

Importance of technology for the future of allergy and immunology

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In writing an editorial on the importance of technology for the advancement of allergy and immunology, pictures say it better than words. The Journal has chosen the clinical immunology laboratory, with the monitor of a flow cytometer showing a pair of histograms displaying T (CD3⁺) cells (*red peak*) and B (CD19⁺) cells (*green peak*) from a normal infant and from an infant with X-linked severe combined immunodeficiency (SCIDX1). These are juxtaposed with a picture of a patient with SCIDX1, David, the Houston "Bubble Boy," when he was 4 years old and confined to the sterile plastic isolator system in which he spent the first 12 years of his life (Fig 1 and the cover of this issue).^{1,2} As seen in the histogram, David lacked the immunologically critical CD3⁺ T-cell population and consequently was at continuous risk for life-threatening infections. There is an increase in the proportion of B cells demonstrated on the histogram, a classic finding in SCIDX1 that should be a definitive clue to the allergist-immunologist evaluating an infant for congenital immunodeficiencies. Monoclonal antibody reagents that distinguish T cells from B cells were just being developed in the late 1970s, and a decade later the emerging technology in flow cytometry permitted rapid and routine identification of T and B cell subsets. David lacked an HLA-matched sibling donor, and the technology to use either haploidentical or matched, unrelated donor bone

Abbreviations used

SCID: Severe combined immunodeficiency disease
TREC: T-cell receptor excision circle

marrow had not yet been developed. National Aeronautics and Space Administration technology designed to contain moon rocks was adapted to create a series of sterile bubbles for David as he grew from infancy to early adolescence.³ The truly extraordinary efforts of hundreds of professionals at Texas Children's Hospital, Baylor College of Medicine, and the Johnson Space Center in Houston clearly outline the extent to which society can go to alter the consequences of a lethal, inherited immune disorder. In this case, the defect affected the common gamma chain (γ_c), a protein that is a member of a family of cytokine receptors that are critical for the normal development of T cells and natural killer cells. The review article on technology by Drs Fleisher and Oliveira⁴ in this issue of the Journal presents an *IL2RG* gene mutation analysis (see Fig 3 on page 232) that demonstrates a single nucleotide substitution producing a deleterious missense mutation that prevents production of the γ_c . In the early 1980s, molecular and cellular technology advanced to circumvent the histocompatibility barrier of bone marrow transplantation. Physical separation (soybean lectin) or elimination (anti-T cell monoclonal antibodies) of mature T-cell populations (capable of inducing lethal graft-versus-host disease) from bone marrow that contained stem cells made it possible to use haploidentical (parent, sibling) bone marrow donors.^{5,6} David was treated with a sibling haploidentical T cell-depleted bone marrow; within months, however, Epstein-Barr virus present in the donor bone marrow infected and activated his CD19⁺ B cells to grow unchecked into oligoclonal malignant B cells.⁷ The technology that has evolved since this heroic child's death in 1984 might have rescued him. *In vitro* expanded Epstein-Barr virus-specific cytotoxic T-cell clones have been used to successfully treat similar tumors arising in immunodeficient or immunosuppressed hosts following bone marrow transplants.⁸ More recently, anti-B cell monoclonal antibody was used with considerable success in eliminating or reducing the B-cell tumor load in transplanted patients.⁹ The beneficial aspects of technology enabling physicians to treat their patients is easily grasped when advances in technology are tied to

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FIG 1. The diagnosis of SCIDX1 in the clinical immunology laboratory. The monitor screen of the flow cytometer shows a control and SCIDX1 lymphocyte histogram with CD3⁺ T cells and CD19⁺ B cells (percent distribution pattern). The picture insert shows David, the “Bubble Boy” (1975), a 4-year-old child with SCIDX1, in a permanent isolator system built by the National Aeronautics and Space Administration at the Johnson Space Center in Houston, Tex. Seen in the foreground are reproductions of a SCIDX1 B-cell histogram and nucleotide patterns.⁴

human experiences, such as with David, who was born before technology developed that could have spared his life.

Other advancements in technology that have enabled the clinical immunologist to better characterize immune responses of patients with abnormal immunity include intracellular cytokine production by separated CD4⁺ and CD8⁺ T cells,¹⁰ IFN- γ -secreting (cytotoxic) T cells by the ELISPOT assay,¹¹ T-cell receptor excision circle (TREC) and analysis that measures recent thymic emigrants,¹² frequency of antigen specific T cells through use of peptide tetramers,¹³ and T-cell receptor diversity (spectratyping).¹⁴ A recent illustration of the power of the clinical application of the last-mentioned technology can be seen in the report by Markert et al,¹⁵ who demonstrated that the T-cell repertoires were strikingly oligoclonal in a subgroup of patients with complete DiGeorge syndrome. This “immunoscope” (TREC analysis and spectratyping) technology helps to explain that the variant DiGeorge syndrome demonstrates an abundance of T cells that are derived from a very limited repertoire of T cells, a finding not easily detected by conventional laboratory examination. All of these analy-

tical methods have produced a much clearer picture of the pathophysiology not only of primary and secondary immunodeficiencies but also in disease states that are covered by an overreaction of components of immunity, as seen in allergic and autoimmune disorders.¹⁶

Of all the technological advances made in the last 3 decades, the ability to detect genetic defects in inherited human conditions has been the most revolutionary. In the realm of congenital immunodeficiencies, DNA technology has enabled more than 100 genotypes of primary immunodeficiencies to be uncovered.¹⁷ Moreover, effective gene therapy for these immune disorders offers great promise, though it does pose significant risks, as pointed out by Chinen and Puck¹⁸ in a recent *Molecular Mechanisms* article. The initial success, in 10 infants with SCIDX1, of use of a retroviral vector-delivered gene construct in the hematopoietic stem cell was tempered by the realization that the T-cell leukemia that developed in 2 of these children was due to insertional mutagenesis.¹⁹ Thus, new vectors will be needed to insert corrective genes into defined areas of the human genome that are distant from chromosome hot spots that cause cancer.

Technology offers escape from the confines of imperfect knowledge of clinical-based diagnosis and management of human disease and promises new mechanisms for treating serious diseases that interfere with a normal life. It is appropriate to attempt to peer into the future and try to imagine how the knowledge of the human genetic code will affect the allergist-immunologist of succeeding generations. Will there exist technology to more clearly guide patient life choices in producing offspring that do not bear the burden of inherited conditions that threaten life? Once they have acquired this knowledge, will physicians be able to exert more control over the clinical outcomes of their patients? It is possible for some of us to reflect on the era prior to recent vaccine developments, when *Haemophilus influenzae* meningitis was a concern of every pediatric intern in the emergency room as a febrile and irritable infant was seen. Similar to the technological advances that produced bacterial vaccines, so too will technology facilitate treatment of allergic diseases. Technology will advance and clarify which asthmatic child will benefit from bronchodilator therapy because of an inherited polymorphism of a hydrolytic enzyme.²⁰ Patient cohorts more responsive to immunotherapy with allergen extracts may be identified by the presence of specific lymphocyte signaling molecules in peripheral blood T cells through the use of quantitative PCR to measure mRNA levels or by intracellular flow cytometry.²¹ Antisense oligonucleotides produced by reverse nucleotide transcription may be used to inhibit IL-4 production by the nasal mucosa of ragweed-sensitive patients.²² Production of recombinant protein allergens and chip-based immunoassays may greatly facilitate the diagnosis of allergic diseases, as noted by Hamilton and Adkinson.²³ Positional cloning will enable physicians to characterize patients early in the causes of their allergic or immunologic diseases and to be more judicious in the selection of treatments and medications that will improve their patients' lives.

Of the accelerating pace of discovery of genetic and molecular lesions, the creation of new reagents and medications, and the clinical trials used to evaluate these new discoveries, one can be assured by the survey of advances made in basic and clinical immunology published in this issue of the Journal.²⁴ We are living in an age of excitement and wonder made possible by the advancement of technology that has brought dreams to reality. The best may be yet to come!

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