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ORIGINAL ARTICLE

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ABSTRACT

BACKGROUND

Women with cystic fibrosis are at increased risk for mucoid conversion of *Pseudomonas aeruginosa*, which contributes to a sexual dichotomy in disease severity.

METHODS

We evaluated the effects of estradiol and its metabolite estriol on *P. aeruginosa* in vitro and in vivo and determined the effect of estradiol on disease exacerbations in women with cystic fibrosis.

RESULTS

Estradiol and estriol induced alginate production in *P. aeruginosa* strain 01 and in clinical isolates obtained from patients with and those without cystic fibrosis. After prolonged exposure to estradiol, *P. aeruginosa* adopted early mucoid morphology, whereas short-term exposure inhibited bacterial catalase activity and increased levels of hydrogen peroxide, which is potentially damaging to DNA. Consequently, a frameshift mutation was identified in *mucA*, a key regulator of alginate biosynthesis in *P. aeruginosa*. In vivo levels of estradiol correlated with infective exacerbations in women with cystic fibrosis, with the majority occurring during the follicular phase ($P<0.05$). A review of the Cystic Fibrosis Registry of Ireland revealed that the use of oral contraceptives was associated with a decreased need for antibiotics. Predominantly nonmucoid *P. aeruginosa* was isolated from sputum during exacerbations in the luteal phase (low estradiol). Increased proportions of mucoid bacteria were isolated during exacerbations occurring in the follicular phase (high estradiol), with a variable *P. aeruginosa* phenotype evident in vivo during the course of the menstrual cycle corresponding to fluctuating estradiol levels.

CONCLUSIONS

Estradiol and estriol induced mucoid conversion of *P. aeruginosa* in women with cystic fibrosis through a mutation of *mucA* in vitro and were associated with selectivity for mucoid isolation, increased exacerbations, and mucoid conversion in vivo. (Funded by the Molecular Medicine Ireland Clinician–Scientist Fellowship Programme.)

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Pseudomonas aeruginosa is a gram-negative opportunistic pathogen associated with cystic fibrosis,[1] a multisystem genetic disease characterized by defects in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which results in recurrent infective exacerbations. Median overall survival among patients with cystic fibrosis in the United States is 39 years, although a sexual dichotomy persists in disease severity,[2-4] with women having a survival disadvantage and poorer lung function.[5-7]

The Republic of Ireland has the highest incidence and carrier rate of cystic fibrosis worldwide[8] and a survival disadvantage for women.[9] Patients with cystic fibrosis become colonized and infected with nonmucoid *P. aeruginosa;* these strains convert to mucoid strains, on average, at a younger age in women than in men.[3,10,11] In an attempt to explain these observations, we and others have investigated the effect of the female sex hormone estradiol on airway biology. Women with cystic fibrosis appear to be at greater risk for exacerbations during periods of high circulating levels of estradiol, when airway-surface liquid is diminished and antimicrobial peptides are scarce and there is a blunted inflammatory response to microbial agonists.[12-14] However, these observations have not been confirmed in vivo. In addition, the role of estradiol-derived metabolites in cystic fibrosis remains largely unknown, with limited data linking estradiol with pseudomonas infection.[14]

The persistence of *P. aeruginosa* in cystic fibrosis has been attributed to hypermutability, drug resistance, loss of virulence, reduced bacterial killing, and defective CFTR.[14-16] Antibiotics delay but do not prevent mucoid conversion. Of equal significance is the capacity of *P. aeruginosa* to form biofilms and convert to mucoidy in vivo.

Alginate, the predominant polysaccharide produced by *P. aeruginosa*, defines its mucoid conversion; it is implicated in pathogenicity and can induce parenchymal damage.[17] Nonmucoid *P. aeruginosa* can be induced to release alginate under conditions characteristic of the lung milieu in patients with cystic fibrosis.[18,19] The production of biofilms is influenced by alginate, but the presence of the polysaccharide is not essential for biofilm formation.[20,21] The principal gene controlling alginate biosynthesis, *mucA,* encodes an antisigma factor that normally prevents *algT* from inducing expression of *algD*.[22,23] Alginate production and mucoid conversion are attributed to loss-of-function mutations in *mucA,* resulting in derepression of *algT* and concomitant induction of *algD*.

The sex-specific effects of *P. aeruginosa* in women with cystic fibrosis may be attributed to estradiol.[12-14] Here we evaluate the effect of estradiol and its metabolite estriol on laboratory and clinical isolates of *P. aeruginosa* and prospectively assess the effect of estradiol on infective exacerbations in women with cystic fibrosis.

METHODS

LABORATORY TESTING

Full details with respect to the *P. aeruginosa* strain 01 (PA01),[24] clinical isolates, culture conditions, bronchoscopy, cell culture, quantitative reverse-transcriptase–polymerase-chain-reaction assay, DNA sequencing of the *P. aeruginosa* phenotype,[25] and assays of catalase, hydrogen peroxide, alginate,[26] and estradiol are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

PATIENTS

From July 2008 through July 2010, we recruited all female patients with cystic fibrosis who presented to Beaumont Hospital in Dublin (44) with a total of 139 infective exacerbations, as defined by the criteria of Fuchs et al.[27] (Table S3 in the Supplementary Appendix). All patients provided written informed consent, and appropriate approval was obtained from the hospital's institutional review board.

STATISTICAL ANALYSIS

Data were analyzed by descriptive, exploratory (comparative), and modeling approaches and methods, including a technique of generation of random numbers. All results are expressed as absolute numbers and percentages, as well as means ±SD or means ±SE. We used the chi-square test or Fisher's exact test to compare categorical data distributions and Kolmogorov–Smirnov or Shapiro-Wilk methods, as appropriate, to test continuous data for normality of distribution. We used parametric methods and tests (e.g., Student's t-test) to analyze normally distributed data and two-group nonparametric Wilcoxon signed-rank and Mann–Whitney U tests to analyze data deviating from a normal distribution. We used the nonparametric Kruskal–Wallis test for the comparison of more than two independent groups. To describe exist-

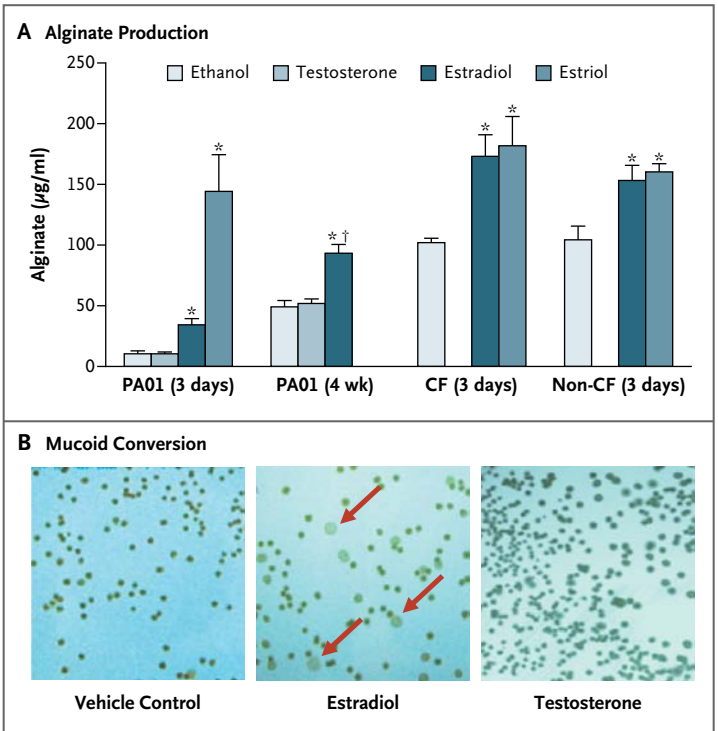

Figure 1. Alginate Production and Mucoid Conversion in *Pseudomonas aeruginosa* Induced by Estradiol and Estriol.

In Panel A, *P. aeruginosa* strain 01 (PA01) or *P. aeruginosa* clinical isolates from patients with and those without cystic fibrosis (CF) were treated for either 3 days or 4 weeks with daily subculture in fresh broth containing ethanol, testosterone, estradiol, or estriol (10 nM for each subculture). Alginate was measured with the use of the uronic acid–carbazole reaction method in three experiments. Data are expressed as means, with T bars indicating the standard deviation. The asterisk indicates P<0.05 for the comparison with ethanol or testosterone; the dagger indicates P<0.01 for the comparison between 3 days and 4 weeks of treatment with estradiol in PA01. Alginate levels were not determined in PA01 at 4 weeks in response to estriol or in CF and non-CF isolates at 3 days in response to testosterone. In Panel B, PA01 was plated onto blue agar after daily subculture for 4 weeks in fresh broth cultures containing ethanol (vehicle control), estradiol, or testosterone (10 nM of each substance). Representative culture plates are shown for three experiments. Mucoid colonies are indicated by arrows.

ing linear trends over years, we calculated the coefficients of the models (intercepts, slopes, and squares of the correlation coefficient [R^2]). Statistical analyses were performed with SPSS software, version 18 (IBM); GraphPad Prism, version 4.0 (GraphPad Software); and modeling tools as integrated in Microsoft Office Excel 2007, version SP2 MSO (Microsoft). All reported P values are two-sided, and a P value of 0.05 was considered to indicate statistical significance, unless stated otherwise.

RESULTS

EFFECT OF ESTROGEN ON ALGINATE PRODUCTION

At baseline, PA01 is nonmucoid. However, it can be induced under appropriate conditions to produce alginate.[24,28] We examined the effect of estradiol and estriol on alginate production in PA01 and in clinical isolates of *P. aeruginosa* obtained from patients with and those without cystic fibrosis (Fig. 1A). Testosterone (an estradiol precursor) and ethanol were used as controls. After 3 days of exposure, 10 nM of estradiol induced more alginate production by PA01 (33.5 μg per milliliter) than did the same amounts of testosterone (9.6 μg per milliliter) and ethanol (9.5 μg per milliliter) (P<0.05 for both comparisons). The increase in alginate production was time-dependent, with increased levels (92.2 μg per milliliter) produced after 4 weeks in continued subculture with estradiol (P<0.01 for both comparisons). Testosterone had no effect on alginate production at 4 weeks. After 3 days, estriol induced significantly more alginate production (143.1 μg per milliliter) than an equal amount of estradiol (P<0.005). Nonmucoid clinical isolates of *P. aeruginosa* similarly produced more alginate after treatment with 10 nM of estradiol or estriol for 3 days (P<0.005). Estradiol up-regulates the expression of *algD*, *algT*, and *mucA* in PA01 (Fig. S1 in the Supplementary Appendix), and estriol was detected in samples of estradiol-treated, immortalized bronchial epithelial cells obtained from patients with and those without cystic fibrosis and in bronchoalveolar-lavage fluid from patients with cystic fibrosis (Fig. S2 and S3 in the Supplementary Appendix).

MUCOID CONVERSION OF *P. AERUGINOSA*

Blue agar plates were used to detect mucoid colonies.[25] After daily subculture for 3 days in fresh broth containing estradiol, testosterone, or ethanol with daily replenishment, no differences in morphology were observed. However, after 4 weeks, early mucoid colony formation was noted in the estradiol-treated cultures (Fig. 1B). More mucoid colonies were detected in the estradiol-treated cultures (5.5×10^8 colony-forming units [CFU] per milliliter for mucoid colonies vs. 2.9×10^9 CFU per milliliter for nonmucoid colonies), than in ethanol-treated cultures (3.3×10^8 CFU per milliliter for mucoid colonies vs. 5.0×10^9 CFU per milliliter for nonmucoid colonies) or testosterone-treated cul-

tures (3.1×10^8 CFU per milliliter for mucoid colonies vs. 4.53×10^9 CFU per milliliter for nonmucoid colonies), equating to 15.9%, 6.2%, and 6.4% mucoid colonies, respectively.

GENETIC MUTATIONS AND MUCOID CONVERSION

Conversion to mucoidy within the lungs of patients with cystic fibrosis results from spontaneous mutations in *mucA*, which was sequenced in both mucoid and nonmucoid colonies grown in estradiol and ethanol, respectively. In 80% of mucoid colonies with exposure to estradiol, a deletion of the nucleotide adenine at position 60 of *mucA* occurred, resulting in a frameshift mutation of glutamic acid (E) to aspartic acid (D) at residue 20 of the MucA protein and the introduction of a premature stop codon (Fig. S4 in the Supplementary Appendix). One mechanism by which this occurs involves estradiol-induced inhibition of catalase activity and increased production of hydrogen peroxide in *P. aeruginosa* (Fig. S5 in the Supplementary Appendix).

ESTRADIOL LEVELS AND INFECTIVE EXACERBATIONS

To assess whether fluctuations in estradiol levels during the menstrual cycle are associated with infective exacerbations, we performed a 24-month prospective clinical study in which we assessed 139 exacerbations in 44 women with cystic fibrosis. We excluded patients who reported having irregular menstrual cycles or the use of oral contraceptives. We plotted 72 exacerbations related to a regular menstrual-cycle pattern in 23 women according to the stage of the cycle when the exacerbation occurred. A serum estradiol curve was prospectively generated from data obtained from 6 women with cystic fibrosis who had a regular menstrual cycle. Exacerbations closely mirrored in vivo estradiol levels. Significant increases in the number of exacerbations occurred during the follicular phase, when estradiol levels peak ($P<0.05$) (Fig. 2A, and Table S3 in the Supplementary Appendix).

To validate the relationship between elevated estradiol levels and the number of infective exacerbations, we assessed serum from 172 patients with cystic fibrosis during a 36-month period (Fig. 2B, and Table S4 in the Supplementary Appendix). The mean serum estradiol level was significantly higher in women with exacerbations than in those with stable disease (777 pmol per liter vs. 303 pmol per liter, $P<0.008$). This pattern was absent in women receiving oral contraceptives and in men. The exacerbation rate per year was significantly lower in women receiving oral contraceptives than in other groups (Fig. S6 in the Supplementary Appendix).

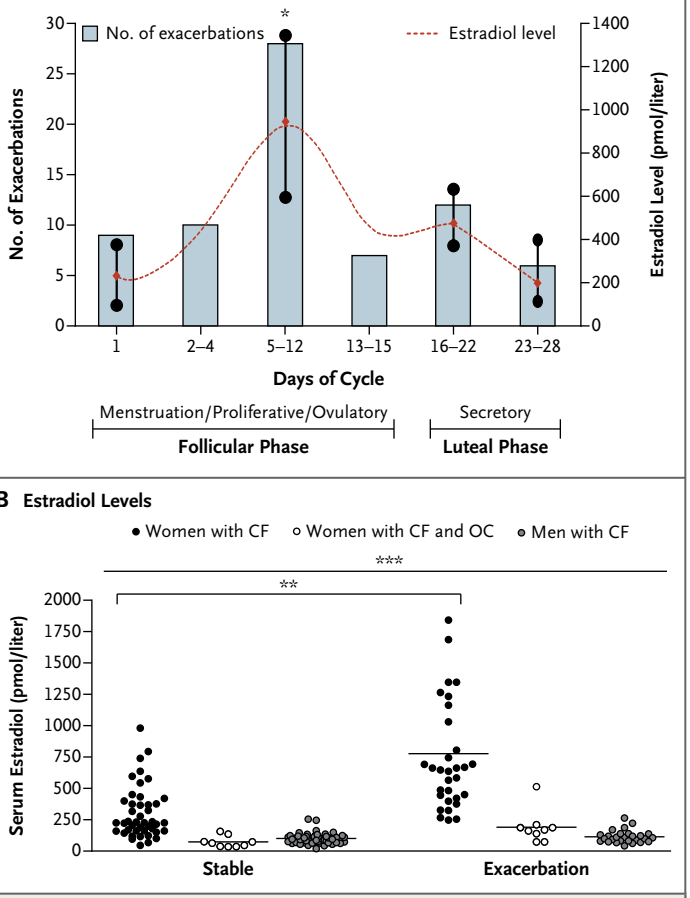

Figure 2. Exacerbations and Estradiol Levels in Women with Cystic Fibrosis.
Panel A shows the number of infective exacerbations according to the time of the menstrual cycle, as recorded for 44 women with cystic fibrosis and plotted against circulating estradiol levels (as measured in 6 women with cystic fibrosis). Shown are means and the range of values. The asterisk indicates $P<0.05$ for the comparison between the proliferative phase of the menstrual cycle (the highest estradiol level) and the menstrual, ovulatory, or secretory phase. Panel B shows serum estradiol levels for 172 patients with cystic fibrosis (CF) classified according to sex and use of oral contraceptives (OC). The double asterisks indicate $P<0.01$, and the triple asterisks indicate $P<0.001$.

IRISH REGISTRY DATA

Next we analyzed data from the Cystic Fibrosis Registry of Ireland during years when the rate of

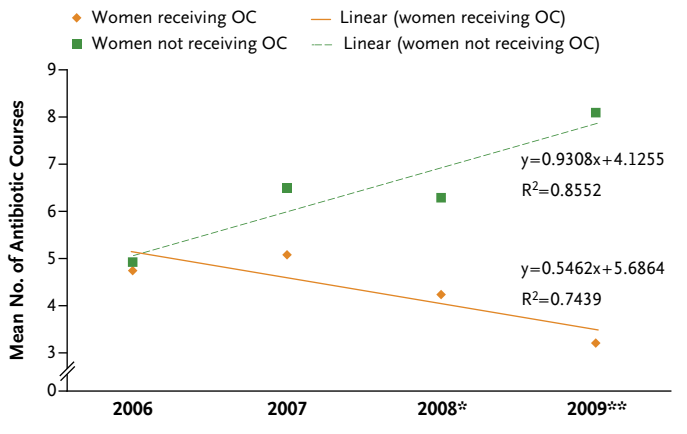

Figure 3. Effect of Oral Contraceptives on the Number of Courses of Antibiotics Required per Year in Women with Cystic Fibrosis.

Available data from the Cystic Fibrosis Registry of Ireland for 239 women (≥18 years of age) were obtained for the years 2001 through 2010, but only years when registry ascertainment was more than 55% (2006–2009) are shown. The mean numbers of courses of antibiotics (oral or intravenous) that were received for clinical deterioration, as determined by the local attending physician, are shown for 36 women receiving oral contraceptives (OC), as compared with a random sample of 41 women not receiving oral contraceptives (approximately 20% of the sample), for the respective time periods. Opposing trends were detected between the two groups. The model equations present the coefficients of linear regression: intercept *a*, slope *b*, and the square of the correlation coefficient (R^2) for the decreasing tendency of women receiving oral contraceptives to require antibiotics (orange line) versus the increasing tendency of women not receiving oral contraceptives to require antibiotics (green line). On the basis of annual comparisons by means of the Mann–Whitney nonparametric test, a single asterisk indicates P=0.07, and double asterisks indicate P=0.002.

ascertainment (i.e., the proportion of registered patients vs. the census population of persons with cystic fibrosis) was more than 55% (2006 through 2009) among women 18 years of age or older, according to their use of oral contraceptives (Table S8 in the Supplementary Appendix). Of the 239 patients whose data were analyzed, 15.1% used oral contraceptives, although the type of oral contraceptive was not recorded. For standardization and because definitions of exacerbations vary among centers, we assessed the mean number of antibiotic courses (either oral or intravenous) required for the treatment of clinical deterioration and its relationship to the use of oral contraceptives. In order to compare the 36 women receiving oral contraceptives with those not receiving oral contraceptives in an approximate ratio of 1:1, we selected a random sample of 41 control women from the remaining 203 women in the registry (approximately 20%). We found a decreasing tendency to require antibiotics in women receiving oral contraceptives, as compared with those not receiving oral contraceptives (Fig. 3). These differences were most significant where registry ascertainment was highest (Table S8 in the Supplementary Appendix). There was a significant reduction in the mean number of antibiotic courses required during times when individual women were receiving oral contraceptives, as compared with times they were not receiving oral contraceptives (Fig. S7 in the Supplementary Appendix).

SEX DIFFERENCES IN COLONIZATION AND MUCOID CONVERSION

In patients with stable cystic fibrosis who were colonized with *P. aeruginosa*, mucoid *P. aeruginosa* was isolated at twice the frequency in women as in men as a proportion of total culture counts (26.2% mucoid cultures in 6 women vs. 10.0% in 4 men) (Table S6 in the Supplementary Appendix). To assess rates of *P. aeruginosa* colonization and mucoid conversion, we explored all data from the Irish registry up to December 31, 2010 (involving 455 women and 554 men) (Table S9 in the Supplementary Appendix). Chronic colonization was defined as isolation of *P. aeruginosa* from at least two sputum samples obtained 6 months apart (in ≥50% of samples) in consecutive years, the first of which was recorded as the year of acquisition. In this analysis, 375 of 1009 patients (37.2%) were colonized by *P. aeruginosa*, with a mean (±SD) age of acquisition of 20.8±9.0 years, as defined by the modified Leeds criteria.[29] The female patients had significantly higher colonization rates (absolute difference, 11.9 percentage points; P<0.001) and acquired it 2.3 years earlier (19.7 vs. 22.0 years, P=0.01) (Table S9 in the Supplementary Appendix). *P. aeruginosa* converted to mucoidy in significantly more female patients (Fig. S8 in the Supplementary Appendix), and the conversion occurred 1.8 years earlier in women than in men (Table S9 in the Supplementary Appendix).

P. AERUGINOSA PHENOTYPE ACCORDING TO MENSTRUAL CYCLE

We recorded the predominant *P. aeruginosa* phenotype in sputum samples obtained from patients in the prospective study. Of the 72 exacerbations in 23 patients who were included in the final analysis, *P. aeruginosa* was not isolated from 4 patients in

15 exacerbations. Using data from the remaining patients, we plotted the percentage of nonmucoid versus mucoid *P. aeruginosa* according to the menstrual-cycle phase when the exacerbation occurred. Mucoid *P. aeruginosa* accounted for 63.0% of the isolates in the follicular phase (high estradiol), as compared with 16.7% nonmucoid isolates. In the luteal phase (low estradiol), we isolated no mucoid *P. aeruginosa*, whereas 72.2% of the samples contained nonmucoid *P. aeruginosa* (Fig. 4A).

To further assess this relationship, we recorded changes in the proportion of mucoid and nonmucoid *P. aeruginosa*, using serially diluted sputum samples obtained from six women with cystic fibrosis and stable menstrual cycles who were known to be colonized by both organisms. In these women, we observed an increase in mucoid isolation at times of high estradiol levels (Fig. 4B, and Fig. S9 in the Supplementary Appendix). This finding explains the lack of mucoid isolation during the luteal phase that was observed in our undiluted sputum samples (Fig. 4A). We also assessed changes in mucoid and nonmucoid proportions in sputum samples during stable and exacerbation states. During exacerbations, nonmucoid counts tripled, as compared with the stable state, whereas mucoid counts increased by a factor of more than 50. These changes occurred in the presence of an increased serum estradiol level (Fig. S10 in the Supplementary Appendix).

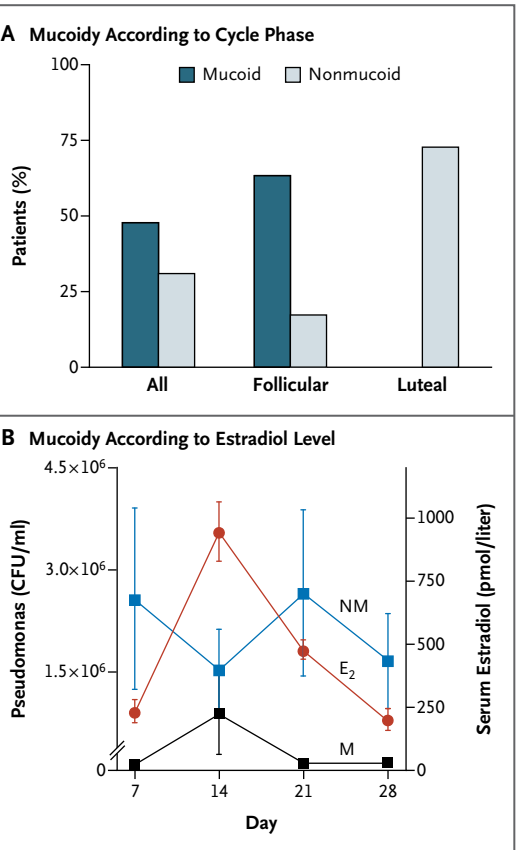

Figure 4. Mucoid and Nonmucoid Pseudomonas Species and Estradiol Levels in Women with Cystic Fibrosis.

Panel A shows the percentage of patients who were colonized with mucoid versus nonmucoid pseudomonas species during exacerbations throughout the menstrual cycle. Pseudomonas species were cultured from undiluted sputum samples obtained from women with cystic fibrosis who were undergoing exacerbations (54 in the follicular phase and 18 in the luteal phase) and identified as either mucoid or nonmucoid. Panel B shows the in vivo variability of the *P. aeruginosa* phenotype in relation to levels of serum estradiol (E_2) during the course of the menstrual cycle in 6 women with stable cystic fibrosis and regular menses, according to whether the sample was mucoid (M) or nonmucoid (NM).

MUCOID CONVERSION AND PREGNANCY

In further support of the hypothesis that estradiol and estriol are important factors in mucoid conversion of *P. aeruginosa* in vivo, we also assessed the physiologically estriol-dominant state of pregnancy. We assessed patients who were colonized with nonmucoid *P. aeruginosa* before pregnancy for the time to mucoid conversion after pregnancy. During a 15-year period (1996–2010), this occurred in 4 of 7 pregnancies (57.1%) within 12 months after delivery and in 5 of 7 pregnancies (71.4%) within 24 months (in a total of 11 pregnancies) (Table S10 in the Supplementary Appendix). Data from the Irish registry from 2001 through 2010 (involving 14 pregnancies) showed that mucoid conversion occurred in 5 of 7 pregnancies (71.4%) within 12 months after delivery and in 6 of 7 (85.7%) within 24 months (Table S10 in the Supplementary Appendix).

DISCUSSION

It has been observed that women with cystic fibrosis acquire *P. aeruginosa* in advance of men and convert to mucoid strains prematurely.[3,5,10,11] In our study, we found that estradiol promoted mucoid conversion of *P. aeruginosa*, increased algi-

nate production in *P. aeruginosa*, and (owing to impaired catalase activity and increased levels of hydrogen peroxide) selected for mutations in *mucA*, a negative regulator of alginate synthesis. In vivo estradiol levels correlated with infective exacerbations among menstruating women with cystic fibrosis, and mucoid *P. aeruginosa* was selectively grown during periods with high levels of circulating estradiol.

Microbial endocrinology suggests that estradiol may directly affect the development of prokaryotes.[30,31] Proteinaceous estrogen-binding receptors have been reported in *P. aeruginosa* and *Escherichia coli*.[32-34] *P. aeruginosa* can actively metabolize steroid hormones[35] and use estradiol as a carbon source.[36] In our study, estriol, although less potently estrogenic than estradiol, was a stronger inducer of alginate. Airway epithelial cells can metabolize estradiol to estriol, which was also detected in samples of bronchoalveolar-lavage fluid obtained from women with cystic fibrosis. Other airway organisms and inflammatory cells may contribute to this pool of estriol. Although estriol was detected in samples of bronchoalveolar-lavage fluid obtained from men with cystic fibrosis, the levels were significantly lower than those obtained from women.

Nonmucoid PA01 can produce alginate[18] and is a suitable model for the study of alginate production.[24,28] Estradiol significantly increased alginate production related to early mucoid morphology on blue agar after 4 weeks.[37] In translating our findings to the airways of women with cystic fibrosis, we found that clinical isolates of *P. aeruginosa* produced higher baseline and estradiol-induced alginate levels than did PA01. Thus, long-term exposure of *P. aeruginosa* to estradiol after menarche from repeated menstrual cycling places women with cystic fibrosis at increased risk for mucoid conversion. The high rates of mucoid conversion among women in the Irish registry and after pregnancy support this finding.

We also identified a mutation in *mucA* that leads to mucoidy. Neutrophils in the lungs of patients with cystic fibrosis release hydrogen peroxide, a DNA-damaging agent that can result in mutations in *mucA*.[38] Estradiol can independently generate hydrogen peroxide and other free radicals.[39,40] Consequently, we assessed the effect of estradiol on hydrogen peroxide generation by *P. aeruginosa*. We found elevated hydrogen peroxide levels after short-term exposure of *P. aeruginosa* to estradiol, and the activity of catalase, the enzyme that detoxifies hydrogen peroxide, was inhibited.

Among patients with cystic fibrosis, mucoid *P. aeruginosa* is observed at higher frequencies in women than in men, and female sex is recognized as a risk factor for early conversion.[10,11] The median age of chronic infection with mucoid *P. aeruginosa* has been reported to be 1.7 years earlier in women, which accelerates the rate of decline in pulmonary function.[3] The age-specific prevalence of mucoid *P. aeruginosa* in patients with cystic fibrosis markedly increases after puberty.[1] In the United States, a case–control analysis of the Cystic Fibrosis Foundation registry confirmed that an earlier age of *P. aeruginosa* infection was associated with increased odds of severe lung disease, a relationship that was found to be stronger in women.[41] Although no significant differences were detected in the prevalence of chronic *P. aeruginosa* infection in a Scandinavian population, the incidence of new infection was higher in women.[5] An assessment of the Irish registry showed that rates of *P. aeruginosa* colonization and mucoid conversion were higher in women than in men, along with earlier ages of acquisition and mucoid conversion.

To investigate the link between estradiol, infective exacerbations, and mucoidy in women with cystic fibrosis, we conducted a clinical study relating infective exacerbations to the day in the menstrual cycle when the exacerbation occurred. A significant relationship between exacerbations and estradiol levels was evident, with the majority of exacerbations occurring during the follicular phase, a stage with the highest estradiol level.

At our institution, women with cystic fibrosis who were receiving oral contraceptives had lower rates of exacerbation. Using registry data, we found that women receiving oral contraceptives required a lower number of antibiotic courses than did women not receiving oral contraceptives and that individual women required less use of antibiotics during the time they were receiving oral contraceptives than during the time they were not receiving oral contraceptives. Although oral contraceptives may be protective against exacerbations, a double-blind, placebo-controlled study of the use of oral contraceptives in women with cystic fibrosis would be necessary to provide proof. Future work should also account for the various subtypes of oral contraceptives, colonization with other organisms, and their antibiotic resistances.

During our clinical study, although mucoid bacteria were predominantly isolated during high-

estradiol phases of the menstrual cycle, as compared with nonmucoid bacteria in low-estradiol phases, proportional differences between the *P. aeruginosa* phenotypes were evident, a finding that illustrates a selective isolation of mucoid *P. aeruginosa* during periods of high levels of circulating estradiol. Although much has been learned about the role of estradiol within the airways of patients with cystic fibrosis, further work is necessary, particularly in the assessment of the use of oral contraceptives and its potential effect on exacerbations and the microbial endocrine effects of estradiol on *P. aeruginosa*.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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