Eng Meng Tan

Edward K L Chan [1], K Michael Pollard,[2] Marvin J Fritzler [3]

Handling editor Josef S Smolen

[1]Oral Biology, Anatomy and Cell Biology, University of Florida, Gainesville, Florida, USA
[2]Molecular Medicine, The Scripps Research Institute, La Jolla, California, USA
[3]Medicine, University of Calgary, Health Sciences Centre, Calgary, Alberta, Canada

Correspondence to
Professor Marvin J Fritzler, Medicine, Health Sciences Centre, Calgary, Canada; fritzler@ucalgary.ca

Received 26 April 2024
Accepted 16 May 2024

To cite: Chan EKL, Pollard KM, Fritzler MJ. *Ann Rheum Dis* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/ard-2024-226025

Eng Meng Tan, MD, Professor Emeritus in the Department of Molecular Medicine of The Scripps Research Institute in La Jolla, California, passed away on 9 March 2024, at the age of 97 years. He was an exceptional clinical research scientist whose lifelong research career was distinguished by numerous seminal studies that uncovered the clinical and diagnostic importance of autoantibodies and their cognate autoantigens in systemic rheumatic and other diseases. Tan, the eldest of a family of eight brothers and two sisters, was born in Malaysia when it was still a British Colony. His intellectual prowess was evident early when he attained the highest score for the Senior English Nationwide Exam in the final year of high school. This became his 'ticket' to the USA for higher education because it ensured a full scholarship for his acceptance to attend Johns Hopkins University where he received a Bachelor of Arts (BA) in 1952 and an Doctor of Medicine (MD) in 1956. He and his family credit the guidance of his paternal grandmother, a midwife nurse by training in Malaysia, with his career choice in medicine. For his generation, an influential grandmother could advise a receptive child on a career pathway and he would be open to the idea and followed through with it.[1]

After completing medical school, he was an intern at Duke University Medical Center followed by 3 years of residency training in Internal Medicine at Case Western University School of Medicine in Cleveland. Following 2 years of research fellowship training in Professor Melvin Kaplan's laboratory in Cleveland (Ohio, USA), he joined the laboratory of Professor Henry G Kunkel at The Rockefeller University (New York, USA) in 1962. It was there that his lifelong interest in the immunology of rheumatic diseases began. While studying antibodies to DNA in systemic lupus erythematosus (SLE), he became interested in cellular autoantigens which are targets of autoantibodies in SLE and other diseases.

He met his wife Lisa while at Case Western and they were married the day before they moved to New York City. (Figure 1). Tan had learnt the relatively new immunofluorescent antibody technique while studying kidney disease in the Kaplan lab[2] as well as a practicum at McGill University in Montreal (Canada). These techniques became important in the subsequent discovery of anti-Sm and other antinuclear autoantibodies (ANA) while at the Kunkel lab.

Tan's publications on anti-Sm[3] and anti-DNA[4] in the Kunkel lab were highly acclaimed and he was recruited as Assistant Professor to the Washington University School of Medicine in St Louis (Missouri, USA) and just 2 years later he was recruited by Dr Frank Dixon to become an Associate Member of the Scripps Clinic and Research Foundation in La Jolla California, where he spent the majority of his efforts on research and up to 1 day per week in clinical practice. Within 3 years, he was promoted to Full Member and Head of Division of Allergy and Immunology (figure 2). In 1977 he was recruited to be Professor of Medicine and Head, Division of Rheumatic Disease at the University of Colorado in Denver. He moved his lab there with a few of his research fellows and a technologist where he continued landmark discoveries for 5 years before being recruited back to Scripps to become Head of the then new W M Keck Autoimmune Disease Center and the Division of Research Rheumatology, in the Department of Basic and Clinical Research; later renamed the Department of Molecular Medicine. He served in this position from 1982 to 2006 when he became Professor Emeritus. Professor Tan was affiliated with Scripps for more than 50 years, during which time he mentored numerous trainees from the USA, Germany, Japan, Sweden, Taiwan, Mexico, Canada, the UK, Brazil, Israel, Australia, China and others (figure 3). Many of his mentees referred to him, respectfully and affectionately, as Dr Tan or simply, 'ET' after the central extraterrestrial character in the beloved 1982 film directed by Steven Spielberg.

ANTINUCLEAR ANTIBODIES: DIAGNOSTIC MARKERS FOR AUTOIMMUNE DISEASES AND PROBES FOR CELL BIOLOGY

Tan published 350 research articles, not including standard review chapters, and accumulated more than 67 000 citations and a Google h-Index of 113. His contributions may be divided into two categories: the discovery and characterisation of autoantibodies as important diagnostic markers of systemic autoimmune diseases, and the use of these autoantibodies as probes to identify and elucidate novel macromolecular cellular structures. Three of the most prominent examples are excellent illustrations of his contributions.

First, and considered one of his most significant discoveries by Tan himself, is the description of anti-Sm autoantibodies which became an extremely important marker for the diagnosis of SLE. The study of anti-Sm and other related anti-small nuclear ribonucleoprotein antibodies was a major contribution to the early understanding of mRNA splicing and the importance of this previously unknown cellular process.[5] The characteristic HEp-2 immunofluorescence assay (HEp-2 IFA) nuclear speckled pattern associated with anti-Sm designated AC-5 by the International Consensus on Autoantibody Patterns (ICAP) is illustrated in figure 4A.

The second example is the report of anti-centromere antibodies in systemic sclerosis[6] which

Figure 1 Dr Tan and his wife Lisa at their wedding. Circa 1962.

has been cited more than 1000 times. To date, the detection of anticentromere antibodies is still clearly linked to systemic sclerosis. Three major antigens were described as CENP-A, CENP-B and CENP-C and they have been extensively studied and reported in more than 2000 papers to date. The distinctive anticentromere HEp-2 IFA pattern, designated AC-3 by ICAP is shown in figure 4B.

The third example is the discovery of antibody to proliferating cell nuclear antigen (PCNA) by Miyachi *et al* which has been cited more than 1240 times.[7] PCNA was found to be a DNA 'clamp' that acts as a processivity factor for DNA polymerase δ in eukaryotic cells and is essential for DNA replication. With optimal fixation, PCNA is associated with proliferating cells and DNA replication and this biomarker is still commonly used in many laboratories studying stem cells and the cell cycle. A literature search for PCNA yielded more than 25 700 citations. Interestingly, when many contemporary investigators are asked whether they know where the term 'PCNA' comes from or how it was discovered, most cannot link the origin to Tan's discovery. The characteristic pleomorphic anti-PCNA HEp-2 IFA pattern designated AC-13 by ICAP is illustrated in figure 4C.

There were many other contributions worthy of mention which also achieved clinical and scientific breakthroughs:

A seminal paper in 1966 from Tan's research in the Kunkel lab showed the sequential appearance of antibody to DNA and DNA antigen in the sera of patients with SLE.[4] Experiments using Ouchterlony double immunodiffusion assays showed that DNA antibody from one time period was reactive with serum DNA at another time period. The sequential appearance of antibody

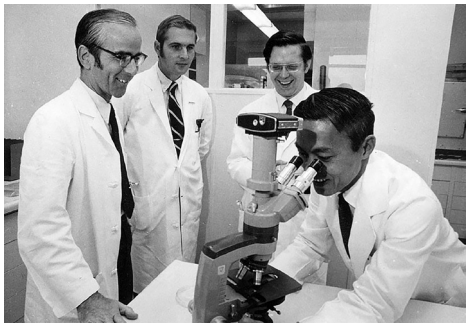

Figure 2 Dr Tan (far right) at a microscope at The Scripps Clinic and Research Institute with colleagues (left to right) Drs John Vaughan, Donald Stevenson and David Matheson. Circa 1971.

Figure 3 A gathering of former trainees, staff, family and colleagues (dubbed The Tan Clan) was a highlight and annual event, typically at the time of the Annual Meeting of the American College of Rheumatology. Eng and Lisa Tan (front row) and their two sons Philip (back row far left) and Peter (back row far right). Circa 2017.

followed by disappearance of free antibody and appearance of DNA antigen had the earmarks of antigen excess associated with circulating immune complexes. At this time, the patient under study had a flare of SLE manifested as high fever and increased proteinuria. Up to that time, experimental animal models of immune complex disease had been described by Frank Dixon and Baruj Benacerraf and their colleagues, but not in human disease. This was the first indication that immune complex formation could also be a factor in human disease and in this case, DNA:anti-DNA immune complexes.

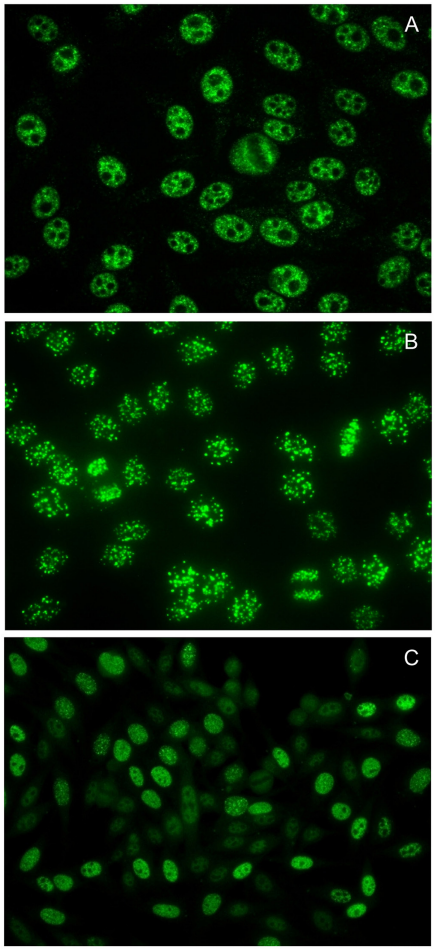

Figure 4 Immunofluorescence patterns on HEp-2 cell substrates reported by by Dr Eng Tan that were the cornerstone of the discovery of anti-Sm (A), anti-centromere (B) and anti-proliferating cell nuclear antigen (anti-PCNA: C).

AUTOANTIBODIES AS CLASSIFICATION CRITERIA

In 1975, Tan *et al* introduced the notion of profiles of ANA as biomarkers in systemic rheumatic diseases.[8] They examined the occurrence of autoantibodies to four different cellular antigens in SLE, rheumatoid arthritis, Sjögren's disease, systemic sclerosis and mixed connective tissue disease, and showed different patterns or profiles of reactivity with these cellular antigens. Anti-Sm was restricted to SLE and antinuclear ribonucleoprotein (RNP) was detected in all patients with mixed connective tissue disease. This notion of autoantibody profiles was further explored in subsequent publications[9–11] and autoantibodies have now been incorporated into classification criteria for SLE, Sjögren's disease, systemic sclerosis, mixed connective tissue disease and others. In systemic sclerosis, the identification of antibodies reactive with centromere proteins[6] was an important breakthrough in establishing that limited cutaneous systemic sclerosis or what at the time was called the CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia) could be primarily defined by the presence of anticentromere antibodies.[12] By comparison, the newly discovered anti-Scl70 (topoisomerase I) antibody was associated with diffuse cutaneous systemic sclerosis.[13] Tan was Chairman of an American College of Rheumatology (ACR) ad hoc committee for establishing a revision of the preliminary SLE classification criteria published in 1982. These were the first classification criteria which included autoantibodies (anti-DNA and anti-Sm as well as generic antinuclear antibodies) in the diagnosis and classification of SLE. It is worth mentioning that the 1982 revised criteria for the classification of SLE[14] is one of the most cited works (16 900 times) coauthored by Dr Tan.

Autoantibodies in cancer

His interest in human autoantibodies in rheumatic diseases eventually led Tan to extend this interest to autoantibodies in cancer. Using similar experimental approaches, his laboratory revealed that there are many autoantibodies in patients with cancer directed against known tumour-associated antigens such as p53 and c-myc as reported by others, but also to other autoantigens such as the recently described p62, a cytoplasmic protein with RNA binding motifs and shown to bind to the 5' untranslated region of insulin-like growth factor II mRNA.[15 16] Together with his previous observations in rheumatic disease and the more recent observations in cancer, he advanced the notion that autoantibodies might be regarded as reporters identifying aberrant cellular mechanisms in tumorigenesis.[17–19]

Tan received numerous honours and awards

A notable recognition was the Senior Distinguished US Scientist Award of the Alexander von Humboldt Foundation in 1986. This award provided opportunity for him to spend several months in Heidelberg where he worked with German scientists/collaborators and cofounded a conference series entitled International Workshops on Autoantibodies and Autoimmunity with Professor E K F Bautz, former head of the Institute of Molecular Genetics in Heidelberg and Professor J R Kalden, University of Erlangen-Nuremberg, and Professor Mitsuo Homma from Keio University in Japan. The first meeting was held in 1989 in Heidelberg and subsequent workshops were held in San Diego, California (USA), the Walter & Eliza Hall Institute in Melbourne (Australia), Chapel Hill (North Carolina, USA), Oslo (Norway), Nagoya (Japan), Berlin (Germany), Gainesville (Florida, USA), Guadalajara (Mexico), Shanghai (China) and others.

The year 1989 was exceptional for Tan as he won three major awards: the Novartis International League of Associations for Rheumatology Prize, the Lee C Howley Sr Award of the National Arthritis Foundation, and Carol Nachman Award for Arthritis Research. He mentioned in his own obituary the special arrangement made in receiving the Carol Nachman Prize in Wiesbaden, Germany, first in the morning of the day and was able to fly to Phoenix in the evening to receive the Lee C Howley Sr Award, the highest honour from the US National Arthritis Foundation.

Tan was also active with other ACR committees and eventually served as its President from 1984 to 1985. He also received major ACR awards including the Distinguished Investigator Award (1991), Master (1992) and the Gold Medal Award (1998).

Tan also received a number of lifetime achievement awards representing acknowledgements of his national and international recognition in clinical immunology. The notable ones include Japan Rheumatism Foundation International Award, Tokyo (2003), European League Against Rheumatism Meritorious Service Award (2005) and Lifetime Achievement Award in Lupus Research at the 8th International Congress on SLE, Shanghai, China (2007).

Also notable, Tan served as the Chairman of a committee sponsored by the International Union of Immunological Societies, WHO and the Centers for Disease Control (CDC, USA) charged with establishing reference sera which could be used as reagents to standardise antinuclear antibody testing. He was Chairman of this committee from 1984 to 2000. Clinician scientists from the USA, Canada, Europe, Japan and Australia were members of this international committee and participated in the establishment of a bank of autoantibody reference sera which were stored by the CDC and distributed by them to research and commercial laboratories.[20] This biobank now provides more than 20 reference sera, each containing an autoantibody of a specific specificity. About 5 years ago, this resource was moved from CDC to the Plasma Service Group (https://www.plasmaservicesgroup.com/) and new reference materials have been added to include other autoantibody specificities. This international committee was involved in the analysis of commercial antinuclear antibody test kits manufactured by different companies in the USA, Europe and Japan. These standard reference sera have been instrumental in helping commercial clinical laboratories reach high standards of performance.

Tan had the highest appreciation of his mentors. This was especially true of Henry G Kunkel who unexpectedly passed away at the age of 67 years. Together with other colleagues, Tan organised and

Figure 5 Eng Tan and wife Lisa at his 90th birthday celebration in La Jolla. Circa 2016.

was the founding President of the Henry G Kunkel Society (1992–1994) established 'to continue his legacy of clinical research based on rigorous observations at the bedside and at the laboratory bench'. Annual meetings are held in New York at The Rockefeller University and there are now several hundred members.

A major event was the celebration of his 90th birthday in La Jolla in 2017 (figure 5). On 6 April 2024, Professor Tan's life and scientific achievements were celebrated by his colleagues, family and friends at the Scripps Research Institute.

Acknowledgements The authors thank Dr Philip Tan (son of Dr Eng M Tan) for assistance in providing information and some photographs. Figure 4 depicting HEp-2 IFA patterns traceable to Dr Tan's seminal research were in the files of coauthors, members of the ICAP (https://anapatterns.org/trees-2021.php).

Contributors All authors contributed to information gathering, compiling, writing, editing and reviewing the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Edward K L Chan http://orcid.org/0000-0003-3938-9503
Marvin J Fritzler http://orcid.org/0000-0003-1652-6608

REFERENCES

1 Tan PK, Chan EKL, Fritzler MJ. Dr. Eng Meng tan August 26, 1926 - March 9, 2024 La Jolla, California. *Lupus* 2024.
2 Tan EM, Kaplan MH. Immunological relation of basement membrane and a serum beta globulin in the mouse. demonstration of renal basement membrane alteration in mice injected with Streptolysin S. *Immunology* 1963;6:331–44.
3 Tan EM, Kunkel HG. Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J Immunol* 1966;96:464–71.
4 Tan EM, Schur PH, Carr RI, *et al*. Deoxybonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. *J Clin Invest* 1966;45:1732–40.
5 Tsokos GC. In the beginning was SM. *J Immunol* 2006;176:1295–6.
6 Moroi Y, Peebles C, Fritzler MJ, *et al*. Autoantibody to centromere (kinetochore) in scleroderma sera. *Proc Natl Acad Sci U S A* 1980;77:1627–31.
7 Miyachi K, Fritzler MJ, Tan EM. Autoantibody to a nuclear antigen in proliferating cells. *J Immunol* 1978;121:2228–34.
8 Notman DD, Kurata N, Tan EM. Profiles of antinuclear antibodies in systemic rheumatic diseases. *Ann Intern Med* 1975;83:464–9.
9 Tan EM. Autoantibodies to nuclear antigens (ANA): their Immunobiology and medicine. *Adv Immunol* 1982;33:167–240.
10 Tan EM, Chan EKL, Sullivan KF, *et al*. Antinuclear antibodies (anas): diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. *Clin Immunol Immunopathol* 1988;47:121–41.
11 Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv Immunol* 1989;44:93–151.
12 Tan EM, Rodnan GP, Garcia I, *et al*. Diversity of antinuclear antibodies in progressive systemic sclerosis. Anti-centromere antibody and its relationship to CREST syndrome. *Arthritis Rheum* 1980;23:617–25.
13 Douvas AS, Achten M, Tan EM. Identification of a nuclear protein (Scl-70) as a unique target of human antinuclear antibodies in scleroderma. *J Biol Chem* 1979;254:10514–22.
14 Tan EM, Cohen AS, Fries JF, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–7.
15 Zhang J-Y, Chan EKL, Peng X-X, *et al*. A novel cytoplasmic protein with RNA-binding motifs is an autoantigen in human hepatocellular carcinoma. *J Exp Med* 1999;189:1101–10.
16 Lu M, Nakamura RM, Dent ED, *et al*. Aberrant expression of fetal RNA-binding protein P62 in liver cancer and liver cirrhosis. *Am J Pathol* 2001;159:945–53.
17 Imai H, Chan EKL, Kiyosawa K, *et al*. Novel nuclear autoantigen with splicing factor motifs identified with antibody from hepatocellular carcinoma. *J Clin Invest* 1993;92:2419–26.
18 Tan EM. Autoantibodies and autoimmunity: a three-decade perspective. A tribute to Henry G. Kunkel. *Ann N Y Acad Sci* 1997;815:1–14.
19 Tan EM. Autoantibodies as reporters identifying aberrant cellular mechanisms in tumorigenesis. *J Clin Invest* 2001;108:1411–5.
20 Tan EM, Fritzler MJ, McDougal JS, *et al*. Reference sera for antinuclear antibodies. I. antibodies to native DNA, SM, nuclear RNP, and SS-B/La. *Arthritis Rheum* 1982;25:1003–5.