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# Cytokine pattern in cystic fibrosis patients during antibiotic therapy and gene therapy using adenoviral vector



## Abstract

Cystic fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Despite improvements in treatment, pulmonary disease still remains the primary cause of death among these patients. In order to introduce a normal CFTR gene copy into airway epithelial cells, adenoviral vectors (AV) have been developed. AV are known to induce an inflammatory reaction that limits transgene expression, and can be potentially harmful. No human study has clearly monitored

simultaneously, systemic and local inflammatory reaction, during AV administration. We report here the levels of C-reactive protein (CRP), interleukin (IL)-6, IL-8, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-1 receptor antagonist (IL-1Ra) in plasma and bronchoalveolar lavage fluid (BALF) from six cystic fibrosis patients receiving AV encoding CFTR (AdCFTR). AdCFTR was administered to three cohorts of two patients into the nose on day 0, at doses ranging from  $10^5$  to  $4 \times 10^8$  plaque-forming units (pfu), followed, on day 1, by aerosolization of  $10^7$  to  $5.4 \times 10^8$  pfu. In order to ensure that patients were in the best clinical condition, and to further attenuate the broncho-pulmonary inflammation secondary to bacterial infection, they received antibiotic therapy, two weeks prior to AdCFTR administration, until 9 to 11 days after. We found that antibiotics markedly decreased CRP, TNF- $\alpha$ , IL-6, IL-1Ra levels in blood. In BALF, antibiotics slightly decreased TNF- $\alpha$  levels but had no effect on IL-8 and IL-1Ra, while IL-6 levels increased. AdCFTR administration did not induce any systemic or local cytokine release. In both blood and BALF, CRP, IL-8, IL-1Ra, TNF- $\alpha$  decreased, while IL-6 levels increased between day -7 and day 3. One patient presented an asymptomatic increase of all parameters in the BALF on day 7. Twenty one days later, he displayed a clinical deterioration suggestive of an exacerbation. In conclusion, this study demonstrates that antibiotic administration tends to attenuate systemic but not local broncho-pulmonary inflammation in CF patients. In the setting of our study, AdCFTR administration did not induce cytokine release. Further studies are necessary to investigate other inflammatory markers and the mechanisms involved during AV-mediated gene transfer for a better understanding of the immune reaction, which continues to hamper the development of gene therapy for CF patients.

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## Full text

### INTRODUCTION

Cystic fibrosis (CF) is the most common recessive genetic disease in Caucasian, and is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [1]. The disease is associated with a progressive decline in lung function, with more than 90% of deaths attributable to pulmonary manifestations [2]. Since 1993, gene therapy for CF has been developed in order to transfer a normal CFTR gene copy into airway epithelial cells. Two different types of vectors can be used to transfer the normal copy of CFTR to target cells. Firstly, non-viral vectors have demonstrated their safety, but transfection is

usually transient and inefficient (review in [3]). Viral vectors, especially adenoviral vectors (AV), are known to be naturally efficient at *in vivo* infection of the respiratory tract where they cause symptoms generally associated with the common cold. Because of their natural tropism for airway cells, AV were considered to be the more obvious way to transport DNA sequences into airway epithelial cells. However, prospects for gene therapy for the treatment of CF depend upon gene transfer efficiency, stable expression of the desired therapeutic gene in airway epithelial cells [4-7] and patient safety [8]. A major limitation to the use of AV is the development of an inflammatory reaction triggered by the vector. In order to attenuate this inflammatory reaction, first generations of AV were adapted by deleting the immediate early genes E1a and E1b, which are initially activated in the adenovirus cell life cycle (review in [3]). Nevertheless, an AV-mediated inflammatory reaction still occurs [9]. This inflammatory reaction has been shown to limit the level of transfection, the expression of the CFTR gene, and can be potentially harmful for the patient [6]. Following lung administration of AV in animal models, an inflammatory response comprising two steps has been described [9-13]. The first step is associated with a lymphocyte, monocyte/macrophage and polymorphonuclear leukocyte infiltration, which has been reported to be the dominant mechanism of AV loss [14]. The second step is characterized by a perivascular and bronchiolar lymphocyte infiltration, and a specific immune response targeting adenoviral epitopes [15]. Soluble mediators such as cytokines are involved in the orchestration of this inflammatory response [11-13]. However, no study in humans has clearly monitored both the local and systemic inflammatory reaction occurring *in vivo* after administration of an AV expressing the normal CFTR cDNA (AdCFTR). We therefore used blood samples to study the systemic response and the bronchoalveolar lavage fluid to sample the inflammatory milieu in the lungs of CF patients included in a phase I clinical trial. Although data obtained in these six patients, in term of transfection efficiency, had previously been published [7], the inflammatory reaction following AdCFTR delivery remained to be reported. To ensure that patients were in best clinical condition before AdCFTR administration, and occasionally because of their poor bronchopulmonary status, patients received appropriate antibiotic treatment. Patients were still receiving antibiotic treatment at the time of AdCFTR delivery. Because of the set up of this clinical trial, we have divided our study into two parts: 1) the antibiotic effect on cytokines, by studying their levels before and after antibiotic treatment and 2) the cytokine situation after AdCFTR administration as patients had already been treated with antibiotics.

Because pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin (IL)-8 have been shown to be produced *in vitro* [16, 17] and *in vivo* after viral infection in animal models [11, 13] and in humans [18, 19], we determined their concentration in our study. In order to complete a comprehensive evaluation of the cytokine network, we also monitored the level of a cytokine receptor antagonist: the interleukin-1 receptor antagonist (IL-1Ra) and the anti-inflammatory cytokine, IL-6.

## MATERIALS AND METHODS

### *Patient characteristics, vector, doses and experimental protocol*

The present study is an extension of a previous phase I clinical trial conducted in 1993/94. The clinical study, approved by the Commission de Génie Génétique, the Commission de Sûreté Biomoléculaire, the Commission de Sûreté Virale and the Comité Consultatif de Protection des Personnes en Recherche Biomoléculaire (CCPPRB) included six CF patients in a phase I clinical trial of AdCFTR administration [7]. The main characteristics of the six CF patients at the time of the study are summarized in table 1.  $1 \times 10^5$  to  $4 \times 10^8$  plaque-forming units (pfu) of AdCFTR (a replication-deficient recombinant, E1-, E3- Ad type 5-based vector containing the human cystic fibrosis transmembrane conductance regulator cDNA) were administered by nasal instillation on day 0, followed the next day by aerosolization of  $1 \times 10^7$  pfu to  $5.4 \times 10^8$  pfu, each in a single escalating dose to three cohorts of two CF patients (Figure 1). Buffer only was administrated into the nose and lungs 9 and 14 days before day 0. Patients received adequate intravenous antibiotic therapy from day -7 to day 9 or 11, but their clinical condition required oral antibiotics from day -14 before entering the study till day -7. Clinical status and respiratory function testing were carefully followed up throughout the trial. Blood biological parameters were analyzed on day -30, -7, 0, 3, 7, 14, 21, 97 and at day -30, -7, 3, 7, 14 in bronchoalveolar lavage fluid (BALF). Because of technical reasons, no data were available from nasal lavage fluid.

### *Bronchoalveolar lavage fluid (BALF)*

Briefly, a segment of lingula or inferior right or left lobe was lavaged with 2 ml/kg of sterile 0.9% sodium chloride, instilled by gravity through a suction channel. Then, the fluid was gently aspirated by side aspiration using negative pressures

of 100-150 mm Hg. The recovered fluid was then pooled and filtered through sterile gauze. The BALF was then centrifuged at 250 x g for 10 min at 4° C. The cell pellet was discarded and the supernatant was further centrifuged at 36,000 x g for 20 min at 4° C. The resultant cell pellet was discarded and, the supernatant was again filtered through a 0.22 µm STAR<sup>®</sup> LB filters (CoStar corp, Cambridge, MA, USA). Filtered BALF were then stored at -70°C for later ELISA analysis.

### ***Cytokine ELISAs***

The concentrations of CRP, IL-8, IL-6, IL-1Ra and TNF-alpha were determined by enzyme-linked immunosorbent assay (ELISA). Commercially available ELISA kits were used to determine the concentration of soluble TNF-alpha, IL-8 (Biosource, Europe S.A, Fleurus, Belgium), IL-6 (Immunotech, Marseille, France) and IL-1Ra (R&D systems, Minneapolis, MN, USA) in blood and in BALF fluid using the procedures provided by the manufacture. Detectable levels of the cytokines were as follow: TNF-alpha, 3 pg/ml; IL-6, < 2 pg/ml; IL-8, 0.7 pg/ml and IL-1Ra, 4 pg/ml. Cytokine levels in BALF are expressed as a concentration per milliliter of BALF.

### ***Data analysis***

The total number of patients is too small (n = 6) to provide sufficient statistical power to discriminate between the variability of the various parameters that were assessed. In this context, the results are represented for each patient at each time point, regarding the dominant trend throughout the trial.

## **RESULTS**

### ***Blood and BALF cytokine levels after antibiotic therapy***

We first monitored the variation of both the local and systemic inflammatory reaction, measuring cytokine levels during antibiotic therapy. We chose to compare two time points: day -30, when none of the six patients had received any antibiotic, with day 7 when all of them had been treated intravenously for at least two weeks with appropriate antibiotics.

In blood, CRP levels decreased after starting antibiotics for patients B, C, E and F, and reached normal levels, below 5 mg/l, by day 7. For patients A and D, although CRP levels showed a slight increase, their levels at day -30 and 7 remained close to the normal biological level of 5 mg/l (respectively 6 and 10 mg/l) (Figure 2A). TNF-alpha levels decreased and reached levels slightly above 10 pg/ml in four out of six patients (B, C, D, E). For patient F, it also decreased, but the level remained elevated at 21 pg/ml on day 7. Finally, for patient A, TNF-alpha levels tended to increase, but day 7 level remained at the moderately elevated level of 17 pg/ml (Figure 2B). IL-6 levels decreased dramatically for patients A and B (55 to 18 pg/ml; 95 to 8 pg/ml respectively), between days -30 and 7. For patients C and D, IL-6 levels stayed below 10 pg/ml. For patient E and F, IL-6 levels slightly increased between the two time points (from 7 pg/ml to 12 pg/ml, for patient E, and from 10 pg/ml to 21 pg/ml for patient F) (Figure 2C). Finally, IL-1Ra decreased considerably in patient F, remained stable for patients A, B, C, E, while it increased in patient D from 187 pg/ml to 536 pg/ml (Figure 2D). IL-8 blood levels remained stable at 7 pg/ml in the six patients during the whole protocol (data not shown).

Data obtained from the BAL study were not as homogenous as those obtained for blood. TNF-alpha levels decreased in patients B, C, D, and F, between the two time points, reaching levels below 20 pg/ml by day 7. For patient A, TNF-alpha levels decreased during antibiotic therapy, but remained markedly elevated at day 7 (146 pg/ml). For patient E, despite antibiotics, TNF-alpha levels clearly increased from 13 pg/ml to 60 pg/ml (Figure 3A). For IL-6, levels in patients A, C, D, F did not go above the range of 20 pg/ml. Patients B and E reached higher levels on day 7 (respectively 75 pg/ml and 85 pg/ml) after two weeks of antibiotic therapy (Figure 3B). For patients A, D and F, IL-1Ra levels decreased after antibiotic treatment was started, whereas it increased for the other three patients B, C and E (Figure 3C). Finally, at the two time points days -30 and 7, IL-8 levels were found to be much higher than 20 pg/ml. Once more, IL-8 levels decreased in half of the patients (B, D, F) after antibiotic were begun, nevertheless remaining at considerably higher levels, and increased for patients A, C, and E (Figure 3D).

### ***Blood and BALF cytokine levels after AdCFTR administration***

We next examined the possible inflammatory effect of AdCFTR administration by comparing cytokine concentrations between days -7 and days 3. Antibiotics had

already been started fourteen days prior to the start of AdCFTR administration (on day 0 by nasal instillation, and the next day by aerosolization), and were continued 9 to 11 days after (Figure 1). Between days -7 and 3, CRP continued decreasing for 4 patients (C, D, E, F) and remained stable for two (A, B) (Figure 4A). TNF-alpha blood level decreased for one patient who received a middle dose of AdCFTR (D) and the two patients receiving a high dose (E, F). For patients treated with a low dose (A, B) and a middle dose (C), TNF-alpha levels increased (from 13 to 19 pg/ml, 15 to 20 pg/ml, and 13 to 14 pg/ml respectively) (Figure 4B). For four out of six patients, IL-6 blood levels demonstrated a similar pattern to CRP. If the IL-6 level increased moderately for patient A (4 to 22 pg/ml), it stayed at a low value for patient C (4 to 11 pg/ml). These two patients respectively received low (patient A) and middle (patient C) doses of AdCFTR (Figure 4C). Finally, IL-1Ra decreased in half of the patients, including the two "middle dose" patients (C, D) and a "low dose" patient (B). IL-1Ra levels increased in the other three patients, the two high dose patients (E, F) and a low dose patient (A) - between day -7 and day 3 (Figure 4D). IL-8 blood levels did not vary during the whole protocol and stayed equal to or below 7 pg/ml (data not shown). In BALF, the overall trend differed from that described in blood. IL-8 and TNF-alpha concentrations decreased in all of the patients, but remained at elevated levels (Figure 5A, 5D). IL-6 levels increased for patients A and B (respectively from 4 to 23 pg/ml, and 29 to 56 pg/ml) and decreased for the other four patients. IL-6 levels in the two patients (E and F) treated with a high dose of AdCFTR decreased although the concentration remained high for patient E. IL-1Ra decreased in patients C, D, E and F (respectively "middle" and "high dose" patients), whereas levels increased in the other two patients, A and B (low dose patients) (Figure 5C).

Out of the six patients, one (patient E) clearly presented both elevated blood and BALF cytokine levels before AdCFTR administration (time point -7). At day 7, he presented a substantial increase in PMNs, TNF-alpha, IL-6, IL-8 and IL-1Ra levels in BALF (data not shown). There was no apparent clinical deterioration or modification of his qualitative or semi-quantitative sputum bacteriological flora. Blood inflammatory parameters did not increase concomitantly. Furthermore, this cytokine and leucocyte increase was transient and levels returned to initial values by day 14. No specific immune response targeting type 5 adenovirus was detected in any of our six patients (data not shown).

## DISCUSSION

Gene therapy in CF patients is still limited by low efficiency of transfection and expression of the CFTR transgene following single administration [4-7]. Although rare, an uncontrolled AV-mediated inflammatory response seems to be responsible for some of the side effects observed in humans [6, 8]. Moreover, the inflammatory reaction, essentially the initial non-specific immune reaction, following AV administration clearly limits transfection levels [14, 20]. Therefore, the understanding or at least the monitoring of this inflammatory reaction is important. Preclinical studies in animal models have demonstrated the early inflammatory changes after first generation adenoviral vector infection [9-13]. Soluble mediators such as cytokines are involved in the orchestration of this early inflammatory reaction [11-13]. Therefore, we studied the evolution of cytokine levels in the serum and BALF of six CF patients included in an AdCFTR aerosolization-administered trial. In order to ensure that patients were in the best clinical condition, and to further attenuate airway inflammation already existing in these CF patients, antibiotics were administered two weeks before AdCFTR administration.

An increasing number of studies have demonstrated that inflammation in the CF lung occurs more as a direct consequence of mutant CFTR protein than bacterial infection [21-23]. After infection, the basal inflammatory imbalance contributes to exert severe damage to the mucosa, paving the way for bacterial colonization and subsequent progress of CF airway disease. The secondary bacteriological colonization is only in part controlled by antibiotic administration [24-28]. Nevertheless, only a few studies have combined the reporting of both blood and BALF cytokines during antibiotic treatment, and to our knowledge the data reported herein represent the first report of the levels of four pro- or anti-inflammatory cytokines following antibiotic treatment. We found, in agreement with previous articles, that after a two-week course of antibiotic therapy, inflammatory markers such as CRP, IL-6, TNF-alpha, decreased in blood [24-26, 28]. We also found that the anti-inflammatory cytokine IL-1Ra remained stable or increased slightly in five out of six patients. Results obtained in BALF were clearly less homogenous than those observed in blood. The results published on this topic are scarce and none of them reports the parallel evolution of TNF-alpha, IL-6, IL-8 and IL-1Ra in both blood and BALF. Moreover, samples were mostly collected from sputum [24, 27] rather than BALF [22]. In our study, the general trend of the cytokine pattern in BALF differs from that observed in blood. Firstly, cytokine levels were basically more elevated than in blood, confirming the well-established concept that CF is fundamentally a broncho-pulmonary

inflammatory disease [21, 22]. Secondly, we did not observe a clear antibiotic effect on cytokine release in BALF. TNF- $\alpha$  levels in our patients clearly decreased in five patients after antibiotics were started, but levels seen after two weeks of therapy were still elevated as has already been reported [24, 27]. For half of the patients, despite the use of antibiotic therapy, the average IL-8 level slightly increased. This is consistent with the data reported by Osika *et al.* in which no significant differences in IL-8 levels were found in the sputum of stable CF patients, patients experiencing an exacerbation or patients receiving antibiotic treatment [27]. Observation by others of a clear decrease in IL-8 levels after antibiotic treatment in BALF is probably a consequence of the younger age of the patient studied [22] and the differences in sampling (sputum *versus* BALF). Another explanation could be the key role played by the nuclear factor-kappa B (NF-kappaB) activity of which is increased in CFTR mutant cells and which is responsible for the high level of IL-8, independently of any infection [23, 29, 30]. The anti-inflammatory cytokine IL-1Ra followed the trend previously described for pro-inflammatory cytokines after antibiotic administration; its levels varied slightly, and remained elevated. This corroborates data published by Kronborg *et al.* and Osika *et al.* who reported a similar pattern [27, 31]. Finally, we also observed that IL-6 levels in BALF tended to increase after antibiotic treatment in five out of our six patients. Others have already reported such an increase in CF patient receiving a two-week antibiotic course [24]. The reason for this increase is unknown, and the design of our study did not allow us to gain any insight in to the mechanism involved.

Because of the decreased inflammation in the airways after two weeks of antibiotic treatment and the continuation of the treatment during the entire protocol, it was possible to speculate that cytokine variations were only the result of AdCFTR administration. The present results are the first to report, albeit in a small group of CF patients, the simultaneous absence of local and systemic inflammatory reactions after AdCFTR administration. Although it is difficult to compare data obtained from preclinical studies involving human trials, studies achieved in animal models corroborated the dose-dependent inflammatory response occurring after AV infection. One major difference between these two types of studies is that the doses used in each species differ by an order of 100 to 1000 less than in humans. For example, the maximal dose used in our lung administration ( $5.4 \times 10^8$  pfu) is equivalent, by weight, to approximately  $5.4 \times 10^5$  pfu in a mouse. Moreover, using aerosol delivery, we estimate that 20 to 40% of the initial AV dose is actually deposited on the lining

of the lower airways [7, 32]. By extrapolation, the number of pfu available to the airways in our trial would be respectively,  $2.5 \times 10^6$  pfu,  $2.5 \times 10^7$  pfu and  $1.4 \times 10^8$  pfu for the three cohorts. Therefore, our results in terms of inflammatory response are consistent with the one observed in previous animal studies. Using human equivalent doses below  $1 \times 10^9$  pfu, no local or systemic cytokine release was observed in rodents [12, 13], non-human primates [11], or humans using nasal instillation [5] or bronchofiberoptic delivery [6]. It is only when higher doses are administered, up to  $1 \times 10^9$  pfu, that animals or humans may have local release of IL-1beta [11-13], TNF-alpha [12, 13], IL-6 [5, 12, 13] or IL-8 [5, 11]. In addition, elevated plasma levels of the inflammatory cytokines IL-1beta, TNF-alpha and IL-6, have been observed following airway administration of AV in humans [6], and rodents [12]. Similar dose-dependent inflammation patterns have been reported in animal models in which aerosol-mediated delivery was performed [32]. Finally, the decrease of IL-8 levels following AdCFTR administration could be the result of the partial correction of the CFTR dysfunction, since it has been reported that *in vitro* IL-8 levels substantially decreased in CF cells transfected with the normal CFTR copy [30].

On day 7, out of the six patients, one patient (E) presented a substantial increase in TNF-alpha, IL-6, IL-8, IL-1Ra and PMN in BALF (twice the level or more than at the previous time point at day 3) without any clinical, radiological, bacteriological or spirometric change. This patient displayed a clinical deterioration 28 days after AdCFTR administration. Although it is not possible to definitively exclude the adenoviral vector as a causal agent, certain facts make this hypothesis unlikely. Firstly, he presented with the most critical illness, and received an aerosolized dose of  $5.4 \times 10^8$  pfu, which is not known to induce any inflammatory reaction in animal models [9-13]. Secondly, this transient cytokine increase and later clinical deterioration appeared some time after the AdCFTR administration. Indeed, in previous reports, systemic and local cytokine release occurs early, usually between day 1 and day 3 [13]. Harmful clinical effects have been observed 12 to 24 hours after AV administration [6, 8]. Moreover, the clinical symptoms observed in patient E were more suggestive of an exacerbation of CF than a viral infection. Finally, the immune status against AV did not change, and the clinical condition improved with a second course of antibiotic.

Our study is limited by the small number of patients, given it was a phase I clinical trial. However, it demonstrates that antibiotic therapy tends to decrease

inflammatory markers in blood but not in BALF. Among our six CF patient, none of them displayed a cytokine increase secondary to adenovirus-mediated gene transfer using aerosolization of low doses of vector. Nevertheless, our study had certain limits. Firstly, inflammatory responses do not consist only of cytokine release; many other mediators are involved. Secondly, cytokine concentrations determined by ELISA do not reflect biological activity. Both quantification of mRNA expression and bioassays would have been a more precise tools. However, because the inflammatory response seems to be the dominant mechanism involved in harmful effects and vector loss [6, 8, 14, 20], further studies will be necessary to investigate the role of these mediators and others in the adenovirus-mediated inflammatory response in humans.

## CONCLUSION

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Figures



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Table 1  
Patient characteristics

| Patient                             | A                    | B                   | C                   | D                   | E                     | F                     |
|-------------------------------------|----------------------|---------------------|---------------------|---------------------|-----------------------|-----------------------|
| Symbol                              | ○                    | ●                   | □                   | ■                   | △                     | ▲                     |
| Age in years                        | 22                   | 21                  | 21                  | 18                  | 26                    | 20                    |
| Shwachman score                     | 90                   | 80                  | 70                  | 80                  | 60                    | 70                    |
| Organisms recovered                 | PA-SA                | SA                  | PA                  | HI-SA               | PA-SA                 | PA                    |
| Antibiotics                         | Ticar-clavu<br>Tobra | Amoxi<br>Clavu      | Imipen<br>Tobra     | Pristin<br>Genta*   | Imipen<br>Tobra       | Ceftazi<br>Tobra      |
| FEV1, ml<br>(% of predicted values) | 4,080<br>(106)       | 4,080<br>(96)       | 2,160<br>(55)       | 2,880<br>(67)       | 2,080<br>(51)         | 2,240<br>(53)         |
| Dose (pfu)                          |                      |                     |                     |                     |                       |                       |
| Nasal                               | 1 × 10 <sup>5</sup>  | 1 × 10 <sup>4</sup> | 1 × 10 <sup>7</sup> | 1 × 10 <sup>7</sup> | 4 × 10 <sup>8</sup>   | 4 × 10 <sup>8</sup>   |
| Aerosol                             | 1 × 10 <sup>7</sup>  | 1 × 10 <sup>7</sup> | 1 × 10 <sup>8</sup> | 1 × 10 <sup>8</sup> | 5.4 × 10 <sup>8</sup> | 5.4 × 10 <sup>8</sup> |

Abbreviations: PA: *Pseudomonas aeruginosa*; SA: *Staphylococcus aureus*; HI: *Haemophilus influenzae*; Ticar-clavu: ticarcillin-clavulanate; Tobra: tobramycin; Amoxi-clavu: amoxicillin-clavulanate; Pristin: pristnamycin; Genta: gentamycin; Imipen: imipenem; Ceftazi: ceftazidime.\* : aerosol administered.

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## Authors

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cytokine, cystic fibrosis, adenoviral vector, antibiotic.

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Stat5, IL-2 receptor, T lymphocytes, CD25.

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A. castellan, cytokine, MGC.

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Interleukin-1 beta, Na<sup>+</sup>K<sup>+</sup> ATPase, NaKCl2, Cl<sup>-</sup> secretion, Na<sup>+</sup> absorption, colon.

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SLE, Interferon gamma, IL-12, IL-15, IL-18, disease activity.

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