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Reply

Sir—We appreciate the comments of Tacconelli and colleagues regarding host factors and the risk of Clostridium difficile–associated diarrhea (CDAD). Although the clinical presentation of CDAD and response to therapy in HIV-infected persons may not differ from that in HIV-seronegative persons [1], data from Tumarello et al. [2] indicate an increased risk of CDAD in HIV-infected persons. This increased risk may be explained partially by frequent and/or prolonged hospitalizations and antibiotic exposures in these patients, but it is possible that HIV-induced immunosuppression may also predispose patients to CDAD by, as yet, unknown mechanisms. The question of a higher incidence of CDAD in HIV-infected patients (2.4%) after HAART therapy compared to HIV-seronegative patients (0.9%) cannot be resolved without knowing the rate of antibiotic use in the two patient groups. Data from Cook County Hospital in Chicago also document an increased risk for CDAD in HIV-infected patients at that institution [3]. HIV coinfection was documented in 40% of the CDAD cases at Cook County Hospital, despite accounting for only 10% of the hospital census. Recent data from Peru suggest that infection due to C. difficile (as detected by a stool toxin assay) may also influence the course of HIV infection [4]. In that study, C. difficile infection, but not CDAD per se, was associated with increased mortality, although it appears that CDAD was not treated in these patients. This finding needs to be confirmed in a patient population in which CDAD is diagnosed and treated in a timely fashion.

What aspects of immunity are important or critical host responses to this pathogen? We and others [5] have looked at serum and mucosal antibodies to C. difficile toxins and nontoxin proteins in patients with CDAD. Although the relation of antibodies to the clinical course of C. difficile infection is controversial, some patients with chronic, relapsing CDAD have responded to intravenous therapy with pooled human immune globulin. Investigation in this area has also resulted in new insights into the role of serum IgA following infection with this mucosal pathogen [6]. Further studies of humoral, mucosal and, potentially, cell-mediated immunity to infection with C. difficile are warranted.

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Metronidazole Resistance in Helicobacter pylori

Sir—Metronidazole is widely used to treat many bacterial and protozoan infections in developing and developed countries. An article in Clinical Infectious Diseases by Dore et al. [1] and an accompanying editorial response by Solnick [2] describe as an “unexpected” finding the observation that metronidazole-resistant and metronidazole-susceptible Helicobacter pylori colonies can be isolated from the same patient, and that these colonies are identical with respect to all other genetic traits. The authors conclude that the mechanism of metronidazole resistance is not known [2] and that “studies addressing drug resistance mechanisms are urgently needed” [1]. In this case, we are quite lucky because while the article and the editorial response were in press, the mechanism of H. pylori resistance to metronidazole was described in an article in Molecular Microbiology [3]. The study shows beautifully that metronidazole is not the active form of the antibiotic and that a bacterially encoded oxygen-insensitive nicotinamide adenine dinucleotide phosphate (NADPH) nitroreductase (rdxA, HP 0954 on the genome sequence, see [4]) is required to reduce metronidazole to the active hydroxylamino-derivative, which is directly responsible for killing H. pylori. The resistance is simply a result of the inactivation of the rdxA gene and therefore a consequence of the inability of the bacterium to transform the metronidazole into the active hydroxylamino-derivative (figure 1). Goodwin et al. [3] also showed that one to three amino acid mutations within the rdxA gene are adequate to inactivate the gene. The role of the rdxA gene in metronidazole resistance was independently confirmed by Debets-Ossenkopp et al. [5], who studied metronidazole resistance in the NCTC11638 strain of H. pylori. In this case, they found that resis-
Figure 1. Schematic representation of the mechanism of action of metronidazole against Helicobacter pylori. (A) Susceptible strains encode a nitroreductase (RdxA) that reduces the nitro group of metronidazole to the hydroxylamino-intermediate that is known to be a powerful antibacterial agent and mutagen. (B) Resistant strains accumulate mutations that inactivate the nitroreductase gene and are unable to reduce metronidazole into the active hydroxylamino-intermediate. R = an imidazole ring. (Only the relevant steps of the chemical reaction are shown).

The acquisition of metronidazole resistance requires the inactivation of a gene (rdxA) that is naturally present in the H. pylori chromosome, instead of the acquisition of a new gene by horizontal transfer, as is the case in the acquisition of many antibiotic resistances. Knowing the above mechanism, it is no longer surprising to isolate a mixed population of metronidazole-susceptible (MtzS) and metronidazole-resistant (MtzR) colonies of the same strain from a patient that has been treated with antibiotics. In fact, the MtrS colonies are natural mutants of the resistant strain that are selected in situ by the antibiotic. The finding that is perhaps worrisome about the mechanism of action of metronidazole is that it is a pro-drug that is transformed into a potent mutagen in the human stomach by resident MtzS H. pylori. This should be carefully evaluated given the established correlation between H. pylori infection and gastric cancer.

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Reply

Sir—Several hypotheses regarding the mechanism of metronidazole resistance in Helicobacter pylori have been suggested. Resistance can occur in organisms with an enhanced DNA repair capability caused by point mutations in the recA protein [1] or by the inability of oxygen scavenging mechanisms to activate metronidazole at the site of metronidazole reduction [2]. As pointed out by Rappuoli et al., recently, Goodwin et al. [3] also reported that mutations of the RdxA protein conferred metronidazole resistance to H. pylori. We found, however, that the rdxA gene was not inactivated in metronidazole resistant H. pylori, but that it was fully repressed in the presence of metronidazole [4]. Hence contrary to Rappuoli et al., we feel that the currently available data on RdxA are insufficient to completely explain the mechanism of metronidazole resistance in H. pylori.

It has been proposed that the mode of action of metronidazole in H. pylori is similar to that of anaerobic bacteria, even though the optimal in vitro culture conditions are microaerobic [5]. The antimicrobial action of metronidazole in anaerobic bacteria is thought to be a three-step process: entry of metronidazole into the cell, reductive activation of metronidazole (becoming toxic metronidazole), and damage to the cell by the toxic reduced products [6–8]. The most important step is the reductive activation of metronidazole that is dependent on the redox system of the target cell. Any redox system in the cell possessing a reduction potential more negative, or lower, than that of the metronidazole will donate its electrons preferentially to metronidazole (becoming toxic metronidazole), and damage to the cell by the toxic reduced products [6–8]. The direct donors of electrons in anaerobic bacteria are thought to be ferredoxin-linked proteins such as ferredoxin, pyruvate oxidoreductase (POR), ferredoxin oxidoreductase (FOR), ferredoxin-like protein (FdxB), or oxygen-insensitive nitroreductase (RdxA) [3, 10–12].

The H. pylori genome contains several genes that code for such proteins. Recently, using quantitative reverse transcriptase (RT)-PCR analysis of total RNA prepared from H. pylori cultured in the presence of metronidazole, we showed that transcription from the gene encoding RdxA was fully repressed and the genes encoding POR, FOR, and FdxB were all dramatically