A Brief Chronicle of CD4 as a Biomarker for HIV/AIDS: A Tribute to the Memory of John L. Fahey

Jonathan M. Kagan[a,*], **Ana M. Sanchez**[b], **Alan Landay**[c], and **Thomas N. Denny**[b]

[a]Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Department of Health and Human Services, Rockville, MD

[b]Duke Human Vaccine Institute and Center for HIV/AIDS, Duke University, Durham, NC

[c]Rush University Medical Center, Chicago, IL

Abstract

Foundational cellular immunology research of the 1960s and 1970s, together with the advent of monoclonal antibodies and flow cytometry, provided the knowledge base and the technological capability that enabled the elucidation of the role of CD4 T cells in HIV infection. Research identifying the sources and magnitude of variation in CD4 measurements, standardized reagents and protocols, and the development of clinical flow cytometers all contributed to the feasibility of widespread CD4 testing. Cohort studies and clinical trials provided the context for establishing the utility of CD4 for prognosis in HIV-infected persons, initial assessment of *in vivo* antiretroviral drug activity, and as a surrogate marker for clinical outcome in antiretroviral therapeutic trials. Even with sensitive HIV viral load measurement, CD4 cell counting is still utilized in determining antiretroviral therapy eligibility and time to initiate therapy. New point of care technologies are helping both to lower the cost of CD4 testing and enable its use in HIV test and treat programs around the world.

Keywords

biomarkers; CD4; HIV/AIDS; immune monitoring; flow cytometry

I. INTRODUCTION

John L. Fahey, our colleague, friend, and mentor, made enduring contributions in the fields of basic and clinical immunology, cancer, and infectious diseases, but perhaps none more important than his findings on HIV/AIDS, beginning with its discovery at UCLA in 1981. Over the ensuing 33 years, his studies of HIV immunopathogenesis and epidemiology (in the United States and internationally) helped reveal the paradoxical nature of HIV infection as a disease of both immune depletion and immune activation, concepts that have informed and helped shape today's approaches to HIV diagnosis, treatment, and prevention. Among

[*]**Address all Correspondence to:** Jonathan Kagan PhD, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Department of Health and Human Services, 5601 Fishers Lane, Room 4B40, MSC 9820, Rockville, MD 20892-9820; jkagan@niaid.nih.gov.

his lasting findings were those made as part of the Multicenter AIDS Cohort Study, whose foundational research on the natural history of HIV infection, including CD4 as a marker for HIV disease risk, stands as testament to what can be achieved when rigorous laboratory research is integrated within long-term cohort studies. The narrative to follow traces the history of CD4's discovery and development as a biomarker for HIV/AIDS and is dedicated in John's memory with the intent to offer insights (and possibly lessons learned) for immunologic biomarker and immunopathology research, to which he was so passionately committed.

II. INITIAL DISCOVERY OF CD4 DEPLETION IN AIDS

The decade of the 1970s saw rapid advances in understanding of the differentiation, function, and phenotypes of human T-lymphocyte subsets[1–5] at the cellular level. These discoveries, coupled with the advent of hybridoma technology,[6] immunofluorescent antibodies,[7,8] and cell-sorting instrumentation,[9–14] heralded a new era of immune diagnostics and immunopathology research such that the appearance of opportunistic infections and Kaposi's sarcoma in previously healthy gay men in the United States (1979–1981) was rapidly recognized as a cellular immune deficiency and the first human disease to be characterized by the selective loss of a specific T cell subset, namely, CD4+ T-helper/inducer cells.[15–17]

It would be nearly three years (1983–1984) before lymphadenopathy-associated virus/human T-lymphotropic virus type III (LAV/HTLV-III) was discovered as the etiologic agent of AIDS[18,19] and the CD4 (T4) antigen an essential component of its receptor.[20,21] Nearly 10 more years elapsed before quantitative measurement of HIV-1 plasma RNA would become widely available in the United States.[22–24] Meantime, as the numbers of cases of what we now call HIV/AIDS grew, physicians and patients needed access to accurate, reproducible CD4 testing for use in diagnosis and therapeutic monitoring, as well as for use in clinical trials.

III. DEVELOPING CD4 AS A FEASIBLE TEST FOR THE CLINICAL LAB

At the time that the first AIDS cases presented in the United States in the early 1980s, relatively few laboratories had the capacity to perform CD4 testing. Pathology laboratories were gaining proficiency in performing antibody-based assays for tumor cell markers (e.g., alpha fetoprotein, carcinoembryonic antigen) on tissue samples using light and immunofluorescence microscopy. As such, some of these laboratories began providing CD4 and CD8 cell enumeration for AIDS patients. Though early cytometers and cell sorters had begun appearing in research laboratories in the 1970s, they were not designed for use in a clinical setting. It was not before the mid-1980s, with the advent of instruments such as Ortho Spectrum III,[25] the Coulter Epics C and Profile,[26,27] and the Becton Dickinson FACScan[13] and widespread commercial availability of fluorescent-dye conjugated monoclonal antibodies to human T cell subsets,[28,29] that flow cytometers began to become widespread in clinical laboratories. These new instruments, with their advanced fluidics, optics, detectors, and analytic software, represented a new era for the future of clinical

immunophenotyping and made CD4 testing practical and affordable. Yet, the technology alone could not assure quality CD4 measurements for clinical use.

Cytologists, immunologists, and clinicians had good reason to suspect that CD4 measurements, just as most biologic assays, would likely demonstrate not only substantial within-person variation but also variation attributable to the test methods themselves. Substantial differences in CD4 counts obtained by different methods and instruments, in different locations, would compromise not only the accuracy and precision of the measurements, but also diminish the usefulness of CD4 testing in guiding clinical decisions regarding disease staging, therapeutic monitoring, and the potential use of CD4 as a surrogate for clinical endpoints in multicenter therapeutic trials. This forward-looking focus on the quality and reliability of CD4 measurements provided the impetus for Janis Giorgi (recruited by John Fahey to UCLA)[30] and Fred Valentine (at NYU)[31] to initiate two of the earliest proficiency testing programs for CD4 measurement. By sending laboratories masked whole blood samples, and collecting, along with the measured CD4 counts and percentages, information about laboratories' hematology results (WBC and differentials, automated versus manual), sample preparation methods, and analytic techniques, these investigators were able to: (i) reveal the often large inter-laboratory variation in CD4 measurement, and (ii) define the contribution of factors including cell separation (versus whole blood), staining methods, washing, fixation, gating, and compensation on the quantitation of CD4 T cells. As a result of this work, CD4 testing methods were standardized to improve quality and reduce both inter- and intra-laboratory variation, laying the groundwork for CD4 testing as a routine clinical laboratory measurement, and to date still the strongest predictor of disease progression and survival in HIV disease.[32,33] Furthermore, this research provided the data to support several evidence-based guidelines for CD4 immunophenotyping (e.g., NIAID Division of AIDS, CDC, and Clinical Laboratory Standards Institute), which became methodologic standards for good laboratory practice.[34–37] Importantly, this work also supported the ability of laboratories to develop reliable reference ranges for CD4 and other immunophenotypes in adults and children[38–40] and to evaluate new CD4 measurement technologies.[41–43] With increased global access to HIV therapy through UNAIDS, PEPFAR, and GFATM, CD4 proficiency testing and quality assurance programs are now widely available throughout much of the developing world and play an important role in supporting CD4-based assessments of patients' HIV disease status, eligibility for antiretroviral therapy, indications for opportunistic infection prophylaxis, and monitoring therapeutic responses. Programs include the U.S.-based NIAID DAIDS Immunology Quality Assessment (IQA) program,[44] UK-based United Kingdom National External Quality Assessment Service (UKNEQAS),[45] Canada-based Quality Assessment and Standardization for Immunological Measures (QASI),[46] South Africa-based African Regional External Quality Assessment Scheme,[47] Brazil-based Qualilab,[48] and Thailand-based External Quality Assessment Program (EQA).[49]

IV. CD4 AS A MARKER FOR HIV/AIDS

The observation that a laboratory marker deviates from the reference ("normal") range in association with a disease or condition can often be important in pointing to an underlying pathology. In 1981, the CD4 depletion seen in the early AIDS patients, together with the

clinical presentation of opportunistic diseases, were strong indicators that this new disease, whatever the cause, featured profound immune deficiency and/or dysregulation. With CD4 measurement standardized and widely available, the next steps for clinical immunologists were to determine: (i) if CD4 could be used to stage patients' disease severity to predict clinical outcome independent of treatment; (ii) if CD4 could be used to screen for activity of novel anti-HIV therapies; and (iii) if the magnitude of change in CD4 counts seen with anti-HIV therapy could predict the clinical benefit of a drug, and if so, how well.

A. CD4 as a Prognostic Marker

Results from the MACS, WITS (Women and Infants Transmission Study) and WIHS (Women's Interagency Health Study), and other longitudinal cohort studies examining the natural history of HIV disease established the utility of CD4 as a predictor of risk for clinical disease in HIV-infected individuals, independent of treatment, based on its ability to measure the severity of T helper cell depletion.[32,50–52] This demonstrated prognostic power of CD4 counts provided the basis early on for the inclusion of CD4 in HIV infection classification systems,[53] and the CDC AIDS surveillance case definition,[54] and CD4 remains an element of the U.S. and WHO HIV/AIDS treatment guidelines.[55,56]

B. CD4 as a Marker of Therapeutic Activity

The earliest phase I/II trials of antiretroviral drugs in HIV-infected people consistently showed that CD4 counts increased in proportion to antiviral activity.[57–60] This finding was fundamental in supporting the FDA's accelerated approval (1991) of the early nucleoside analog HIV reverse transcriptase inhibitors didanosine (ddI), and zalcitabine (ddC) (1992), and several other anti-HIV drugs including the first protease inhibitor, saquinavir, in 1995.[61] While quantitative plasma HIV-1 RNA is the now the standard for assessing viral load and antiviral therapeutic activity, there can be discordance between immunologic and virologic responses to antiretroviral therapy.[62,63] For this reason, CD4 counts still remain an important biologic marker in the context of early-phase trials to evaluate the activity of new anti-HIV therapies.

C. CD4 as a Surrogate Marker for Clinical Endpoints in Clinical Trials

The ultimate function for a biomarker is to predict clinical outcome and enable the assessment of efficacy of interventions based on marker values. The more fully the marker value reflects the clinical benefit of an intervention, such as the proportion of treatment effect explained,[64,65] the greater its validity in substituting for clinical endpoints in efficacy trials. Several analyses have shown that in HIV infection, despite its utility as both a prognostic and antiviral activity marker, CD4 count is a relatively weak surrogate marker of antiretroviral efficacy in that the observed increase in CD4 only partially explains the clinical benefit seen in patients.[66,67] Furthermore, the increased CD4 counts observed in trials of HIV patients undergoing treatment with interleukin-2 were not associated with clinical benefit.[68,69] This finding reemphasized the need for caution in the interpretation of biomarker changes in the context of different therapies (e.g., antivirals versus immunomodulators) where there may be reason to consider that the intervention may have an effect on the marker independent of mechanism(s) that lead to clinical benefit.

D. CD4 in Infants and Children

CD4, like other biomarkers, can vary in its utility in different populations. CD4 counts in children under five are highly variable due to fluctuations in absolute lymphocyte counts.[70] For this reason, the CD4 percentage is preferred over the CD4 cell count for use in young children[71] at the time of diagnosis of HIV infection, for monitoring children not on anti-retroviral therapy, as well as those on treatment when complete virologic suppression cannot be achieved.

V. CURRENT STATUS AND FUTURE OF CD4 ENUMERATION IN HIV/AIDS

The 2013 WHO consolidated antiretroviral (ARV) guidelines recommend viral load testing as the preferred approach to monitoring antiretroviral therapy response as it is more sensitive and can detect treatment failure earlier than CD4 counts and clinical monitoring.[55] These recommendations are supported by data from multiple trials and observational cohorts showing that HIV-positive patients on antiretroviral therapy whose viral load is well controlled have relatively stable CD4 cell counts.[72–76] Nonetheless, CD4 cell counting is still recommended for determining ART eligibility and time to initiate therapy.[55] In addition, CD4 cell counts are utilized to determine treatment and/or prophylaxis for opportunistic infections such as cryptococcal meningitis,[77] malaria, and bacterial infections.[55]

CD4 testing by flow cytometry can be cost prohibitive in developing countries with instruments typically priced at ~$75K or more, and reagents at $3–$7 per test depending on testing frequency.[78] This has generated interest in developing lower-cost point of care (POC) testing options for CD4 enumeration. The WHO criteria for POC diagnostic tools are defined by the acronym ASSURED: affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end users.[79] Recent studies have demonstrated that CD4 POC testing can reduce loss to follow-up increasing ART initiation rates.[80–82] For example, implementation of POC testing with the Alere Pima at four primary health clinics in Mozambique reduced the percent of patients lost to follow-up before start of ART from 64% to 33%.[80] Prior to the initiation of the POC testing, blood samples were collected once weekly and sent to a nearby laboratory, and patients had to return to the clinic once results were available. With the implementation of the Alere assay, finger stick blood samples were collected and tested generally on the same day.

There are currently four available POC devices with others in development. Table 1 summarizes information on CD4 POC testing options that are currently available or expected in the future.[78] As POC devices are developed they must be compared to reference (e.g., flow cytometry) technologies to determine performance characteristics including bias, precision, misclassifications for treatment decisions, and instrument reporting errors. Peeling et al.,[83] in their recent review of POC testing devices, found a lack of standardized testing schemes and fewer than half of studies included precision analyses. Lessons learned from the history of CD4 flow cytometry, including the importance of internal quality control, standardization, and external quality assurance programs, can play an important role in establishing the acceptability of POC testing for CD4.

VI. AFTERWARD

Looking back on 40 years of CD4 and HIV/AIDS research, a rather lucid picture and a cohesive story emerges from several lines of both competing and converging research. It is never so obvious, except perhaps in hindsight, if or how the pieces of a large scientific puzzle might all fit together. Exactly how John Fahey himself would look back on all of this we cannot be sure. But were he to have reviewed this brief history, we are confident he would have concurred with the telling, and some of us who worked closely with him can probably still hear his timeless and inimitable admonition to "insert the mind," to make use of what we have learned and move the field forward.

Acknowledgments

ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ARV	antiretroviral
CD	cluster of differentiation
CDC	Centers for Disease Control
FDA	U.S. Food and Drug Administration
HIV	human immunodeficiency virus
NIAID	National Institute of Allergy and Infectious Diseases
POC	point of care
UCLA	University of California, Los Angeles
WHO	World Health Organization

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TABLE 1

CD4 Point of care (POC) testing devices summary

Name	Status	Technology	Device price (USD)	Test price (USD)	Key features
Alere Pima Analyser	Available	Multicolor fluorescence-based instrument	$6500–$12,000	$6–$12	Measures absolute CD4 counts, but not % CD4 counts Uses disposable test cartridge with dried reagents Powered by traditional power supply, batter or solar power
Partec CyFlow miniPOC	Available	Flow cytometry-based instrument	$12,000	$4	Measures absolute CD4 counts and %CD4 counts Lyophilized testing reagents Powered by traditional power supply, solar charge or portable battery system
HumaCount CD4 NOW	Available	Flow cytometer and hematology analyzer	$25,000	$10	Measures absolute CD4 counts, %CD4 counts, and hematology Lyophilized testing reagents requiring no manual preparation Powered by traditional power supply, solar charge or portable battery system
BD FACS Presto	Available	Instrument based on fluorescence imaging and absorbance reading	Varies by country	Varies by country	Measures CD4 absolute count, %CD4 and hemoglobin Lyophilized testing reagents are provided as a disposable test cartridge Powered by traditional power supply, solar charge or portable battery system
Millipore Muse and Muse Human CD4 T cell kit	Available for laboratory research use only	Fluorescent-based microcapillary system	$13,000	$4	Measures absolute CD4 counts and %CD4 counts Millipore is seeking CE-IVD marking Requires minimal sample preparation Reagents are stored at 2–8°C
VISITECT CD4 semi-quantitative test	In development	Lateral flow assay	Optional reader est. $1200	Est. $2	Provides semiquantitative results for CD4 counts Disposable lateral flow assay Optional reader will be made available
Daktari CD4 Counter	In development	Microfluidic-based electro-chemical sensing	Est. $8000	Est. $9	Provides Absolute CD4 counts, but not % CD4 counts Label-free; antibodies required for cell separation
MBio CD4 System	In development	Two color fluorescence imaging cytometer	Not Available	Not Available	Measures absolute CD4 counts, but not % CD4 counts Uses disposable test cartridge