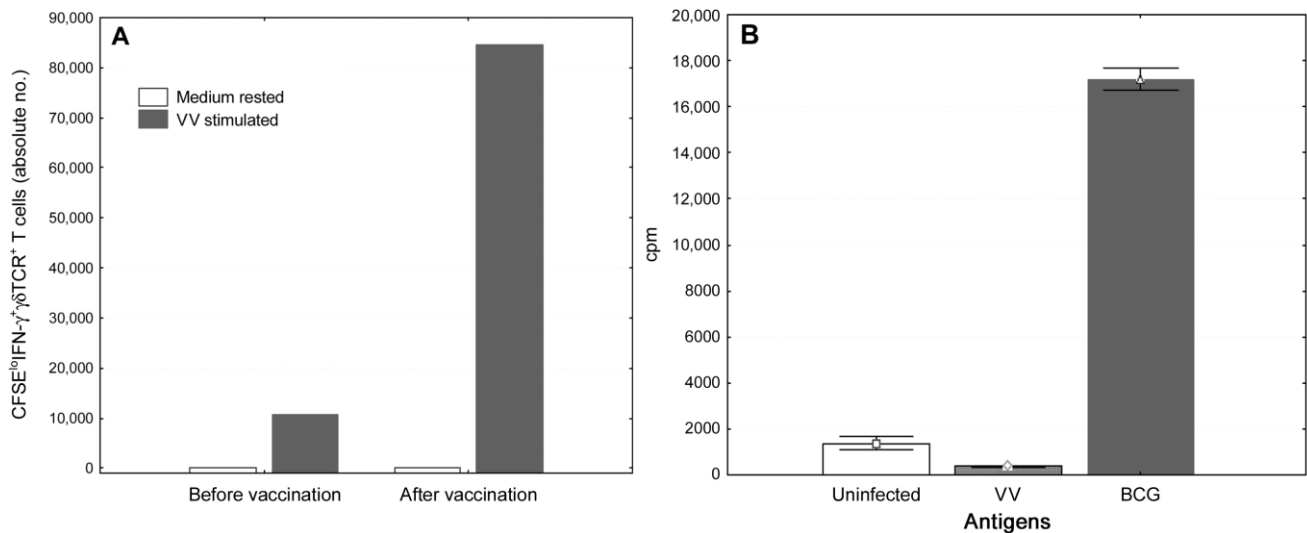


Figure 1. Enhanced vaccinia virus (VV) memory immune responses and $\gamma\delta$ T cell antigen specificity. *A*, Induction of memory immune $\gamma\delta$ T cells by vaccination with VV. $\gamma\delta$ T cells were immunomagnetically purified from peripheral-blood mononuclear cells (PBMCs) that had been harvested from a single volunteer before and 45 days after vaccination and labeled with carboxyfluorescein succinimidyl ester (CFSE). These naive and memory immune $\gamma\delta$ T cells were incubated with $\alpha\beta$ T cells that had been immunomagnetically purified from autologous PBMCs harvested 45 days after vaccination. These cocultures were rested in medium or stimulated with an optimal dose of live VV for 10 days before flow-cytometric analysis of lymphoproliferation and IFN- γ secretion was conducted, as described elsewhere [4]. An 8-fold increase in $\gamma\delta$ T cell proliferation and IFN- γ secretion is seen for the postvaccination (memory immune) $\gamma\delta$ T cells. TCR, T cell receptor. *B*, Development of differential antigen specificities by $\gamma\delta$ T cells. A long-term $\gamma\delta$ T cell line was generated by repeated stimulation of $\gamma\delta$ T cells with bacille Calmette-Guérin (BCG)-infected dendritic cells originally harvested from an individual latently infected with *Mycobacterium tuberculosis*. This $\gamma\delta$ T cell line was stimulated for 4 days with irradiated autologous PBMCs pulsed with nothing or with optimal doses of either live VV or live BCG. On day 4, proliferation was assessed by tritiated thymidine incorporation. This $\gamma\delta$ T cell line responded only to BCG stimulation and not to VV stimulation, demonstrating differential antigen specificity.

combinations of naive and memory immune $\alpha\beta$ and $\gamma\delta$ T cells were mixed and stimulated in culture with live VV. Figure 1A shows the results of naive and memory immune $\gamma\delta$ T cells either rested in medium or stimulated with live VV in the presence of memory immune $\alpha\beta$ T cells from one of the volunteers studied. Despite the presence of memory immune $\alpha\beta$ T cells in both cases, only the memory immune $\gamma\delta$ T cells demonstrated VV-specific lymphoproliferation and interferon (IFN)- γ secretion. These results indicate that the postvaccination $\gamma\delta$ T cells had developed memory responses that were dependent on their previous in vivo priming and that the $\gamma\delta$ T cell responses that we detected after vaccination were not simply the consequence of a bystander effect resulting from growth factors secreted by memory immune $\alpha\beta$ T cells.

Therefore, our results suggest that $\gamma\delta$ T cells, in addition to responding to VV [4], canarypox virus [5], and bacille Calmette-

Guérin (BCG) [6] as innate immune cells, can develop a memory immune phenotype. Innate immune responses by $\gamma\delta$ T cells could be useful for immunotherapy; however, memory immune $\gamma\delta$ T cells could also be useful as immunologic targets for prophylactic vaccines. Furthermore, we have generated additional data demonstrating that $\gamma\delta$ T cells can develop memory immune responses specific for unique pathogens (figure 1B). A BCG-specific long-term $\gamma\delta$ T cell line responded only to BCG-infected and not to VV-infected antigen-presenting cells. These latter results indicate differential antigen specificities for mycobacteria- and poxvirus-responsive $\gamma\delta$ T cells.

Because we have found that V γ 9V δ 2 T cells can develop antigen-specific memory similar to that of $\alpha\beta$ T cells, we believe it is important to determine whether vaccine immunity could benefit from targeting this subpopulation of $\gamma\delta$ T cells. We are currently identifying the unique components

of mycobacteria and poxviruses that induce antigen-specific memory immune V γ 9V δ 2 T cells. In addition, we are focusing our mechanistic studies on elucidating the antigen-specific effects that memory immune V γ 9V δ 2 T cells have on mycobacterial and poxviral replication, rather than the effects of supernatants derived from cultures of $\gamma\delta$ T cells not stimulated by active mycobacterial or poxviral replication.

We agree with Agrati et al. that the induction of $\gamma\delta$ T cells in vivo may result in new prophylactic and/or therapeutic strategies for poxviral infection as well as for mycobacterial infection. In addition, we agree that synthetic phosphoantigens and/or nitrogen-containing bisphosphonates could be used to safely up-regulate V γ 9V δ 2 T cell responses in patients with ongoing poxviral and/or mycobacterial infection, resulting in nonspecific protective effects through the secretion of chemokines and IFN- γ . Our work further suggests that, because we find differential an-

tigen specificities for mycobacteria- and poxvirus-responsive $\gamma\delta$ T cells, the inclusion of specific poxviral and/or mycobacterial antigens in new vaccines may induce potentially more important $\gamma\delta$ T cell responses that have preexposure protective effects.

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Single-Dose Sulfadoxine-Pyrimethamine in Intermittent Preventive Treatment of Malaria

To the Editor—Sulfadoxine-pyrimethamine (S-P), a safe and inexpensive drug, is widely used for intermittent preventive treatment (IPT) of malaria during pregnancy (IPTp) in Africa and is the prime candidate for use for IPT in infants (IPTi) (<http://www.ipti-malaria.org>). Marks et al. report that a single treatment dose of S-P can cause parasitological rebound and selection of drug-resistant parasites for a short period after drug clearance [1]. This conclusion has potentially serious implications for the IPT approach to reducing the burden of malaria during pregnancy and in children.

We are concerned about the study methods and presentation of results by Marks et al.

1. A priori criteria for enrolling the 68 subjects were not reported.
2. There was a substantial imbalance in the number of subjects between the 2 study arms (28 vs. 35—i.e., the placebo arm had 25% more subjects than the S-P arm). A brief note on the randomization is needed for readers to understand the reasons for this.
3. The follow-up rate differed significantly between the 2 study arms (table 1). The mean number of days of obser-

vation per subject was significantly higher in the S-P arm than in the placebo arm (141 vs. 94 days; $P < .05$). Furthermore, it is unclear why the follow-up rates for days at risk were significantly different (93% vs. 57%) while the ratios of observed versus expected numbers of observations were similar (0.77 vs. 0.76) between the 2 arms.

4. Figure 1A of the Marks et al. article shows that the proportion of infants who were parasitemic by week 24 is ~70% in the S-P arm, but in the table it is stated that only 57.1% (16/28) were ever parasitemic. Similarly, in the placebo arm, in the graph it appears that ~48% were parasitemic, whereas in the table it is 40% (14/35). The analysis for repeated episodes would have been better made by comparing incidence rates.

5. The authors state that the incidence of 4 mutations was higher in the S-P arm (rate ratio, 2.1) than in the placebo arm 8 weeks after administration of S-P (an 8-week cutoff was used to exclude the time before S-P had been eliminated from the blood). However, the incidence of 4 mutations over the whole follow-up period (without excluding the first 8 weeks) was 2.21 episodes/year in the S-P arm and 1.56 episodes/year in the placebo arm (rate ratio, 1.4). If the first 8 weeks are included, the rate ratio should be higher and not lower, because subjects treated with S-P

Table 1. Follow-up rate in the 2 study arms.

Indicator of successful follow-up	S-P arm	Placebo arm
Expected days at risk, ^a no.	5096	6370
Expected days of risk to be excluded, ^b no.	868	672
Expected days at risk, corrected for episodes of malaria, ^c no.	4228	5698
Observed days at risk, no.	3955	3276
Follow-up rate, ^d %	93	57
Expected observations, ^e no.	336	420
Observed observations, no.	259	320
Ratio of observed vs. expected observations	0.77	0.76
Days of observation, ^f mean, no.	141.3	93.6

NOTE. S-P, sulfadoxine-pyrimethamine.

^a (No. of subjects in each arm) \times (expected no. of days of follow-up per subject [182 days]).

^b (No. of episodes of malaria) \times (no. of malaria risk-free days after treatment per episode [28 days]).

^c (Expected no. of days at risk) – (total no. of malaria risk-free days after treatment).

^d (Observed no. of days at risk)/(expected no. of days at risk, corrected for episodes of malaria).

^e (No. of subjects) \times (expected no. of observations per subject [12]).

^f (Observed no. of days at risk)/(no. of subjects).

should get infected only by resistant parasites. It is unclear why the rate ratio is higher when the episodes that occurred during the first 8 weeks were excluded.

The results of this study are far from clear. It is not surprising that subjects in the S-P arm had more episodes of infection and S-P resistance, because they were observed longer. The incidence of any malaria infection over the whole study period was similar in the S-P and placebo arms (2.86 vs. 2.67 episodes/year). This may suggest rebound, since one would expect some prophylactic effect in the S-P arm that is not borne out in the overall incidence rates.

The threat and implications of IPTi increasing the frequency of resistant alleles—which are the study's main conclusions as well as the focus of the editorial by Breman and O'Meara [2]—are likely to have been exaggerated for the following reasons. First, the switch to using artesunate combination therapy for most malaria-endemic countries would effectively treat S-P-resistant strains and also relieve most of the selecting drug pressure. Second, in settings where S-P remains the first-line treatment, by far the largest proportion of S-P doses are given as treatment. With regard to the generation of resistant alleles, the effect on drug pressure resulting from IPTp and IPTi would be minimal compared with that resulting from treatment. Third, although mutations in the *dhfr* and *dhps* genes are associated with in vivo drug resistance to S-P, the relationship is not absolute. Evidence from several sites points toward low-level parasitemia with mutation-bearing parasites being effectively treated by S-P. Because IPTi and IPTp are given to apparently healthy individuals, it is unlikely that resistant strains will be passed on. Finally, mathematical models developed to date suggest that the effect of IPT targeted at infants on the rate of change of resistance genes at a population level will be minimal (N. Alexander, personal communication). The IPTi Con-

sortium is looking into many of these unanswered questions.

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Reply to Gosling et al.

To the Editor—Intermittent preventive treatment (IPT) for malaria with sulfadoxine-pyrimethamine (S-P) is, without doubt, a promising control measure for malaria in infants. In addition to the protective efficacy provided, drug safety and clinical rebound are important parameters for assessment of the efficacy of IPT in infants (IPTi). Before mass implementation of IPTi, concerns regarding the development of drug resistance must be addressed. The advantages of S-P for IPTi are indisputable: applicability in infants, single-dose treatment, low cost, wide availability, and broad acceptance. However, a number of publications have described the rapid emergence of S-P resistance after treatment of mild malaria (e.g., [1–3]) or S-P application to clear parasites [4]. In our article, we described selection of resistant *Plasmodium falciparum* strains after prophylaxis with S-P [5] and communicated our observation of parasitological rebound as a note of

caution to be considered in strategies of IPTi implementation. Confusion arose from our erroneous and confounding interchange of 2 numbers in table 1 of our article (see the erratum to [5]). This mistake, however, did not affect any of the statistical calculations presented.

We offer the following responses to the points raised by Gosling et al. [6] in this issue of the *Journal of Infectious Diseases*:

1. The a priori selection criteria were described in the original text—namely, (1) age 9 months (± 3 weeks), (2) no fever, (3) no S-P treatment during the preceding 6 months, and (4) no asexual parasitemia.

2. As mentioned in our article, samples were collected during the course of a clinical trial for which block randomization in blocks of 10 was performed. Only those randomized children fulfilling the aforementioned criteria were enrolled in our substudy. Differences in numbers of participants resulted from the fact that samples were chosen in a double-blinded manner and were analyzed separately. Incidence analyses were not directly dependent on the numbers of individuals included, since they are based on person-time at risk.

3. The reason for the failure to reproduce calculations is given in the erratum. With the corrected numbers, the infection rates given in the text (3.5 for the S-P group and 2.2 for the placebo group) are reproducible. Follow-up rates and mean numbers of observations are similar in both study arms.

4. The graphs of the survivor functions (figure 1 of our article) are based on the number of failures and the number of censored individuals. The deviation of the proportion of individuals who were never parasitemic given in table 1 from that given in figure 1 is a result of censoring. The analysis of repeated episodes refers to comparisons of incidence rates (designated in the text as “incidence rate ratios”).

5. With the correct numbers of days at risk in table 1, the incidence rates are 2.7 for the treatment group and 1.3 for the placebo group, as stated in the text.

Accordingly, the resulting incidence rate ratio is 2.1.

Before the establishment of intervention strategies, data on relevant factors should, at best, be confirmed by independent working groups and not exclusively within a consortium. At the time of publication of this correspondence, neither the rate of resistance after single-dose S-P treatment in Tanzania, where the first IPTi study was conducted 5 years ago, nor conclusive explanatory mathematical models have been published [7]. Exclusive artemisinin-based combination therapies in malaria-endemic areas would relieve most of the selecting drug pressure exerted by S-P but not necessarily reduce the frequency of S-P-resistant *P. falciparum* strains, most likely as a result of good fitness [8].

We do not believe that the number of malaria treatments will necessarily be decreased by IPTi, as hypothesized by Gosling et al. According to the data available from Tanzania, the background incidence rate, as is evident from the placebo group, is 0.43 malaria episodes per person-year at risk [7]. If these episodes were treated with S-P, the number of treatments would be 7 times lower than the 3 doses provided in the first year of life through their IPTi application scheme.

For evaluation of optimal IPTi conditions, the choice and dosage of the drug, periods between applications, linkage of the IPTi schedule to the World Health Organization Expanded Program of Immunization or seasonal conditions, and the risk of drug resistance and parasitological rebound should all be taken into account. This applies especially when risks and unwanted consequences are accepted as the toll paid for effective intervention and improved protection from malaria. If possible risks are ignored, sustainable acceptance of IPTi will be endangered.

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Note added in proof. Most recently, an increase in S-P resistance after drug application was observed in a trial of seasonal IPT in children. Cissé B, Sokhna C, Boulanger D, et al. *Lancet* 2006;367:659–67.

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Gilbert Syndrome and the Development of Antiretroviral Therapy–Associated Hyperbilirubinemia: Genetic Screening Is Unnecessary

To the Editor—We take issue with the suggestion by Rotger et al., in their article “Gilbert Syndrome and the Development of Antiretroviral Therapy–Associated Hyperbilirubinemia” [1], that testing for the A(TA)₇TAA allele is a “good example of how genetic testing” may be used “in the future in the clinical setting” (p. 1386). The authors showed that individuals homozygous for the A(TA)₇TAA allele and receiving atazanavir or indinavir were at increased risk for experiencing hyperbilirubinemia in the jaundice ranges [1]. This finding has been demonstrated previously [2, 3]. Rotger et al. then developed a model for the identification of individuals at risk for hyperbilirubinemia, by genetic screening for the A(TA)₇TAA allele before initiation of antiretroviral therapy. We admire their goal of presenting “a concept for the use of genetic data in clinics” (p. 1384), since this is a proven method for reducing toxicities that cause morbidity and mortality in HIV-infected patients (e.g., glucose-6-phosphate dehydrogenase deficiency). However, we believe that genetic testing should generally be reserved for the prevention of serious or irreversible complications.

The authors state that although “hyperbilirubinemia is not a serious adverse effect, clinical jaundice can stigmatize the HIV-infected individual and result in additional consultations and in treatment modification” (p. 1384). Although we agree with the authors that clinical jaundice can be stigmatizing, the hyperbilirubinemia associated with protease inhibitors resolves completely days after the medication is discontinued, thereby limiting the stigmatizing period. Rather than genetic screening, we suggest that the possibility of developing jaundice should simply be discussed with all patients before initiation of atazanavir or indinavir therapy, just as the risk for more medically important ad-

verse events, such as hyperlipidemia or hyperglycemia, should be discussed before initiation of all protease inhibitor therapy. Importantly, protease inhibitor-associated unconjugated hyperbilirubinemia is not associated with hepatotoxicity and is completely reversible with discontinuation of the protease inhibitor therapy [4, 5]. Therefore, additional consultation should not be necessary in the setting of protease inhibitor-associated hyperbilirubinemia. Furthermore, in large trials, treatment discontinuation for hyperbilirubinemia occurs rarely (<1%) [5, 6].

Rotger et al. do not explore the financial cost of adding genetic screening before antiretroviral therapy initiation, which would be considerable if applied to the entire HIV-infected population. The charge for characterizing the UDP-glucuronosyltransferase 1A1 (*UDPGT1A1*) promoter can be ~\$300. Cost-effectiveness analyses are needed to determine the utility of such screening measures. There are limited data on the cost-effectiveness of genetic screening for benign medical conditions in HIV infection or other conditions. In addition to its financial constraints, genetic testing for the A(TA)₇TAA allele is almost completely unavailable in resource-limited settings. The authors also do not discuss the burden of unnecessarily excluding from taking atazanavir or indinavir substantial numbers of individuals who are homozygous or heterozygous for the A(TA)₇TAA allele and will not experience clinical jaundice.

Finally, the authors' statement that the A(TA)₇TAA allele is associated with the same "physiological effect" in nonwhite populations should be made with caution. Data from population studies indicate that jaundice and hyperbilirubinemia are multifactorial in any given subject. For instance, studies have indicated that Asian infants have a higher—and African American infants a lower—incidence of hyperbilirubinemia, compared with their white counterparts [7]. In work referred to by Rotger et al., it was speculated that the prevalence of the A(TA)₇TAA variant

UDPGT1A1 promoter would therefore be highest in Asian subjects, intermediate in white subjects, and lowest in African American subjects [8]. Contrary to this expectation, the A(TA)₇TAA variant was most common among African Americans and least common among subjects of Asian origin [8]. Thus, although there is a relationship between *UDPGT1A1* promoter repeat number and *UDPGT1A1* activity (and jaundice) within a racial group, this correlation does not appear to hold across ethnic groups.

In conclusion, although genetic screening offers much promise for decreasing the frequency of adverse events related to medications, we feel that such testing should be reserved for the prevention of serious or irreversible complications. Genetic screening for predisposition to a well-characterized, rapidly reversible adverse event that is not associated with an undesirable medical outcome seems unwarranted.

**Richard E. Nettles, Michael J. Child,
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Reply to Nettles et al.

To the Editor—Nettles et al.'s [1] comments are consistent with the discussion on the genetic predisposition to unconjugated hyperbilirubinemia in the article by Rotger et al. [2]. However, the mechanism leading to jaundice in newborns that Nettles et al. refer to is not the appropriate example for discussing the genetics of Gilbert syndrome across ethnic groups. In addition, I disagree with 2 of their statements: that stigmatizing a patient is a minor issue as long as the treatment is discontinued and that genetic screening should be reserved for the prevention of serious, irreversible complications.

In the management of a disease that necessitates long-term (lifelong) treatment, "minor" adverse effects are frequent and important [3]. A bout of diarrhea, some nausea, and the occasional jaundice are not to be minimized in importance, in particular when tolerance and toxicity currently constitute the main reasons for treatment discontinuation and change [4].

The cost of genetic testing is rapidly decreasing [5], and our understanding of pharmacogenetics is increasing [6]. This should allow for the development of tests for genetic prediction of toxicity and ef-

ficacy to guide treatment choice among the multiple available antiretroviral drugs. *UGT1A1* is just one of the various genes that could be included in a testing panel. There is limited interest in interrogating any single gene or in aiming analysis at any particular drug.

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Heterosubtypic Immunity to Influenza: Right Hypothesis, Wrong Comparison

To the Editor—We read with interest Epstein's analysis of influenza in Cleveland families [1]. We do not believe that the results convincingly demonstrate heterosubtypic immunity—that is, immunity that is elicited by influenza virus infection

and that partially prevents reinfection by different influenza virus subtypes. Epstein's main conclusions are drawn from the difference in influenza attack rates, during the 1957 (pandemic) study year, between children and adults who had had influenza during earlier years (16/29 [55%] vs. 1/18 [6%]; $P = .002$). Yet a strong difference was also observed between children and adults who had not been infected during earlier years (11/66 [17%] vs. 39/75 [52%]; $P < .001$), whereas no difference was noted, in either adults (17% vs. 6%; $P = .28$, Fisher's exact test) or children (55% vs. 52%; $P = .94$), between individuals who had had influenza and those who had not. Our interpretation of these findings is that there was a difference, irrespective of prior exposure, between the attack rate in children and that in adults. This is not surprising [2]. Heterosubtypic immunity to human influenza infection is supported by both biological evidence [3] and epidemiological theory [4, 5], although, in our view, it cannot be deduced from Epstein's comparison of pandemic attack rates in children versus those in adults.

Fabrice Carrat and Audrey Lavenu

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Reply to Carrat and Lavenu

To the Editor—Carrat and Lavenu comment that the Cleveland Family Study data do not convincingly demonstrate heterosubtypic immunity [1]. My analysis of the subgroup of individuals who had had influenza during the earlier years of that study showed an apparent protective effect of prior illness in adults but not in children; however, my article pointed out that the difference in outcome in adults with such episodes versus those without such episodes was not statistically significant [2, p. 51]. Carrat and Lavenu show this another way, and I accept their point. They and I reached essentially the same conclusion—that “[t]hese historical data alone cannot prove the existence of cross-protection” [2, p. 52]. I felt that the limited data available, although not statistically adequate, were important to describe, for the following reasons:

1. Pandemic influenza caused illness in a 3-fold-lower percentage of adults with prior influenza during the study years than those without (5.6% vs. 16.7%), suggesting an impact of the prior infections, while in children the percentage if anything was slightly higher (55.2% vs. 52.0%). Also note that the overall difference between adults and children in the study population was pronounced only in the pandemic year [2, table 1].

2. The study is valuable for the richness of clinical and laboratory details, and it demonstrates that the low rates of illness in adults were not due to lack of exposure; most of these adults were exposed to the pandemic virus within their own families.

3. These data describe experience during a pandemic; the article does not

make indirect inferences from other kinds of studies. In fact, both the overall difference between adults and children and the small number of adult cases are likely consequences if there is heterosubtypic immunity. This means that a much larger data set is necessary to allow a conclusion to be drawn; however, an informative, larger data set with similar clinical and laboratory details is not available from a pandemic.

Studies of human T cell reactivities and epidemiological modeling studies—such as those which Carrat and Lavenu mention—provide valuable indirect support, but they cannot prove heterosubtypic immunity either. Cross-reactive human T cells are found (references 21 and 22 in the paper and their reference 3) but would not necessarily prevent illness, and their potential use for the control of a pandemic is much debated.

Many investigators have shown that vaccine candidates based on one influenza A subtype are effective in protecting mice against challenge with viruses of various other subtypes [3–6], including H5N1 [7–9]; however, these results have been challenged as being unlikely to translate to humans, and clinical testing has been very limited. One purpose of my article's description of the historical data was to show that the findings suggest an impact of heterosubtypic immunity—and to call for further investigation. Future studies could test the question in adults—and, if the results warrant, could later explore whether there are ways to induce cross-protection in children.

Thank you to Carrat and Lavenu for their attention to both the issue and the data. I hope that this discussion will encourage prospective clinical trials and epidemiological surveillance studies to provide more definitive information, which they have also advocated [10].

Suzanne L. Epstein

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Excretion of Oral Poliovirus Vaccine Strains: Effect of Vaccination Schedule

To the Editor—In the 15 December 2005 issue of the *Journal*, Laassri et al. report

their study of gastrointestinal mucosal immunity after different polio-vaccine schedules. The major focus of the study was the influence that previous vaccination with inactivated poliovirus vaccine (IPV) has on excretion of oral poliovirus vaccine (OPV) administered as the third dose in a sequential vaccine schedule [1].

In a 1997 issue of the *Journal*, we and our colleagues published an article that described the influence that 5 different combinations of IPV and OPV have on subsequent excretion of polioviruses after a challenge dose of trivalent OPV has been administered [2]. The IPV that we employed was identical, in both potency and immunogenicity, to the IPV that currently is distributed in the United States and that was used in the study by Laassri et al. [3]. As did Laassri et al., we measured OPV virus excretion, by type, at weeks 1 and 3 after challenge. We did not study infants given just 1 dose of OPV, because previous published reports had amply demonstrated that >90% of infants shed OPV viruses in feces after their first dose of OPV had been administered [4–6].

For the groups of major interest (i.e., infants given only IPV and infants given only OPV before challenge), the excretion rates observed by Laassri et al. are quite similar to those that we had reported. Therefore, we are gratified to see our results confirmed by their study.

It is important to remember that the data from OPV challenge studies have greater comparative than absolute value. For virtually all reported studies, the OPV challenges have been scheduled within a few months after completion of the vaccine schedule(s) under study. Because intestinal immunity induced by IPV probably wanes with time, as it does after an OPV series has been administered [7], one cannot rely on the data from these studies to predict the degree of intestinal immunity induced by IPV years after completion of the IPV series. This may be an important consideration when one is assessing the influence that IPV vaccination has on the circulation of live polioviruses

after cessation of routine OPV immunization globally; however, the fact that, despite repeated introductions of live polioviruses by travelers, there is no detectable circulation of wild- or vaccine-type polioviruses in IPV recipients in countries where IPV has been used for many years provides some reassurance that this will not be a major problem [8].

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