Category: Tools and technologies for systems biology

Title: Virtual Patients and Virtual Drug Targets: A Systems-Level Approach to Pharmaceutical R&D

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Introduction

Although researchers in the biopharmaceutical industry have made major breakthroughs in genomics and combinatorial chemistry, the time required for the development and release of a new disease treatment has not changed significantly. One might assume that the rate-limiting factor is a lack of data necessary for pursuing further research. But modern drug discovery and development efforts have been lagging for precisely the opposite reason. The enormous yield of data from the explosive fields of genomics and combinatorial chemistry has flooded the development pipeline of the biopharmaceutical industry. Faced with this ever-growing deluge, researchers are frequently stalled by the difficulty of determining its informational value.

This glut of data has challenged the scientific community to rediscover the importance of understanding a disease from a systems perspective—that is, a top-down view of the entire network of physiological pathways that play a role in the disease. The ability to gain this perspective can lead to valuable insight about the underlying cause and mechanism of a disease that would be very difficult to achieve by studying an isolated pathway. From a systems perspective, researchers can more readily identify the most relevant leads provided by genomics technologies and evaluate them effectively.

Recently developed simulation systems can help the researcher to achieve this broader perspective. Use of this technology facilitates the design of experiments that pinpoint specific aspects of a disease while taking into account interactions with related physiological pathways. Here, we describe Entelos® PhysioLabs™, computer-based mathematical models of human physiology that allow scientists to simulate hypotheses in order to test virtual drug targets and therapeutic strategies in virtual patient types.

Current Drug Development Paradigm

Pharmaceutical and biotechnology companies engage in a constant challenge to discover, patent, and develop innovative products to alleviate human suffering. The largest firms spend more than $1 billion per year in R&D alone, adopting the latest drug discovery systems to push greater and greater numbers of compounds through the research pipeline. Advances in cell and molecular biology have created a data explosion, greatly accelerating the pace of identifying potential new drug targets. Therefore, most companies find themselves with too many unvalidated drug targets to prioritize and push through the drug development pipeline.

About 80% of experimental compounds still fail in clinical testing, and chronic diseases are proving to be especially difficult to tackle. On average, it takes roughly $500 million and 12 years for an experimental compound to move from the lab bench to drug approval. Among the most important therapeutic areas targeted by companies today (e.g., arthritis, asthma, diabetes, and obesity) are chronic, complicated conditions that depend on multiple mechanisms, specific patient factors, and environmental effects. To optimize the quality of decision-making during the development of drugs to treat such conditions, pharmaceutical and biotechnology companies require a new approach that weaves together the threads of information they have accumulated to achieve a broader perspective of a disease. Computer simulation systems offer the promise of filling this need.

Using a Systems-Level Approach for Pharmaceutical R&D

Computer modeling and simulation have a rich, successful history in many industries, for example, automobile and airplane design, semiconductors, and oil-and-gas exploration. Simulation systems have only recently been adopted by the pharmaceutical industry. Several biological models applicable for use in various aspects of the pharmaceutical R&D process are now available. Entelos PhysioLabs, for example, are comprehensive models that simulate the physiology of an entire disease system. To date, these simulation systems include models of asthma and obesity. The asthma and obesity PhysioLabs consist of three main components: 1) a graphical "disease map," representing important physiological interactions within a disease system; 2) an extensive reference database, linking scientific papers and experts' comments to specific structures on the disease map; and 3) a simulation engine allowing researchers to conduct "what if" experiments to test new intervention strategies in different patient types.

PhysioLab Model Development

PhysioLabs are developed with Entelos' proprietary modeling and simulation platform. Development is an iterative process of: 1) identifying the necessary biological scope, 2) creating the mathematical model based on known biological data and engineering design and control principles, and 3) calibrating and validating the model by comparing simulation results with experimental results. PhysioLab modelers take a top-down approach, starting first with clinical signs and symptoms, then identifying

relevant physiologic systems, and finally approaching the cells, signals, and pathways involved in healthy and disease states. When completed, the mathematical model consists of several hundred nonlinear, coupled ordinary differential equations. Simulations are achieved by solving the system of equations numerically on a computer using an adaptive step-size algorithm.

PhysioLabs are designed so that users can easily access the mathematical equations and parameters representing the physiology, and the data and rationale used to create the model. An intuitive interface allows users to create and run simulated experiments and view their results.

Virtual Patient Example—Obesity PhysioLab Case: Leptin Deficiency

Every experiment in a PhysioLab involves a virtual patient. The user defines a patient type on which to carry out the experiment. The PhysioLab makes it convenient for users to do so by providing a baseline patient representing a healthy individual in the obesity PhysioLab or an asymptomatic but mildly asthmatic individual in the asthma PhysioLab. Users can readily model the desired patient characteristics by entering alternative values to substitute for baseline parameters.

Here we describe a virtual experiment modeled after a study reported in the literature—the treatment of a leptin-deficient human with an infusion of leptin.[1] We used Entelos® Obesity PhysioLab™, which simulates appetite regulation, nutrient consumption, and the metabolic and physiologic processes associated with nutrient oxidation and storage. The results of the experiment proved to be comparable to those reported, demonstrating the value of the PhysioLab for exploring the effects of a potential therapy before committing expensive wet lab and clinical resources.

Leptin is a hormone[2] demonstrated to regulate food intake and weight homeostasis.[3] It alters the milieu of brain neuropeptides such that the signals which stimulate feeding are inhibited.[4] Leptin deficiency was first discovered in mice carrying a homozygous mutation in the leptin gene (ob/ob).[5] These animals were hyperphagic (inclined to overeat) to the extent that their adipose tissue stores and total body weight far exceeded those of wild-type control mice.[6]

Leptin deficiency has been observed in humans as well. Two children in one family were discovered to have a homozygous frame-shift mutation in the leptin gene, resulting in virtually undetectable levels of circulating leptin.[7] The clinical progress of one of these children, a nine-year-old girl, was recently described by Farooqi *et al.*[1] Reported to be hyperphagic and massively obese, the child suffered a decreased quality of life. Following the identification of leptin deficiency, daily treatment by subcutaneous injection of leptin was initiated. The child's food consumption decreased, and she experienced rapid weight loss and a greater sense of normalcy.

Simulation of this scenario in the PhysioLab required the creation of a virtual patient with a leptin deficiency. We began with an experiment provided as part of the PhysioLab product, in which a virtual individual, through self-controlled food intake, maintains a stable weight of 70 kg, with 20% body fat. (Because the PhysioLab is designed to represent the physiology of an adult, the virtual individual is an adult; therefore, the results of a simulation might not precisely replicate clinical studies of children.) We

imposed a total leptin deficiency on the 70 kg individual by setting to zero the parameter associated with adipose leptin production (Fig. 1A, B). The lack of production resulted in virtual leptin concentrations of 0 ng/ml. The PhysioLab was then set to simulate one year of leptin deficiency, an experiment that takes about one hour in real time, depending on the speed of the computer. In addition, we simulated one year of normal metabolism as a control (not shown).

The results showed that food consumption increased by about 25% (data not shown) in the virtual leptin-deficient patient, as compared with the control; and body weight increased by 18 kg (Fig. 2). The increased body weight could largely be attributed to an increase in adipose tissue mass, amounting to about 15 kg (Fig. 2). The changes in body weight and adipose tissue mass are comparable to those observed in the leptin-deficient girl, who gained about 20 kg of total body weight in the year before beginning leptin treatment.

To simulate leptin therapy, we took advantage of parameters built into Entelos Obesity PhysioLab representing the delivery of exogenous plasma leptin. We entered values for these parameters specifying a start time, stop time, delivery rate, and a repeat interval to simulate a constant infusion of therapeutic leptin (Fig 3A, B). With respect to pharmacokinetics, this mode of delivery differed from the daily subcutaneous leptin injection administered to the leptin-deficient girl, which peaked and decayed to a lower value daily. However, the average concentration to which the girl was exposed was about the same as that delivered by the simulated infusion (70 ng/ml).

We set the PhysioLab to simulate one year of therapy for the virtual leptin-deficient patient from the preceding simulation (Fig. 4). The results showed that the patient's body weight and adipose mass declined substantially, by about 13 kg and 10kg, respectively (Fig.5 and data not shown). These results compare favorably with the reported changes brought about by treating the leptin-deficient girl. Her hyperphagia diminished, and she lost 16.4 kg of total body weight, of which 15.6 kg were adipose tissue. The PhysioLab also simulated weight gain and loss at rates similar to those observed *in vivo*, even though neither the metabolic effects of leptin nor contributions from growth processes were taken into account. This observation suggests that further exploration might shed light on the role these factors play.

Entelos Obesity PhysioLab accurately reproduced the changes associated with leptin deficiency and treatment in the human observed by Farooqi *et al.*[1] The simulations demonstrate how researchers can use the PhysioLab to model obesity with a specified etiology and test potential therapies. Because the PhysioLab is a whole-body model, this capability is not limited to leptin deficiency but extends to many other etiologies.

Virtual Target Example—Asthma PhysioLab Case: Interleukin-5 (IL-5) Antibody

Because a PhysioLab simulation solves for the entire system of mathematical equations within a given model, profound yet unexpected effects of modulating an isolated drug target pathway within the context of an entire disease may be observed. Here, we describe the evaluation of a virtual drug target using Entelos® Asthma PhysioLab™.

Asthma is a chronic disease characterized by airway hyperresponsiveness and obstruction, and eosinophilic inflammation of airway tissue. When triggered, an asthma attack typically consists of immediate airway obstruction that resolves in about an hour. Several hours after this early phase, many patients also experience a late phase of airway obstruction that usually resolves within a day.

Particularly during the late phase of an asthma attack, the number of eosinophils in airway tissues increases. Eosinophils are leukocytes involved in allergic and parasitic inflammatory responses. The eosinophils release granule proteins, cytokines, and inflammatory mediators that all contribute, in some measure, to hyperresponsiveness and inflammation of airway tissue, and airway obstruction. Because of the apparent correlation between increased eosinophilic presence in airway tissues and asthmatic severity, eosinophils have been a focus of asthma research. The correlation of asthma with eosinophilia and increased IL-5 in humans, and the data supporting this correlation from animal models, led to the hypothesis that blocking IL-5 activity could be an effective asthma therapy.[8]

A humanized monoclonal antibody that recognizes and neutralizes IL-5 had been shown to effectively prevent airway hyperresponsiveness in several species, including mice, guineas pigs, and monkeys.[9, 10, 11] These animal data supported the hypothesis that an anti-IL-5 therapy could be used to prevent asthma pathology in humans.

To investigate the effect of inhibiting IL-5 activity in an asthmatic patient, we performed a simulation using Entelos Asthma PhysioLab. This experiment was carried out on a virtual asthmatic patient who had the baseline conditions of chronic mild allergic asthma. The asthmatic patient received anti-IL-5 therapy for 7 days to block IL-5 activity, including IL-5's role in eosinophil differentiation and maturation. On day 8, the patient received an inhaled antigen challenge, and the effects of the challenge were measured for 24 hours.

Figure 6A shows the number of eosinophils measured in the airway tissue of the patient who received the anti-IL-5 treatment and in that of a control patient. As expected, the anti-IL-5 treatment produced a dramatic reduction in eosinophil numbers. Figure 6B shows the airway conductance measured for each patient following the antigen challenge. When the antigen challenge is administered, airway conductance drops significantly in each patient and then recovers, as is typical during the early-phase response. Several hours later, airway conductance drops again, marking the late-phase response. The patient receiving anti-IL-5 therapy has only slightly improved airway conductance throughout the simulated experiment. These results suggest that eosinophils are not the primary contributors to late-phase airway obstruction in asthma. Very similar to recent findings of clinical studies[12], this outcome of the virtual experiment contradicts the results of studies using animal models.[9, 10, 11]

Results concerning eosinophils are not the only interesting outcome of this experiment, however, because simulations in the PhysioLab take into account all the pathways in the disease system. The PhysioLab measures changes in all active system components over the course of the experiment and makes these results available to the user. Consequently, users can identify unexpected mechanisms and gain new insight as to why a treatment may or may not be clinically effective. For example, having removed

eosinophils from the system, we can examine contributions to the late-phase asthmatic response from other factors in asthma such as macrophages, basophils, epithelial cells, and sensory nerves.

Results from the same anti-IL-5 simulation show that macrophages (Fig. 6C) and basophils (data not shown) were not affected by the anti-IL-5 treatment and were still able to produce inflammatory mediators such as cysteinyl leukotrienes (Fig. 6D), which contribute to airway closure during the late-phase asthmatic response. Furthermore, these results show that both activated macrophages and cysteinyl leukotrienes increase during the late-phase response, indicating that macrophage activation may be a greater contributor to airway obstruction than eosinophilia.

Conclusion

Advances in genomics technologies have dramatically increased the rate at which researchers acquire new biological information. Integrating this information with a systems perspective will facilitate the translation of this information into knowledge about the intricacies of complex biological systems. Physiology simulation technology is a valuable new approach to pursuing this system-level understanding of biological systems. Entelos PhysioLabs, for example, are predictive simulation systems that enable scientists to test hypotheses generated from laboratory and clinical studies. The use of predictive simulation systems in conjunction with experimental data helps streamline the process of investigating disease systems and identifying better targets for therapeutic intervention. With this technology, scientists can simulate and test questions such as: How do genetics or environment interact with therapies to ameliorate or exacerbate symptoms? How do dosing and patient compliance affect overall clinical effectiveness? Among the many possible points of intervention, which mechanisms have the greatest impact on disease progression? Which patient types appear to be the best candidates for clinical trials? The ability to tackle such questions before moving a compound into large-scale clinical trials helps researchers in the pharmaceutical industry select better drug candidates to increase their chances for success.

Acknowledgements

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Figure 1A

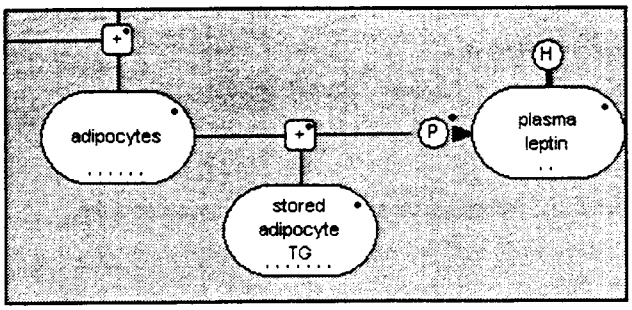

Figure 1B

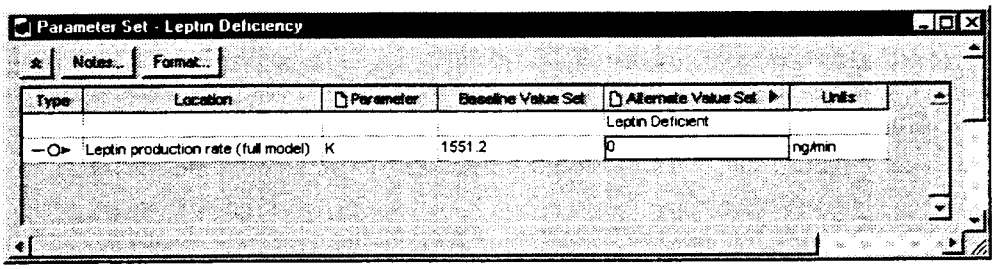

Figure 1. (**A**) Detail of the Entelos Obesity PhysioLab disease map, showing the three nodes that represent the production of leptin by fat cells (adipocytes). The entire disease map contains over 300 nodes. (**B**) To create a leptin-deficient virtual patient, the leptin production rate is set to zero.

Figure 2

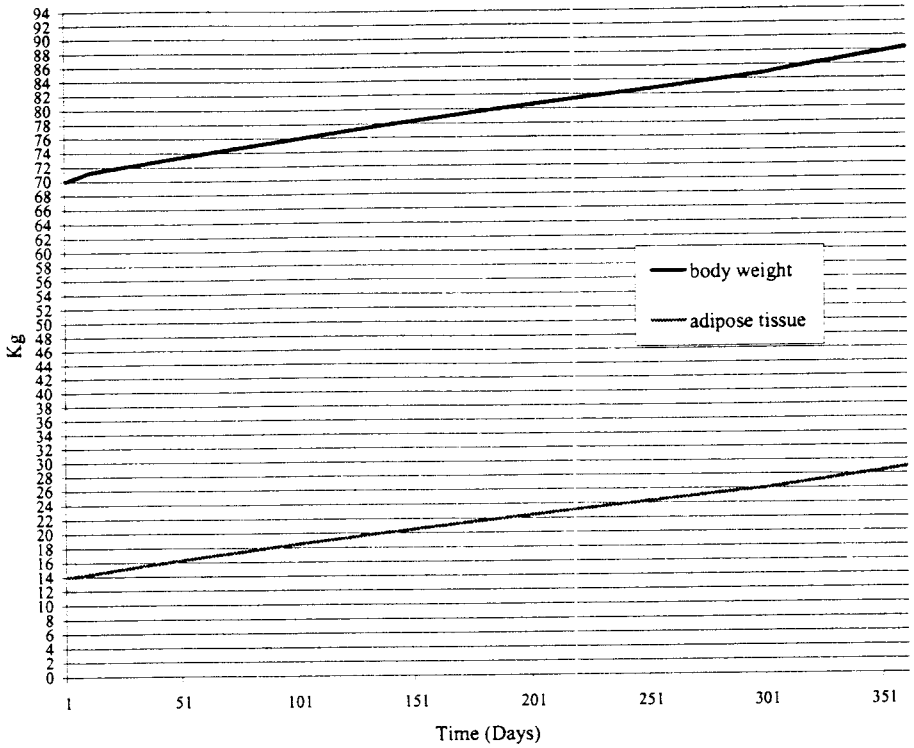

Figure 2. PhysioLab simulation results for leptin-deficient patient showing total body weight and mass of the adipose tissue. Over the duration of the experiment, the patient gained 18 kg, of which 15 kg were adipose tissue.

Figure 3A

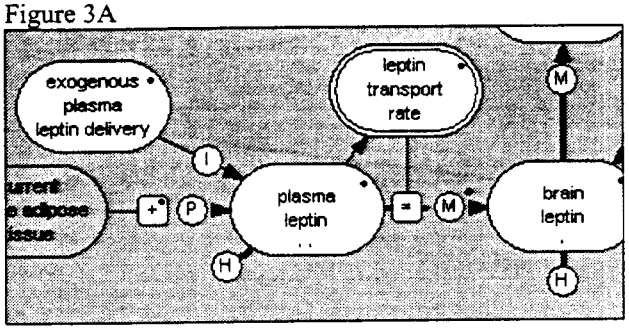

Figure 3B

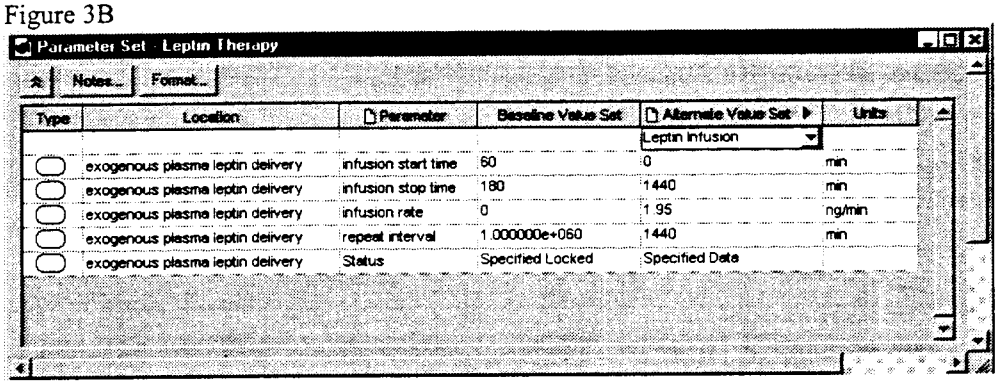

Figure 3. (**A**) Detail of the Entelos Obesity PhysioLab disease map, showing the node that represents exogenous plasma leptin delivery. (**B**) The "exogenous plasma leptin delivery" node contains parameters that specify delivery conditions. We entered values to specify a constant infusion of leptin over an entire day (start = 0, stop = 1440 minutes; 1 day = 1440 minutes). The infusion was repeated every day for the duration of the simulation.

Figure 4

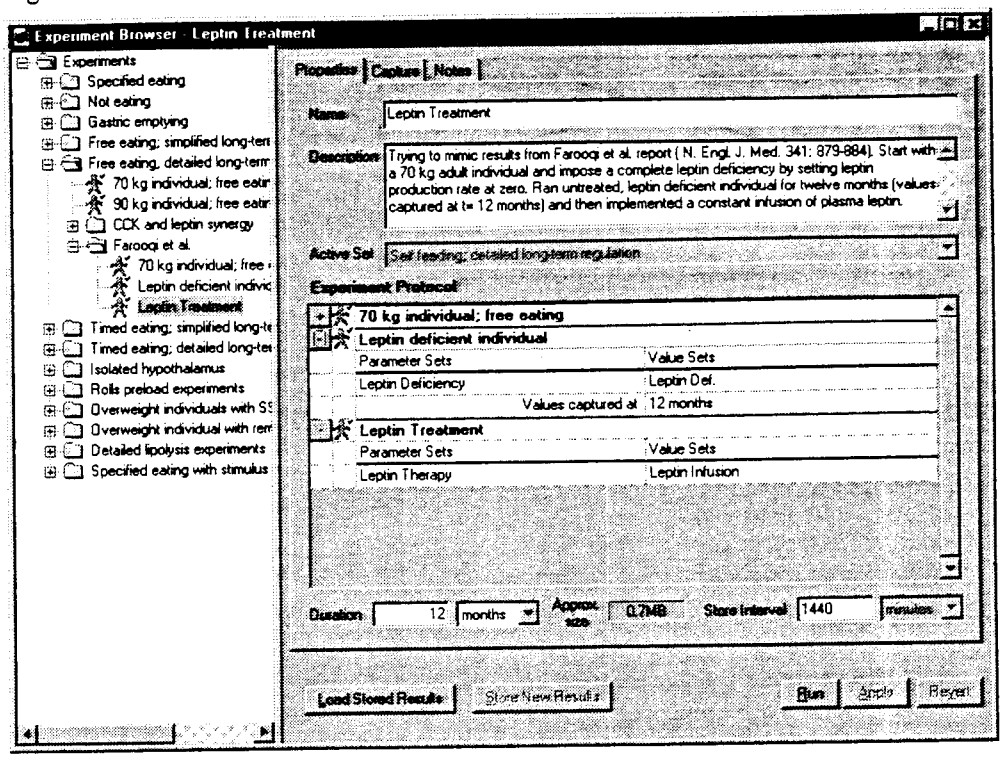

Figure 4. Experiment protocol window in Entelos Obesity PhysioLab defining the conditions for simulating leptin therapy for a leptin-deficient virtual patient. Sets of parameters specifying no leptin production ("Leptin deficient individual") and a constant leptin infusion ("Leptin treatment") are in the experiment protocol. The duration of the experiment is 12 months (about one hour in computer simulation time).

Figure 5

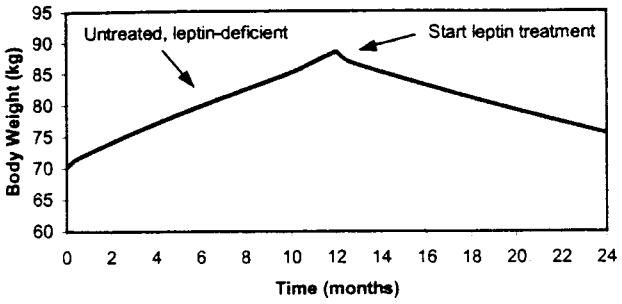

Figure 5. Results of an obesity PhysioLab experiment that simulates the treatment of a leptin-deficient individual.

Figure 6A

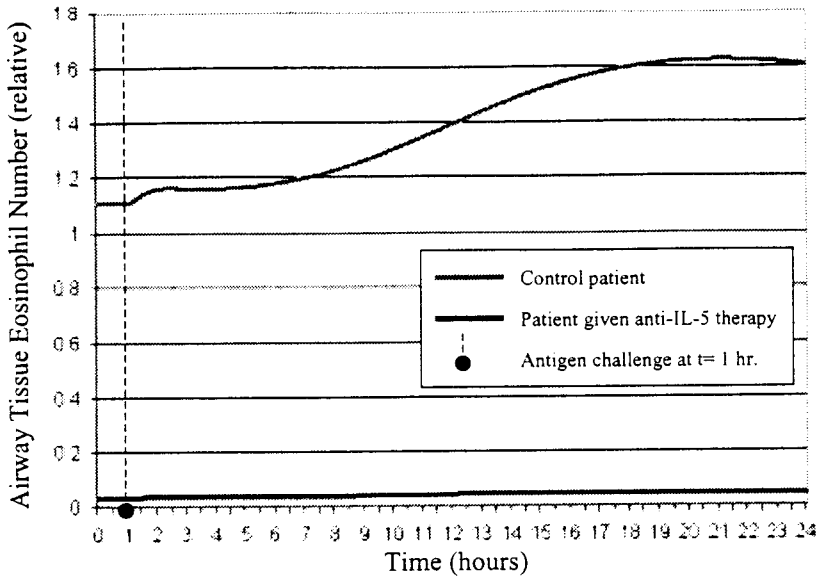

Figure 6B

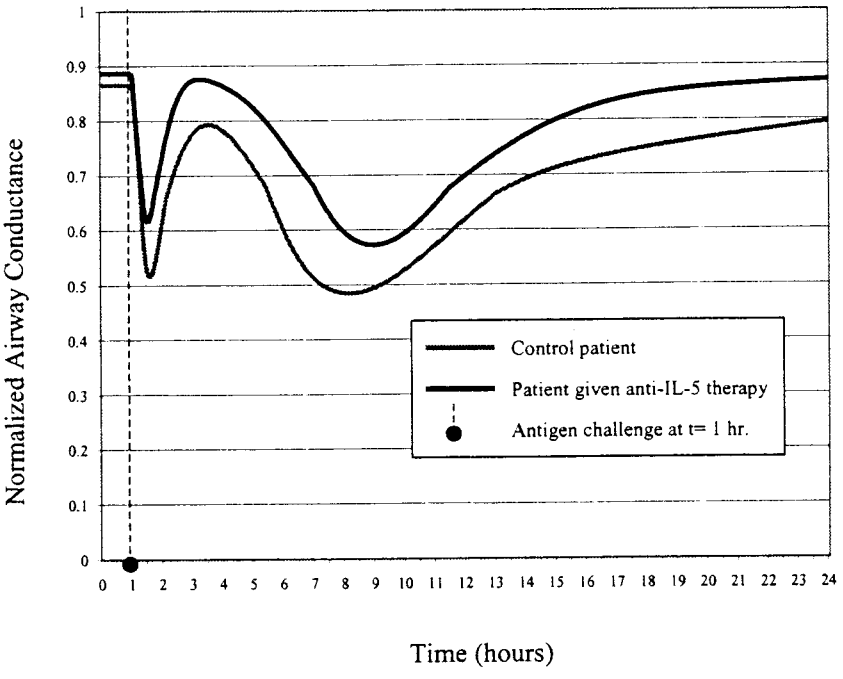

Figure 6C

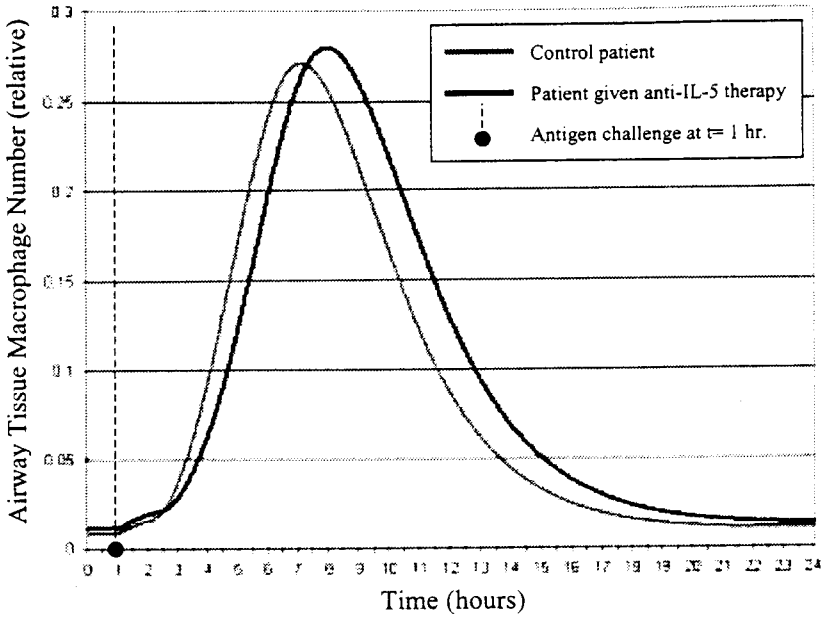

Figure 6D

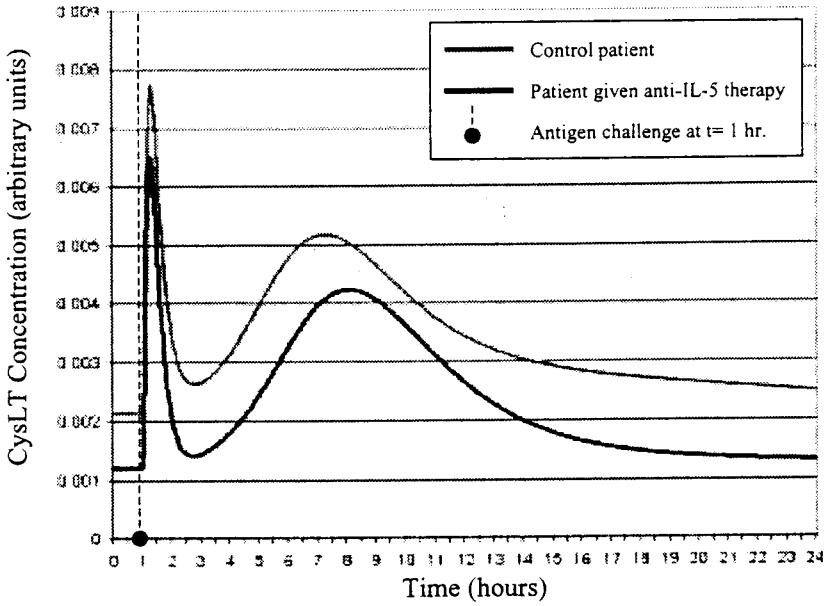

Figure 6. Simulation results from an antigen challenge experiment performed on a mildly allergic asthmatic control patient (gray curve) and a mildly allergic asthmatic patient given an anti-IL-5 treatment for 7 days (black curve). An inhaled antigen challenge was given one hour into the simulation.