Richard R. (Randy) Hardy 1952–2016

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Widely recognized for his expertise in flow cytometry and B cell development and for the development of the 'Hardy scheme' of B cell differentiation, Randy Hardy died on May 29, 2016, of complications associated with ALS. Most recently a senior member of the Fox Chase Cancer Center in Pennsylvania, Hardy completed his doctoral studies in biochemistry at the California Institute of Technology in 1981, where he received the Herbert Newby McCoy Award for outstanding achievement in research. He then spent the next three years as a fellow of the California division of the American Cancer Society, working in the Herzenberg laboratory in the Department of Genetics at the Stanford University School of Medicine.

At Stanford, Randy met and ultimately married his long-term collaborator, Kyoko Hayakawa, then a postdoctoral fellow in the Herzenberg lab who had trained with Tomio Tada at the University of Tokyo. Toward the end of 1984, Randy and Kyoko left Stanford and joined the Kishimoto laboratory at Osaka University, where they worked until 1987, when Randy was appointed to the faculty of the Fox Chase Cancer Center. Kyoko initially worked with Randy in his new laboratory, but within two years she was independently appointed a member of the Fox Chase Cancer Center faculty, a position she still holds today.

Shortly after arriving at Stanford in 1981, Randy began working with David Parks, Kyoko and the Herzenbergs to complete the development of, and demonstrate the power of, what soon became the first dual-laser flow cytometry instrument to be put into routine use in biomedical research. Randy also played a key role in developing the use of fluorescence-labeled monoclonal antibody reagents to define and quantitate 'cluster of differentiation' (CD) markers. This included the introduction of phycoerythrin and other fluorescent proteins as reagents for both fluorescence microscopy and flow cytometry. Randy was one of the first to recognize the importance of these techniques, which revolutionized the way research in immunology and other disciplines is conducted.

More recently, Randy combined this flow cytometry expertise with a series of molecular analyses related to B cell development. He helped organize the network-based ImmGen Project Consortium, and he became the *de facto* go-to person for the molecular analysis of gene expression in flow-cytometry-sorted B cell subsets. We well remember his excitement when the ImmGen app appeared, giving researchers easy access to molecular expression data for many flow-cytometry-sorted subsets, including the B cell subsets that he and his colleagues defined.

Randy also had a formative role in the development and application of multicolor (and, later, high-dimensional) fluorescence-activated cell sorting technologies in lymphoid studies. In a series of highly insightful collaborative analyses of murine B cell subsets, he used the two-laser flow cytometer to demonstrate the existence of characteristic B cell subsets that differ between adult BALB/c and CBA/N mice. He showed that these subsets are genetically and developmentally determined rather than (as thought at the time) reflective of successive stages of the B cell response to antigenic stimulation. This seminal work initiated an incredibly fruitful, lifelong collaboration with Kyoko. They ultimately published more than 70 papers together as they pursued their joint quest to more fully understand B cell development and selection.

Follow-up studies that Randy and Kyoko began while still at Stanford yielded another first: they uncovered the existence of a B cell subset in mice that expresses low but clearly detectable levels of the Ly-1 surface marker (now known as CD5), which had previously been detected only on T cells. Further, they showed that this novel 'Ly-1 B' cell subset, currently known as B-1a cells, is present in normal mice and expanded in autoimmune mice.

In the ensuing years, the collaboration between Randy and Kyoko flourished. Randy paid particular attention to early events in the B-2 cell development pathway and to the relationship between B-1a cells and the development of chronic lymphoid leukemia in humans. Kyoko researched the mechanisms and consequences of the self-reactivity of antibodies produced by B-1a cells and demonstrated the dependence of B-1a cell autoantibody production—specifically that of anti–Thy-1—on the expression of the gene encoding the Thy-1 glycoprotein.

In 1991, within 4 years of arriving at Fox Chase, Randy and Kyoko published what has become one of the most influential papers on B cell development. In a key series of studies charting the developmental pathway of B-2 (so-called conventional B) cells in bone marrow and in neonatal organs, they introduced what is now widely known as the Hardy scheme, a flow-cytometry-based phenotypic delineation of the B cell developmental pathway—from early precursors to fully matured B cells—in bone marrow. Paul Kincade, in his commentary on the republication of this landmark paper in the *Journal of Immunology*, stated, "If nature abhors a vacuum, Randy Hardy was the ideal person to fill it." We could not agree more!

Despite being very interactive scientists, Randy and Kyoko were very private people. Randy built and thoroughly enjoyed an extensive collection of tropical fish, which he kept in a series of tanks at home and at the laboratory. In addition, he (and sometimes his family) often joined his long-term colleague David Parks (Herzenberg Laboratory) on hiking and camping trips in the United States and Japan.

Randy's death came all too soon. He will be sorely missed by his wife and by his daughter Naomi, who is just finishing medical school. And of course Randy will also be missed by all who had the privilege of studying or working with him.

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