Chapter 23

Immunopathology

Atul K. Bhan, Robert B. Colvin,
and Harold F. Dvorak

The application of immunology to clinical medicine and the development of immunopathology as a specialized field stem largely from the pioneering work of the great immunologist/pathologist Paul Ehrlich, who received the 1908 Noble Prize for his work on immune responses in infection (1). The introduction of immunofluorescence techniques by Dr. Albert Coons in the 1940s set the stage for establishment of immunohistochemical techniques and development of flow cytometric analysis of cells. Dr. Coons, who had been trained in internal medicine at the Massachusetts General Hospital (MGH), performed most of his work at Harvard Medical School (HMS), initially in the Department of Microbiology and Immunology and later in the Pathology Department. Over the years, immunopathology has applied the knowledge obtained from basic immunology research to achieve a better understanding of disease pathogenesis, develop sophisticated tests for accurate clinical diagnosis, and implement new therapeutic strategies.

The history of immunopathology at MGH will be described for three periods: (1) before 1975, when immunopathology concepts were being developed and when the Immunopathology Laboratory was established; (2) from 1975 to 1991, when the Immunopathology Unit was formed and flourished under the leadership of Dr. Robert T. McCluskey (chapter 14) to become the dominant research unit in MGH Pathology, and when, beginning in the 1980s, the unit rapidly implemented diagnostic immunohistochemistry into the routine diagnostic service; and (3) from 1991 to the present, when the unit continued to develop new immunological diagnostic tests and conduct basic research.

Early Years: Before 1975

The earliest manifestations of the then-nascent field of immunopathology at MGH were the studies of Dr. Louis Dienes, a bacteriologist who was recruited to the Pathology department in 1930 (see figure 21.3; chapters 5 and 21). He showed that simple proteins (ovalbumin) could elicit a "tuberculin reaction" in the skin if the animal (guinea pig) had previously been sensitized by inoculation of the protein into a tuberculous lesion (2). At the time only products of organisms were thought to trigger this type of response, termed a delayed-type hypersensitivity reaction and later shown to be a T cell mediated reaction. With Dr. Tracy Mallory (chapter 6) in 1932 he characterized the morphology of the lesions and contrasted them with antibody-mediated reactions (Arthus reaction). Tuberculin sensitivity could also be produced by sensitization with protein alone (no tuberculous infection), although it was milder (Jones-Mote reaction). Also with Dr. Mallory, in 1937, he showed that tuberculin hypersensitivity developed simultaneously with the mononuclear infiltrate surrounding tuberculous lesions.

Starting in 1951, Dr. Byron Waksman (figure 23.1), a neurologist at MGH, showed that analogous delayed immune reactions to autologous proteins (myelin) could produce a rapid-onset form of disseminated encephalomyelitis in rabbits that had been immunized with rabbit spinal cord, characterized by a perivenous infiltration of "round" cells and neuronal demyelination, which had pathologic similarities to multiple sclerosis (3). Dr. Waksman devoted most of his career to an understanding of the pathogenesis of delayed hypersensitivity as it occurred, not only in the brain, but also in joints (a model of rheumatoid arthritis) and skin of animals of several species. His laboratory was on Warren 3, above the Pathology department, and he attracted several outstanding pathologists into the field of immunopathology (chapter 17).

One of those attracted was an HMS student, Harold F. ("Hal") Dvorak, who was impressed by Dr. Waksman's lectures and delighted to accept his joint offer, with Dr. Benjamin Castleman (chapter 8), to take a post-sophomore pathology fellowship in 1959–1960, splitting time between diagnostic pathology and research. Dr. Dvorak, who worked on cell transfer studies and development of in vitro immune assays, considered the fellowship one of the most formative parts of his life. Dr. Waksman had a coterie of international postdoctoral fellows in his lab at that time, including Branislov Jankovich (Yugoslavia), Barry Arnason (Canada), and Timo Kosunen (Finland), all of whom were working on aspects of cellular immunity. Dr. Kosunen made use of the recently available isotope tritiated thymidine to perform autoradiography and demonstrated for the first time that a substantial fraction of cells accumulating in tuberculin-type reactions were actively dividing (4). Dr. Arnason was one of the first to demonstrate that neonatal thymectomy severely compromised the immune system, so that rats were able to "tolerate" and not reject grafts of foreign tissues. Dr. Robert B. Colvin (chapter 25) was another HMS student attracted to the group on Warren 3, in his case by Dr. Arnason's lectures, and he spent the summer of 1966, after his second year at HMS, studying the antigens of the hippocampus in the laboratory directly above Dr. Castleman's office.

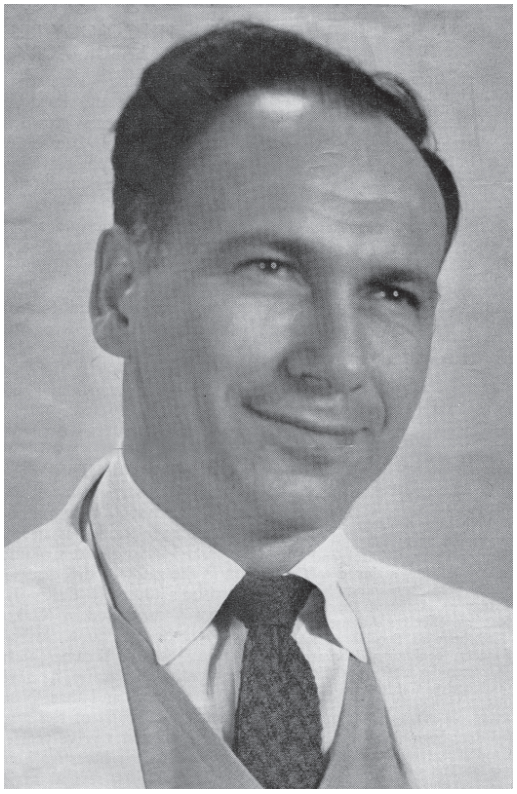

Figure 23.1 *Byron Waksman*

Recognizing the increasing importance of immunology in the understanding and treatment of many diseases, Dr. Castleman decided to form an Immunopathology Laboratory in 1959 under the direction of Dr. Martin H. Flax (figure 23.2), a protégé of Dr. Waksman. The concept of immune mediated injury was extended to other organs by Drs. Flax and Waksman in 1963 studies on adjuvant disease (an autoimmune arthritis) and in 1963–1966 studies on experimental allergic thyroiditis (5) with Drs. Stuart Sell and James Billote; on allergic contact dermatitis with Dr. James Caulfield; and on beryllium granulomatous hypersensitivity with Dr. Sidney Leskowitz in Medicine (6). As the early focus of the laboratory was diagnostic renal biopsies, a close

relationship developed between the Immunopathology Laboratory and nephrologists as well as the Renal Transplantation Unit.

Dr. Vivian Pinn started working with Dr. Flax in 1967, when she joined MGH as a resident in Pathology (see figure 7.26). Dr. Pinn moved with Dr. Flax to Tufts University Medical School in 1970, when Dr. Flax was made the Chairman of the Department of Pathology there. She remained involved in immunopathology of the kidney and subsequently became Professor and Chairman of Pathology at Howard University College of Medicine, the first African American woman to chair an academic pathology department.

Dr. Colvin, who at that time was a resident in Pathology, worked on renal and transplant pathology with Drs. Pinn and Flax and became the resident expert in this area when they left for Tufts. Dr. Colvin was able to resuscitate an ancient Reichert fluorescent microscope for immunofluorescence, which had to be done at night, since the room on Warren 1 had no shades on the windows.

Dr. Dvorak had come back to MGH for an internship and residency in Pathology in 1963 and then, working with Dr. Flax, made important observations regarding immunological unresponsiveness induced by intravenous tolerizing antigen. After two years of research at NIH, Dr. Dvorak returned to the MGH in 1967 and began developing and directing immunopathology research. Dr. Dvorak used elegant one-micron Giemsa-stained Epon sections, prepared by his research assistant, Eleanor Manseau, to study the cellular details of delayed-hypersensitivity reactions by light microscopy (7). He was, therefore, for the first time able to appreciate infiltrating basophil leukocytes in tissues and their participation in a type of delayed-hypersensitivity reaction (Jones-Mote reaction) induced by immunization with protein antigens in incomplete Freund's adjuvant (which were probably what Drs. Dienes and Mallory had created by injecting ovalbumin without tubercle bacillus). Because of its high basophil content, the reaction was called cutaneous basophil hypersensitivity (CBH). Similar, basophil-rich reactions were found in other organs and tissues in response to an immune challenge: for example, the eye in ocular hypersensitivity and human kidneys undergoing acute allograft rejection (8, 9). Dr. Dvorak remembers showing one-micron Epon slides to Dr. Castleman; he was impressed but asked for a hematoxylin and eosin (H&E) section of the same lesions and had no trouble detecting basophils!

These studies were extended to human cellular immunity when Dr. Dvorak developed poison ivy on Martha's Vineyard. He biopsied his rash and found it was swarming with basophils. He secured the help of Dr. Colvin and Dr. Martin C. Mihm Jr. of Dermatopathology (chapter 18) to extend these studies to other contact allergens, employing volunteers who were biopsied, including the investigators. Dr. Ann Dvorak (Hal's

Figure 23.2 *Martin Flax*

wife), who had done a postdoctoral fellowship in electron microscopy with Dr. Morris Karnovsky at HMS and had helped demonstrate basophils as an important feature of other cellular immunity in guinea pigs, also participated in the studies. Dr. Ann Dvorak showed through ultrastructural studies the activation of microvascular endothelial cells and pericytes in the venules from which lymphocytes, basophils, and other inflammatory cells were extravasating, and the degranulation of both basophils and mast cells as features of cellular immunity. She found that basophils and mast cells in cellular immune reactions underwent not the explosive degranulation characteristic of anaphylaxis, but, rather, a partial type of piecemeal degranulation in which granule contents were released gradually over time by a vesicular transport mechanism. Her concept of piecemeal degranulation is now widely accepted not only in basophils but also in degranulating eosinophils, which she subsequently studied after moving to Beth Israel Hospital in 1979. One important observation made in these studies was that fibrin deposited outside the vessels was related to the induration, a characteristic feature of tuberculin hypersensitivity (10). Proof that fibrin was responsible for causing induration came from subsequent studies by Dr. Colvin in two patients with congenital afibrinogenemia; both developed erythematous delayed reactions with a typical cellular infiltrate but lacked fibrin deposition and induration.

1975–1991: The McCluskey Era

As Dr. Dvorak's studies were progressing, Dr. Robert McCluskey succeeded Dr. Castleman as Chief of Pathology (chapter 13). During Dr. McCluskey's tenure at Children's Hospital in Boston (1971–1974), he established immunopathology research and developed collaborations that continued after his move to MGH. In 1975 Dr. Atul K. Bhan, who had been a postdoctoral fellow with Dr. McCluskey following completion of Chief Residency in Pathology and a postdoctoral research fellowship at Children's Hospital, moved to MGH to pursue immunopathology research. The Immunopathology Laboratory at that time was located on the first floor of the Warren Building. Dr. Elizabeth Hammond, who graduated from the University of Utah School of Medicine in 1967, joined the lab first as a research fellow in 1972 and subsequently as a staff member in 1974. Dr. Stephen J. Galli, who graduated from HMS in 1973, joined the lab as a research fellow in 1977. Both Dr. Hammond and Dr. Galli had been residents in Pathology at MGH. At that time as well, Dr. Ann Dvorak was recruited from Tufts to establish a clinical and research electron microscopy laboratory.

In the 1970s MGH administration committed new resources to the Pathology department, adding much needed space for research on the fifth and eighth floors of the recently built Cox Building. A committee composed of Drs. Hal and Ann Dvorak, Bhan, Hammond, and Mihm designed the Cox space for research as well as for diagnostic immunopathology and electron microscopy. The highly functional open design has been copied by the other major research laboratories at MGH (e.g., the Wellman/Thier, Martin, and Simches buildings). The fifth-floor Immunopathology Unit was completed by January 1976 and the eighth-floor animal facilities in April 1977. The only glitch was that the electron microscopes were installed over the massive ventilation fans bolted to the ceiling of the fourth floor; the solution was to suspend the microscopes on bungee cords attached to a 3-inch-thick iron plate.

The new laboratories in the Cox Building provided the infrastructure for the development of the nationally recognized Immunopathology Unit. The unit, directed by Hal Dvorak, was staffed initially by Drs. Bhan, Hammond, John Long, Galli, Neil Orenstein, and Colvin, who had returned to MGH after working as an experimental pathologist at Walter Reed Army Institute of Pathology from 1972 to 1975. Dr. Hammond studied macrophage migration inhibitory factor,

Figure 23.3 *Harold Dvorak working in the Immunopathology Laboratory, Cox 5, late 1970s*

and she and Dr. Colvin got their first R01 grant together on the clotting system in delayed-type hypersensitivity. She soon left to accompany her husband to Salt Lake City, however, where she became Professor of Pathology at the University of Utah and Chair of the Department of Pathology at the LDS Hospital. Dr. Galli and his wife, Anne, continued the work on basophils with Hal Dvorak, demonstrating that purified guinea pig basophils were able to synthesize histamine. Dr. Galli subsequently focused his research on mast cells, and he became one of the world's leading authorities on mast cell biology; he moved from Beth Israel to become Chair of the Department of Pathology at Stanford University School of Medicine in 1999.

Dr. Hal Dvorak (figure 23.3) extended his studies of the immune response to tumors and collaborated with Dr. W. Hallowell Churchill at Brigham and Women's Hospital (9). Dr. Dvorak found that supernatants from nearly all human and animal tumors generated intense blue spots in the Evans blue dye permeability assay, whereas those from several normal cells did not (figure 23.4). He called this tumor supernatant permeabilizing activity *vascular permeability factor,* or VPF (11). Subsequently, VPF was shown to be a weak endothelial cell mitogen, was renamed vascular endothelial growth factor (VEGF), or, more specifically, VEGF-A, and became a therapeutic target in cancer and macular degeneration. Subsequent studies revealed that VPF/VEGF was overexpressed by most malignant tumors and at lower levels in a number of normal adult tissues. After moving to Beth Israel Hospital in 1979 (taking with him his wife, Ann, and Stephen Galli), Dr. Dvorak continued his work and found that VPF/VEGF had a central role in wound healing and chronic inflammatory diseases such as psoriasis, rheumatoid arthritis, and induced lymphangiogenesis as well as angiogenesis; others discovered that it was essential for the development of the normal vasculature. Thus, Dr. Dvorak's discovery of VPF resulted not only in the finding of a new molecule but also in the elucidation of a process—one that, although not yet completely understood, is responsible for initiating stroma formation and tissue repair following injury. Unfortunately, this same process also facilitates the growth and spread of malignant tumors, as well as having central roles in diabetic retinopathy and several types of inflammatory diseases.

Because of Dr. McCluskey's research interest in renal diseases, the immunopathogenesis of kidney diseases became a major research and diagnostic focus of the Immunopathology Unit (figure 23.5). He built a strong research team, including Dr. Colvin, Dr. Bhan, and Bernard Collins to study mechanisms involved in renal diseases. Dr. Eveline E. Schneeberger, who had worked previously with Dr. McCluskey at Children's Hospital, joined the team in 1979. Drs. Bhan and McCluskey explored novel mechanisms of

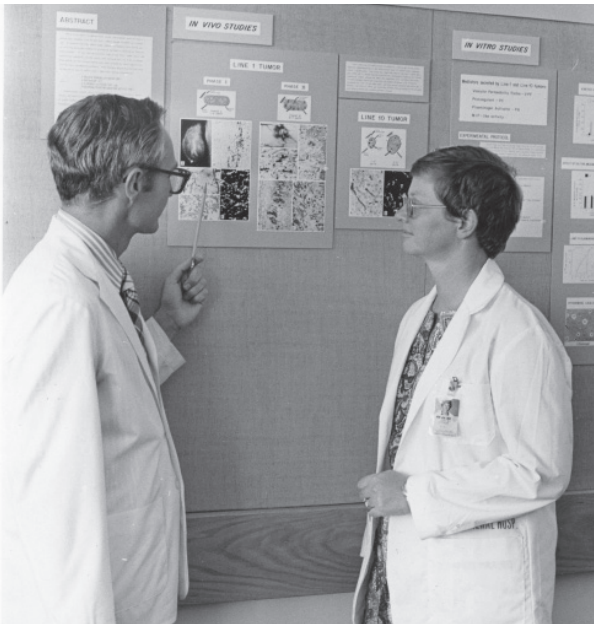

Figure 23.4 *Harold and Ann Dvorak in front of the research poster describing tumor secreted mediators, Cox 5, late 1970s*

glomerulonephritis. By adoptive transfer experiments, they conclusively showed that T cells could induce glomerular injury against a planted glomerular antigen, challenging the dogma that T cells had no role in glomerulonephritis (12). Dr. McCluskey recruited Dr. Lynn Baird to identify the antigen of membranous glomerulonephritis, a goal he had long pursued. These studies and a subsequent collaboration with Dr. John Smith led to the identification and cloning of an autoantigen located in rat renal tubules and podocytes (megalin), which was the target in Heyman nephritis (13), a rat model of membranous glomerulonephritis (MGN); however, this antigen did not prove to be the human autoantigen they were seeking. Dr. McCluskey continued to work on this theme after his retirement as chief in 1991 and even secured an NIH grant to support his studies. Dr. Collins and Colvin continue working on the target antigens of MGN in 2011. Collins also took a major initiative in developing and expanding the application of immunofluorescence in human renal biopsies and developed many tests that were offered as part of the departmental reference laboratory in renal pathology, including the Western blot assay for the detection of antibodies to glomerular basement membrane (GBM), which is the definitive diagnostic test for anti-GBM antibodies. Immunofluorescence studies were also applied for the diagnosis of skin disorders, including indirect immunofluorescence tests for the detection of circulating autoantibodies in patients with bullous lesions of the skin.

Dr. John Niles, a nephrologist, started postdoctoral research training with Dr. McCluskey in 1985. Together they investigated the role of antineutrophil cytoplasmic autoantibodies (ANCA) in the pathogenesis of Wegener's granulomatosis. These studies led to identification of one of the ANCA autoantigens, proteinase-3, and the development of quantitative diagnostic assays. Dr. Niles has achieved international recognition as an expert in ANCA-related diseases and directs the MGH Pathology reference laboratory for ANCA testing (14, 15).

Robert Colvin became the Director of the Immunopathology Unit in 1979. In Dr. Colvin, Dr. McCluskey found a colleague with a common interest in renal pathogenesis and developing a strong diagnostic renal pathology program. While a house officer in Pathology at MGH, Dr. Colvin had become interested in renal and transplant pathology. Dr. Paul Russell, a distinguished surgeon who established the MGH Transplant Service, became a mentor and longtime friend in this field. From 1971 to 1972, Dr. Colvin was an NIH Research Fellow under the mentorship of Dr. Harold Dvorak, working on the role of the clotting system in T cell mediated immune reactions. Dr. Colvin extended the studies to the role of fibronectin in inflammatory reactions and wound healing with a fellow, Dr. Richard Clark; together they defined the concept of a "provisional matrix" (16). Dr. Colvin worked on the pathogenesis of autoimmune tubulointerstitial diseases, continuing the work on anti-tubular

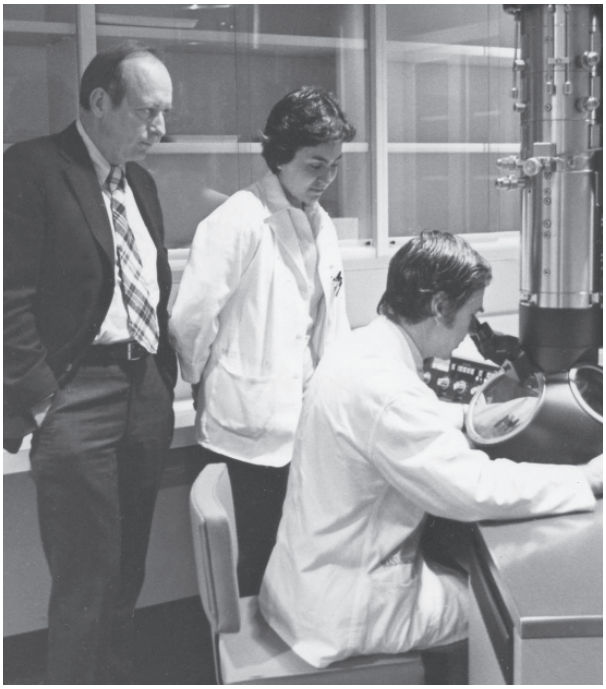

Figure 23.5 *Robert McCluskey (left) with Richard Dickersin (looking through electron microscope) and Ann Brescia (electron microscopy technician), Cox 5, late 1970s*

basement membrane disease and in transplantation. His close interactions with Dr. Benedict Cosimi and other members of the renal transplant group resulted in seminal observations and publications and recognition of MGH as a major center of renal transplantation. Dr. Colvin was a key member of the team that tried the first therapeutic monoclonal antibody, OKT3, in a patient who developed acute renal allograft rejection on October 20, 1980 (17). The team gathered around the prototype flow cytometer operated by Dr. Colvin the evening after the first dose and observed that all the T cells had disappeared from the circulation within an hour of treatment. Someone in the flow cytometry room quoted Dr. John Collins Warren (chapter 1) and said, "Gentlemen, this is no humbug!"

Atul Bhan had been trained in anatomic and experimental pathology at the All India Institute of Medical Sciences (AIIMS), New Delhi, under Dr. Vulimiri Ramalingaswami, who had had close associations with members of the MGH Pathology staff (chapter 7). Both Benjamin Castleman and Walter Putschar had visited AIIMS to help the development of the Pathology department. Dr. Bhan's research experience while at Children's Hospital from 1972 to 1975 and collaborative studies with Dr. Stuart Schlossman at Dana-Farber Cancer Institute set the foundation for his future work on lymphoid cells in normal and diseased states. One of the important findings of these studies was the demonstration that T cells, but not B cells, migrate to rejecting allografts (18).

The development of hybridoma-derived monoclonal antibodies in 1975 had a profound influence on all aspects of biological sciences, including immunopathology. One of the immediate effects was on the characterization of normal and neoplastic hematopoietic cell lineages. Dr. Bhan performed important early work to identify and characterize lymphoid cell differentiation and dendritic cells in the lymphoid tissues and at the inflammatory sites (19, 20). Dr. Sibrand Poppema, a visiting fellow from the University of Groningen, participated in the initial studies to identify stages of T cell and B cell location and differentiation in the lymphoid tissues (19); Dr. Poppema, internationally recognized for his research on Hodgkin's disease, became Professor of Pathology at the University of Groningen in 1995. Dr. George Murphy (chapter 18), a resident in Pathology at MGH and a trainee of Dr. Mihm's, carried out studies on the analysis of Langerhans cells and histiocytosis X with monoclonal antibodies in collaboration with Drs. Bhan and Terence Harrist (21).

In 1981 the Diagnostic Immunoperoxidase Laboratory was established by Dr. Bhan with the help of Bruce Kaynor, a talented and dedicated technician. Dr. Nancy L. Harris (see figure 24.9), who had joined the Immunopathology Unit in 1978 as a research fellow, published several important papers with Dr. Bhan on immunohistochemical characterization of lymphoid cells for the diagnosis and classification of

lymphoid malignancies (22). She continued to apply immunohistochemistry to hematological malignancies and achieved national and international recognition (chapter 16). Subsequently, the application of immunohistochemical techniques extended to all specialties of surgical pathology. Dr. Salim Kabawat, who joined the Immunopathology Unit as a research fellow in Dr. Colvin's laboratory in 1983, described the reactivity of a monoclonal antibody, OC125, produced against an ovarian tumor by Dr. Robert Bast at Dana-Farber Cancer Institute (23). (As a medical student, Dr. Bast had spent time in MGH Pathology; chapter 7.) The antigen CA125 recognized by the antibody has become an established marker of ovarian tumors, and the serum levels of this antigen are used for diagnosis and monitoring of patients with some forms of ovarian cancer.

The refinement of immunohistochemical techniques and introduction of antigen retrieval methods in the 1980s made it possible to identify an increasing number of tumor markers with antibodies that were preferentially selected for their ability to recognize antigens in routinely fixed specimens in surgical pathology. As Director of the Immunohistochemistry (Immunoperoxidase) Laboratory, Dr. Bhan took a leading role in integrating immunohistochemistry into surgical pathology and selecting panels of antibodies that allowed characterization of tumor cells, not only by their morphologic features, but also by their molecular profile. His interactions with the Surgical Pathology staff led to the development of strategies for the characterization of a wide spectrum of tumors, including hematopoietic, soft tissue, endocrine, and metastatic epithelial tumors (unknown primary); perhaps the most significant effect was in the diagnosis of poorly differentiated malignant tumors.

The availability of monoclonal antibodies to functionally distinct lymphoid cells had an immediate influence on the immunohistochemical characterization of a wide variety of inflammatory conditions. The analysis also included allograft rejection and tumor infiltrating lymphocytes. Many of Dr. Bhan's studies were supported by an NIH grant to study immunocompetent cells infiltrating human breast cancer.

Dr. James T. Kurnick joined the staff of the Immunopathology Unit in 1980, following pathology training at the University of Colorado and postdoctoral work in immunology in Denver and Sweden. His work cloning human T lymphocytes, including the first propagation of helper T cells in any species, brought him to the MGH; here he extended his work with normal T cell responses to soluble and cellular antigens, including work propagating activated T lymphocytes from sites of inflammation in transplants, myocarditis, rheumatoid arthritis, pyelonephritis, and cancers. The work led to functional and genetic studies on cells isolated from inflamed tissues, including the demonstration of clonal dominance of T cell infiltrates in several conditions, such as rheumatoid arthritis and tumors (24, 25). Dr. Ivan Stamenkovic (see below) participated in the studies of rheumatoid arthritis. Dr. Richard Kradin, who had a joint clinical appointment in both Pathology and Medicine (Pulmonary Unit), joined Dr. Kurnick's research and developed a protocol in which patients with lung cancer were treated with in vitro expanded tumor infiltrating lymphocytes (figure 23.6). This led to the treatment of advanced melanomas and non–small cell lung cancers with infusion of autologous tumor infiltrating lymphocytes (26). Dr. Kurnik's work on the immune response to cancers has continued in recent years with the realization that tumors can escape immune detection and destruction by the reversible loss of targeted differentiation antigens (27). Following a decade in the Immunopathogy Unit on Cox 5, Dr. Kurnick led a departmental expansion to the new research facilities (Pathology Research at MGH East) in Charlestown.

Dr. Kradin continued his work in pulmonary immunology and interstitial lung diseases (28, 29), addressing the T cell, dendritic cell, and

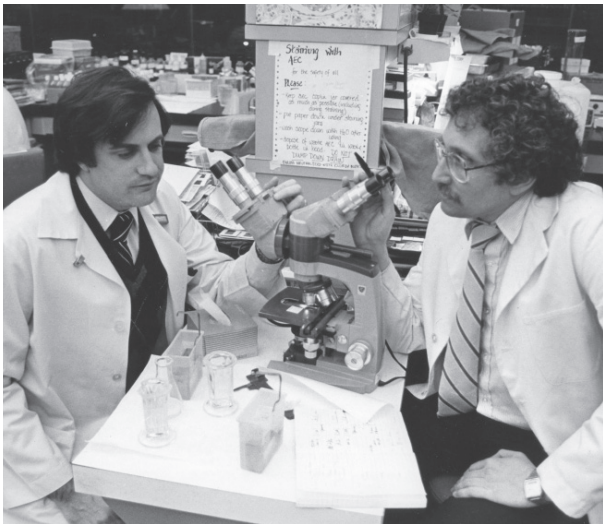

Figure 23.6 *Richard Kradin (left) and James Kurnick, Immunopathology Laboratory, Cox 5, 1980s*

macrophage responses to inhaled antigens and migratory properties of dendritic cells, especially to local lymph nodes, and edited a textbook, *Immunopathology of Lung Disease.* Five of Dr. Kradin's laboratory research fellows, including Drs. Patricia Finn and Steven Dubinett, subsequently pursued successful research careers and are currently department chairmen in the United States and internationally.

The Flow Cytometry Laboratory was started in 1978 by Dr. Colvin with a gift of a FACS II cell sorter from the Arthur D. Little Company. Initially it was housed in an unused animal room on Cox 8 before it moved to new facilities on Warren 5. Flow cytometry was used initially in the evaluation of tumor ploidy as well as for detection of lymphocyte surface antigens with monoclonal antibodies. In the early 1980s, through collaboration with Ortho Diagnostics, a prototype automated flow analyzer was installed and used for pioneering work to characterize circulating T cell subsets with the new monoclonal antibodies. Among the research fellows working with Dr. Colvin whose careers were influenced by their flow cytometry studies were Dr. Andrew Lazarovits, who created the monoclonal antibody Act-I to α4β7 integrin and later became a Professor of Medicine in Ottawa; Robert Burton, collaborator in the early OKT3 studies, who went from being a fellow at MGH to Professor of Surgery at Newcastle, Australia; and Anthony Warrens, now a Professor of Medicine at Hammersmith Hospital in London. Dr. Frederic I. Preffer, who had research training at Roswell Park Memorial Institute at Buffalo, joined the Immunopathology Unit as Dr. Bhan's postdoctoral fellow in 1982. He made flow cytometry his field and found the technology fascinating (30); he later described his experience as "love at first sight." He was chosen to direct the Clinical Flow Cytometry Lab, which was established 1985, and he directs it to this day. He also directs the Research Flow Cytometry Core, which has numerous state-of-the-art flow sorters and analyzers, and a premier research/cell sorting lab in the MGH research campus in Charlestown as well as a facility in the Simches Building on the main campus (31, 32).

Dr. Nadine Cerf-Bensussan, a postdoctoral research fellow from France, joined Dr. Bhan's laboratory in 1981 to pursue research in mucosal immunology. She identified a molecule expressed exclusively by intraepithelial lymphocytes (IEL) in rats (33) and subsequently in human IEL (αEβ7, CD103), an integrin that is involved in the interaction with intestinal epithelial cells by binding to E-cadherin. Dr. Cerf-Bensussan is now an internationally recognized mucosal immunologist. Dr. Gary Russell, a pediatric gastroenterologist working with Dr. Bhan, extended these studies by making several similar antibodies against mucosal lymphocytes propagated from intestinal biopsies from patients with celiac disease (34). Monoclonal antibodies specific to rat Kupffer cells made in Dr. Bhan's laboratory with the help of Drs. Richard Moscicki (who had been a research fellow in the unit) and Kurt Bloch, Director of Clinical Immunology, confirmed the hypothesis that cells express unique functionally important molecules depending on their location and environment. Dr. Masafumi Maruiwa, a postdoctoral fellow from Japan in Dr. Bhan's

laboratory, demonstrated in collaboration with Dr. Amin Arnaout of the MGH Renal Unit that one of these antibodies recognized a unique complement receptor on Kupffer cells (35).

The influence of immunopathology on diagnostic pathology grew in the 1970s and 1980s. The U.S. and Canadian Academy of Pathology (USCAP) invited Drs. McCluskey and Colvin to organize a special course entitled "Immunopathologic Techniques in Diagnostic Pathology." The course was first given by the members of the unit at the annual meeting of the society in Boston in 1976 and subsequently every two years from 1977 to 1987. The success of the course led to the publication in 1988 of a textbook entitled *Diagnostic Immunopathology,* edited by Drs. Colvin, Bhan, and McCluskey. A second edition of the book, containing a more comprehensive review of immunopathologic mechanisms and diseases, transplantation, immunohistochemistry of tumors, and techniques, was published in 1995 (36).

Immunopathology was approved by the American Board of Medical Specialties in 1983. Dr. McCluskey was among the first to be certified and become a member of the test committee. Subsequently, both Drs. Colvin and Bhan were certified and served on the test committee. By 1999 the American Board of Pathology stopped issuing Special Certification in Immunopathology; since every trainee in pathology needed to know immunological mechanisms and immunology-based laboratory tests, the Board decided to include immunopathology as a part of Anatomic and Clinical Pathology certification.

The NIH general postdoctoral research training program that started in the Pathology department in 1957 served as a forum for enlisting trainees in immunopathology as well as other areas. Support for research training in immunopathology beginning in 1980 was largely through an NIH institutional training grant titled "Immunology and Tumor Biology," submitted originally by Harold Dvorak; Dr. Colvin was the first program director. The five concurrent trainees were selected primarily from the MGH Pathology Training Program and clinical services, but there were also individuals trained at other institutions. Early trainees included Drs. Galli, Harris, James Faix, Raymond Sobel, Dobri Kiprov, Theodore Mayer, Kradin, Andrew Rosenberg, Moscicki, Preffer, Niles, and Johnson Wong. Dr. Sobel (chapter 17) joined the Immunopathology Unit in 1981 as a research fellow with Dr. Colvin, after completing neuropathology training at Stanford. The focus of his research was immune responses in the brain, including experimental allergic encephalomyelitis (EAE), which extended Dr. Waksman's work (37). Dr. Sobel returned to Stanford in 1992.

1991 to Present

In 1991 Dr. Colvin succeeded Dr. McCluskey as Chief of the Pathology Service at MGH (figure 23.7). Dr. Colvin appointed Dr. Bhan the Director of the Immunopathology Unit, which at this time was composed of research laboratories staffed by Drs. Bhan (mucosal immunology), Kradin (pulmonary immunology and inflammation), Niles (ANCA), Schneeberger (permeability properties of the air/blood barrier in the lung), Preffer (flow cytometry), Wong (immunotherapy of HIV infection and transplant rejection), and Colvin (transplantation immunology). Dr. G. Richard Dickersin, who had succeeded Ann Dvorak as Director of the Diagnostic Electron Microscopy Laboratory (chapter 16), shared the electron microscopic area of the Cox 5 laboratory space with Dr. Schneeberger.

Dr. Colvin continued to be involved in transplant research; among his notable research contributions were the identification of the diagnostic criteria for acute antibody-mediated rejection and the discovery of chronic antibody-mediated rejection, using peritubular capillary deposition of the complement fragment, C4d, as a key marker (38, 39, 40, 41). His criteria have been accepted by the Banff schema and have become incorporated into standard clinical practice. Dr.

Figure 23.7 *Farewell to Robert Colvin as Director of Immunopathology Unit upon becoming Chief of Pathology, Cox 5 staff, 1991. Front row, seated, left to right: Atul Bhan, Robert Colvin, Richard Dickersin, Eveline Schneeberger. First row standing: Bruce Kaynor, Chen-Kwen Hsuing, Guoli Pan, Wei Lin. Second row standing: Luba Zugachin (behind Atul Bhan), Ruth Manozzi, Wei Jia Xia, Colleen Hagan (partially hidden), Marcia Levy, unidentified, Chris Howard. Third row standing: John Niles, Masafumi Mauiwa, Gary Russell (partially hidden behind Wei Jia Xia), Richard Kradin, Bernard Collins, Volker Nickelit (behind Marcia Levy), Malgorzata Stronska, unidentified, Gertrude Fondren (behind Chris Howard). Back row: Fred Preffer (behind Gary Russell), Ray Sobel (behind and right of M. Stronska).*

Colvin was one of the initial authors on the Banff classification in 1993, and he continues as a leader of this biannual international meeting that promotes scientific progress and standardization of practice in transplantation. In studies with Dr. Paul Russell, Dr. Colvin showed that antibody is sufficient to initiate chronic vascular rejection in mouse heart transplants, without a necessary participation of T cells (42). More recently, Dr. Colvin has collaborated with Drs. Sachs and Cosimi on a series of preclinical trials of induction of tolerance to kidney allografts by combined donor bone marrow transplantation. He also has an NIH-funded program on regulatory T cells in mouse kidney graft acceptance and in vivo imaging of graft-infiltrating cells.

The establishment of liver transplantation program in 1983 extended Dr. Bhan's collaboration with Dr. Jules Dienstag (MGH Gastrointestinal Unit), with whom he had investigated the immunopathogenesis of primary biliary cirrhosis and viral hepatitis (43). This collaboration also led to Dr. Bhan's participation in an NIH-sponsored multicenter clinical trial to evaluate therapy for hepatitis C.

The development of knockout and transgenic mice in the early 1990s revolutionized the way immunologically mediated reactions and diseases could be studied. Dr. Bhan's collaboration with Dr. Peter Mombaerts in Dr. Susumu Tonegawa's laboratory at MIT led to the recognition that colitis could develop spontaneously in mice

deficient in T cells (44). Similar observations in other immunodeficient mice formed the basis for developing models of human inflammatory bowel disease (IBD) and contributed to the formulation of the widely accepted hypothesis that the two major forms of IBD, namely, Crohn's disease and ulcerative colitis, develop owing to dysregulated immune responses to normally resident enteric microflora. Dr. Atsushi Mizoguchi and his wife, Dr. Emiko Mizoguchi, postdoctoral fellows in Dr. Bhan's laboratory, performed studies in these T cell receptor knockout mice, which resulted in long-term research support from NIH. The most significant findings included the recognition of a pathogenic role of cytokines in colitis, the demonstration of a suppressive effect of appendectomy on colitis development (45), and the identification of regulatory B cells and their suppressive role in chronic mucosal inflammation (46). Dr. Bhan helped Dr. Daniel Podolsky, head of the MGH Gastrointestinal Unit, establish an NIH-funded Center for the Study of Inflammatory Bowel Disease (CSIBD) at MGH in 1990. This is a nationally and internationally recognized research center that helps investigators in their IBD-related research and provides pilot feasibility grants.

Dr. Schneeberger's research focused on two themes supported by NIH grants. The first relates to examination of the molecular structure and regulation of tight junctions as they pertain to permeability properties of the air/blood barrier in the lung (47). The second area is the biology of dendritic cells in the lung (48). The studies include analysis of tricellulin in regulating the migration of dendritic cells across airway and alveolar epithelium in an ovalbumin-induced model of asthma in mice. She also has participated in the diagnostic renal pathology service for many years.

Dr. Johnson Wong, who had been trained in Immunology/Allergy at MGH, joined the Immunopathology Unit as Dr. Colvin's research fellow from 1983 to 1985. He became an independent investigator and continued his research in the new immunopathology facilities on Warren 5 until 2002, when he returned to the Clinical Immunology Unit. He developed anti-CD3:anti-CD8 bifunctional antibodies to eliminate the replication component of virus and improvement of immunologic deficit in HIV-1 infected patients, and as a therapeutic protocol for prolongation of allograft survival with low systemic toxicity. Dr. James A. MacLean, who had also been trained in Immunology/Allergy at MGH, participated in these studies as a research fellow in the Immunopathology Unit from 1989 to 1992 (49).

In 1994 the Residency Review Committee for the Accreditation Council for Graduate Medical Education approved the Clinical Training Program in Immunopathology, directed by Dr. Bhan. The comprehensive training program included rotations through the Clinical Immunology Laboratory under the supervision of Dr. Kurt Bloch and, for molecular biologic training, the Pathology Research Laboratory at MGH East under the supervision of Ivan Stamenkovic. The first trainee of the program was Dr. R. Neal Smith, who entered after his residency in Pathology at MGH. He is now Associate Professor of Pathology; his primary interests are in renal pathology and transplantation. As a research fellow he had participated in the IBD studies in Dr. Bhan's laboratory and helped in the identification of regulatory B cells. As a faculty member he became an investigator at the Harvard Center for Islet Transplantation and provided pathology services to the MGH Transplantation Biology Research Center. Dr. Shamila Mauiyyedi, who was a postdoctoral fellow with Dr. Colvin, working on research related to transplantation, completed immunopathology training in 1999, after which she joined the faculty of the University of Texas at Houston Medical School.

Dr. Stamenkovic, who had graduated from the University of Geneva School of Medicine in 1978, and had been a research fellow in the Immunopathology Unit and Molecular Biology Department

Figure 23.8 *Robert McCluskey's eightieth birthday celebration, 2003. Left to right: Atul Bhan, Bernard Collins, Robert McCluskey, Eveline Schneeberger.*

from 1985 to 1988, carried out work on CD44's effect on tumor growth and migration (50) and the role of CD22 in lymphocyte adhesion and activation (51). He was appointed Director of Pathology Research at MGH East in 1994. He went to Switzerland in 2001 as Professor of Experimental Pathology at the University of Lausanne, where he became chairman of the department in 2009. In the late 1990s immunopathology research at the Pathology research laboratory was carried out by Drs. McCluskey (figure 23.8), Giuseppe Andres, and James Kurnick. Dr. Andres, a visiting Professor of Pathology, had a long collaborative research relationship with Dr. McCluskey dating from their days together in Buffalo (chapter 14). Their collaborations continued at MGH East and involved the study of pathogenic mechanisms in a model of anti-GBM nephritis in rats.

In 2000 the Immunopathology Unit was moved to the renovated fifth floor of the Warren Building. Dr. Schneeberger's lab moved to the Pathology Research Laboratory at Charlestown. Dr. James Stone, who had been recruited from Brigham and Women's Hospital to lead Cardiovascular Pathology (chapter 16), joined the group. In 2005 Drs. Bhan's and Stone's research laboratories moved to the eighth floor of the Richard B. Simches Research Center. This move allowed Dr. Atsushi Mizoguchi to develop an independent research laboratory. Dr. Colvin's laboratory expanded and, as the Immunopathology Research Unit, moved to newly renovated space on the eighth floor of the Thier Building.

Over this period, the Immunopathology Unit, with its busy immunohistochemistry, immunofluorescence, flow cytometry, and ANCA laboratories, became primarily engaged in diagnostic testing. These diagnostic activities grew dramatically in volume and sophistication. The clinical volume of the Clinical Flow Cytometry Laboratory increased from 44 to 9,000 cases per year. These studies include eight-color analyses of lymphoid cells, leukocytes, and stem cells in normal, immunodeficient, and malignant states.

The immunofluorescence studies, both direct and indirect (for detection of circulating autoantibodies), continued to expand; they now involve more than 1,000 cases each year. The renal biopsy reference laboratory, under Dr. Colvins's direction and Collins's technical supervision, provides light, immunofluorescence, and electron microscopy services for 400–500 renal biopsies per year, and the unit performs serological testing on over 8,000 ANCA and 800 anti-glomerular basement membrane antibodies samples. The Immunohistochemistry (Immunoperoxidase) Laboratory, under Dr. Bhan's direction for most of this period, grew from a menu of fewer than 10 immunoperoxidase stains in 1981 to over 200 stains in 2007, when the laboratory became fully automated. The most significant effect of immunohistochemistry on tumor diagnosis and classification happened in the 1990s and led to numerous publications and presentations by both staff and trainees at national and international meetings.

Conclusion

The Immunopathology Unit has functioned for more than 30 years as a center that has brought together physicians and researchers interested not only in basic research but also in developing diagnostic tests. Perhaps its most important legacy has been the training of young pathologists and clinicians who have succeeded in both research and clinical practice. Their efforts will no doubt keep the field of immunopathology a vibrant component of patient care and medical inquiry for many years to come.

References

1. Gill TJ III, Ward PA. Immunopathology. Hypersensitivity or tolerance? JAMA 238:1921–1923, 1977.
2. Swartz MN, Dvorak HF. Obituary: Dr. Louis Dienes. J Infect Dis 130:89–91, 1974.
3. Waksman BH, Adams RD. Infectious leukoencephalitis. A critical comparison of certain experimental and naturally-occurring viral leukoencephalitides with experimental allergic encephalomyelitis. J Neuropathol Exp Neurol 21:491–518, 1962.
4. Kosunen TU, Waksman BH, Flax MH, Tihen WS. Radioautographic study of cellular mechanisms in delayed hypersensitivity. I. Delayed reactions to tuberculin and purified proteins in the rat and guinea pig. Immunology 6:276–290, 1963.
5. Flax MH. Experimental allergic thyroiditis in the guinea pig. II. Morphologic studies of development of disease. Lab Invest 12:199–213, 1963.
6. Dvorak HF, Simpson BA, Flax MH, Leskowitz S. The fate of antigen in delayed hypersensitivity skin reactions. J Immunol 104:718–727, 1970.
7. Dvorak HF, Dvorak AM, Simpson BA, Richerson HB, Leskowitz S, Karnovsky MJ. Cutaneous basophil hypersensitivity. II. A light and electron microscopic description. J Exp Med 132:558–582, 1970.
8. Dvorak HF, Mihm MC Jr., Dvorak AM, Barnes BA, Manseau EJ, Galli SJ. Rejection of first-set skin allografts in man. The microvasculature is the critical target of the immune response. J Exp Med 150:322–337, 1979.
9. Dvorak HF, Dvorak AM, Churchill WH. Immunologic rejection of diethylnitrosamine-induced hepatomas in strain 2 guinea pigs. Participation of basophilic leukocytes and macrophage aggregates. J Exp Med 137:751–775, 1973.
10. Colvin RB, Johnson RA, Mihm MC Jr., Dvorak HF. Role of the clotting system in cell-mediated hypersensitivity. I. Fibrin deposition in delayed skin reactions in man. J Exp Med 138:686–698, 1973.
11. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219:983–985, 1983.
12. Bhan AK, Schneeberger EE, Collins AB, McCluskey RT. Evidence for a pathogenic role of a cell-mediated immune mechanism in experimental glomerulonephritis. J Exp Med 148:246–260, 1978.
13. Raychowdhury R, Niles JL, McCluskey RT, Smith JA. Autoimmune target in Heymann nephritis is a glycoprotein with homology to the LDL receptor. Science 244:1163–1165, 1989.
14. Niles JL, McCluskey RT, Ahmad MF, Arnaout MA. Wegener's granulomatosis autoantigen is a

novel neutrophil serine proteinase. Blood 74:1888–1893, 1989.
15. Niles JL, Pan GL, Collins AB, Shannon T, Skates S, Fienberg R, Arnaout MA, McCluskey RT. Antigen-specific radioimmunoassays for anti-neutrophil cytoplasmic antibodies in the diagnosis of rapidly progressive glomerulonephritis. J Am Soc Nephrol 2:27–36, 1991.
16. Clark RA, Lanigan JM, Della Pelle P, Manseau E, Dvorak HF, Colvin RB. Fibronectin and fibrin provide a provisional matrix for epidermal cell migration during wound reepithelialization. J Invest Dermatol 79:264–269, 1982.
17. Cosimi AB, Colvin RB, Burton RC, Rubin RH, Goldstein G, Kung PC, Hansen WP, Delmonico FL, Russell PS. Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. NEJM 305:308–314, 1981.
18. Bhan AK, Reinisch CL, Levey RH, McCluskey RT, Schlossman SF. T-cell migration into allografts. J Exp Med 141:1210–1215, 1975.
19. Bhan AK, Reinherz EL, Poppema S, McCluskey RT, Schlossman SF. Location of T cell and major histocompatibility complex antigens in the human thymus. J Exp Med 152:771–782, 1980.
20. Bhan AK, Nadler LM, Stashenko P, McCluskey RT, Schlossman SF. Stages of B cell differentiation in human lymphoid tissue. J Exp Med 154:737–749, 1981.
21. Murphy GF, Bhan AK, Sato S, Harrist TJ, Mihm MC Jr. Characterization of Langerhans cells by the use of monoclonal antibodies. Lab Invest 45:465–468, 1981.
22. Harris NL, Nadler LM, Bhan AK. Immunohistologic characterization of two malignant lymphomas of germinal center type (centroblastic/centrocytic and centrocytic) with monoclonal antibodies. Follicular and diffuse lymphomas of small-cleaved-cell type are related but distinct entities. Am J Pathol 117:262–272, 1984.
23. Kabawat SE, Bast RC Jr., Bhan AK, Welch WR, Knapp RC, Colvin RB. Tissue distribution of a coelomic-epithelium-related antigen recognized by the monoclonal antibody OC125. Int J Gynecol Pathol 2:275–285, 1983.
24. Kurnick JT, Kradin RL, Blumberg R, Schneeberger EE, Boyle LA. Functional characterization of T lymphocytes propagated from human lung carcinomas. Clin Immunol Immunopathol 38:367–380, 1986.
25. Stamenkovic I, Stegagno M, Wright KA, Krane SM, Amento EP, Colvin RB, Duquesnoy RJ, Kurnick JT. Clonal dominance among T-lymphocyte infiltrates in arthritis. Proc Natl Acad Sci 85:1179–1183, 1988.
26. Kradin RL, Kurnick JT, Lazarus DS, Preffer FI, Dubinett SM, Pinto CE, Gifford J, Davidson E, Grove B, Callahan RJ, et al. Tumour-infiltrating lymphocytes and interleukin-2 in treatment of advanced cancer. Lancet 333:577–580, 1989.
27. Kurnick JT, Ramirez-Montagut T, Boyle LA, Andrews DM, Pandolfi F, Durda PJ, Butera D, Dunn IS, Benson EM, Gobin SJ, van den Elsen PJ. A novel autocrine pathway of tumor escape from immune recognition. Melanoma cell lines produce a soluble protein that diminishes expression of the gene encoding the melanocyte lineage melan-A/MART-1 antigen through down-modulation of its promoter. J Immunol 167:1204–1211, 2001.
28. Kradin RL, Divertie MB, Colvin RB, Ramirez J, Ryu J, Carpenter HA, Bhan AK. Usual interstitial pneumonitis is a T-cell alveolitis. Clin Immunol Immunopathol 40:224–235, 1986.
29. Xia W, Pinto CE, Kradin RL. The antigen-presenting activities of Ia+ dendritic cells shift dynamically from lung to lymph node after an airway challenge with soluble antigen. J Exp Med 181:1275–1283, 1995.
30. Preffer FI, Colvin RB, Leary CP, Boyle LA, Tuazon TV, Lazarovits AI, Cosimi AB, Kurnick JT. Two-color flow cytometry and functional analysis of lymphocytes cultured from human renal allografts. Identification of a Leu-2+3+ subpopulation. J Immunol 137:2823–2830, 1986.
31. Preffer FI, Dombkowski D, Sykes M, Scadden D, Yang YG. Lineage-negative side-population (SP) cells with restricted hematopoietic capacity circulate in normal human adult blood. Immunophenotypic and functional characterization. Stem Cells 20:417–427, 2002.
32. Preffer F, Dombkowski D. Advances in complex multiparameter flow cytometry technology. Applications in stem cell research. Cytometry Part B, Clinical Cytometry 76:295–314, 2009.

33. Cerf-Bensussan N, Guy-Grand D, Lisowska-Grospierre B, Griscelli C, Bhan AK. A monoclonal antibody specific for rat intestinal lymphocytes. J Immunol 136:76–82, 1986.
34. Russell GJ, Parker CM, Cepek KL, Mandelbrot DA, Sood A, Mizoguchi E, Ebert EC, Brenner MB, Bhan AK. Distinct structural and functional epitopes of the alpha E beta 7 integrin. Eur J Immunol 24:2832–2841, 1994.
35. Maruiwa M, Mizoguchi A, Russell GJ, Narula N, Stronska M, Mizoguchi E, Rabb H, Arnaout MA, Bhan AK. Anti-KCA-3, a monoclonal antibody reactive with a rat complement C3 receptor, distinguishes Kupffer cells from other macrophages. J Immunol 150:4019–4030, 1993.
36. Colvin RB, Bhan AK, McCluskey RT. *Diagnostic Immunopathology,* 2nd ed. New York: Raven Press, 1995.
37. Sobel RA, Blanchette BW, Bhan AK, Colvin RB. The immunopathology of experimental allergic encephalomyelitis. I. Quantitative analysis of inflammatory cells in situ. J Immunol 132:2393–2401, 1984.
38. Colvin RB, Cohen AH, Saiontz C, Bonsib S, Buick M, Burke B, Carter S, Cavallo T, Haas M, Lindblad A, Manivel JC, Nast CC, Salomon D, Weaver C, Weiss M. Evaluation of pathologic criteria for acute renal allograft rejection. Reproducibility, sensitivity, and clinical correlation. J Am Soc Nephrol 8:1930–1941, 1997.
39. Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Williams WW, Tolkoff-Rubin N, Cosimi AB, Colvin RB. Complement activation in acute humoral renal allograft rejection. Diagnostic significance of C4d deposits in peritubular capillaries. J Am Soc Nephrol 10:2208–2214, 1999.
40. Mauiyyedi S, Della Pelle P, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin NE, Williams WW, Cosimi AB, Schneeberger EE, Colvin RB. Chronic humoral rejection. Identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. J Am Soc Nephrol 12:574–582, 2001.
41. Smith RN, Kawai T, Boskovic S, Nadazdin O, Sachs DH, Cosimi AB, Colvin RB. Four stages and lack of stable accommodation in chronic alloantibody-mediated renal allograft rejection in Cynomolgus monkeys. Am J Transplantation 8:1662–1672, 2008.
42. Hirohashi T, Uehara S, Chase CM, Della Pelle P, Madsen JC, Russell PS, Colvin RB. Complement independent antibody-mediated endarteritis and transplant arteriopathy in mice. Am J Transplantation 10:510–517, 2010.
43. Wands JR, Dienstag JL, Bhan AK, Feller ER, Isselbacher KJ. Circulating immune complexes and complement activation in primary biliary cirrhosis. NEJM 298:233–237, 1978.
44. Mombaerts P, Mizoguchi E, Grusby MJ, Glimcher LH, Bhan AK, Tonegawa S. Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. Cell 75:274–282, 1993.
45. Mizoguchi A, Mizoguchi E, Chiba C, Bhan AK. Role of appendix in the development of inflammatory bowel disease in TCR-alpha mutant mice. J Exp Med 184:707–715, 1996.
46. Mizoguchi A, Mizoguchi E, Takedatsu H, Blumberg RS, Bhan AK. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. Immunity 16:219–230, 2002.
47. Hou J, Renigunta A, Konrad M, Gomes AS, Schneeberger EE, Paul DL, Waldegger S, Goodenough DA. Claudin-16 and claudin-19 interact and form a cation-selective tight junction complex. J Clin Invest 118:619–628, 2008.
48. Schneeberger EE, Vu Q, LeBlanc BW, Doerschuk CM. The accumulation of dendritic cells in the lung is impaired in CD18-/- but not in ICAM-1-/- mutant mice. J Immunol 164:2472–2478, 2000.
49. MacLean JA, Su Z, Guo Y, Sy MS, Colvin RB, Wong JT. Anti-CD3:anti-IL-2 receptor bispecific monoclonal antibody. Targeting of activated T cells in vitro. J Immunol 150:1619–1628, 1993.
50. Bartolazzi A, Peach R, Aruffo A, Stamenkovic I. Interaction between CD44 and hyaluronate is directly implicated in the regulation of tumor development. J Exp Med 180:53–66, 1994.
51. Sgroi D, Koretzky GA, Stamenkovic I. Regulation of CD45 engagement by the B-cell receptor CD22. Proc Natl Acad Sci 92:4026–4030, 1995.