

40-year career focuses on research, collaboration

By K. Frank Austen, MD, FAAAAI

A particularly important aspect of my investigative interests over the past 40 years has been the shared experience with post-doctoral fellows that often extended into their initial faculty appointments. My initial appointment in the Department of Medicine at Massachusetts General Hospital (MGH) was as a member of the Infectious Disease Unit. I subsequently obtained a larger laboratory as Chief of the Pulmonary Unit of the Department of Medicine at MGH. We demonstrated that slow reacting substance of anaphylaxis (SRS-A) in the rat could be generated either by an IgE or an IgG_a dependent pathway with the former but not the latter requiring mast cells.

When the laboratory moved in 1966 to Robert B. Brigham Hospital (RBBH), I also served as Chief of a Harvard Medical School Department of Medicine. As the mission of that Institution was to care for patients with rheumatologic diseases, we had a training program for that subspecialty. Through collaboration with the practice of **Albert L. Sheffer, MD, FAAAAI**, we also provided a training program for certification in allergy.

This was also a period when I pursued a third area of interest, complement. I had a postdoctoral fellowship equivalent at Walter Reed Army Institute for Research as part of my military duty and a separate fellowship in the Department of Microbiology after completing my medical residency at MGH. With a team of researchers, we defined the activation steps of the properdin pathway and the mechanism by which these same proteins serve to amplify either classical or alternative pathway activation. With **Irma Gigli, MD, FAAAAI**, and **Nicholas A. Soter, MD, FAAAAI**, who were already trained in dermatology, cutaneous necrotizing vasculitis was appreciated to present as urticaria rather than palpable purpura in some patients.

In 1977, the laboratory moved to the Seeley G. Mudd Building on the quadrangle of Harvard Medical School. At this time, several of our researchers generated SRS-A by calcium ionophore stimulation of a mast cell line that had been labeled for sulfur and for arachidonic acid. After purification, the composition revealed a structure composed of a metabolite of arachidonic acid and glutathione. A collaboration with the Harvard University Department of Chemistry identified three components of SRS-A, leukotriene C₄ (LTC₄) with the glutathione adduct, LTD₄ from which the glutamic acid had been cleaved and LTE₄ from which the glycine had been deleted. Through this collaboration, we were able to do a first in human study of the response to intradermal injections. As compared to saline or the prostanoid, prostaglandin D₂ (PGD₂), the wheal and flare elicited by the cysteinyl leukotrienes (cysLTs) was larger and of greater duration. A second study with biopsies revealed that the cysLTs elicited a pure cutaneous edema without any cellular influx, whereas LTB₄ recruited neutrophilic leukocytes into an abscess-like collection.

Based on the knowledge that fish oil ingestion led to an attenuated form of thromboxane, **Tak H. Lee, MD, FAAAAI**, joined our laboratory and studied the effects of these oils on the leukotriene pathway products. Eicosapentaenoic acid was processed by cellular 5-lipoxygenase/LTC₄ synthase (LTC₄S) to generate a fully active LTC₅, whereas the LTB₅ was markedly attenuated in chemotactic function *in vitro*. Ingestion of fish oils by humans led to incorporation of docosahexaenoic acid into the cell membrane and attenuation of leukocyte activation. Taken together, these studies suggested that with a normal intake of fish oil, there might be a dampening of inflammatory responses.

Collaboration with researchers from Vanderbilt University revealed the generation of PGD₂ with activation. This was followed by the recognition that patients with systemic mastocytosis and intense flushing with syncope excreted unusual amounts of a urinary metabolite of PGD₂ and that the hypotension could be controlled with aspirin administration to block biosynthesis at the cyclooxygenase step. **Stephen T. Holgate, MD, DSc, FAAAAI**, examined nucleotide responses *in vitro* and later explored this area in humans. With **Lawrence B. Schwartz, MD, PhD, FAAAAI**, human lung tryptase was purified to homogeneity and shown to be a tetramer.

Howard R. Katz, PhD, had noted that within a family of mAb prepared against mouse peritoneal macrophages, there were three that also identified peritoneal mast cells. He entered the postdoctoral program to identify the mast cells epitopes and described the biosynthesis of each. Later with **Jonathan P. Arm, MD, FAAAAI**, the cDNAs and gene for one of the epitopes known as gp49 was cloned. Subsequently, Mariana C. Castells, MD, PhD, together with other researchers, cloned

the second gene. The recognition that the gp49B gene product, gp49B1, contained tandem immunotyrosine inhibition motifs in the cytosolic domain led to the demonstration that co-ligation of this protein to IgE/FcεR1 during cross-linking prevented the latter from activating bone marrow-derived mast cells. Targeted disruption revealed that the gp49B1 null strain could be sensitized for a passive cutaneous anaphylactic reaction with one-tenth the IgE mAb to dinitrophenol as the wild-type littermates. That the mast cells lacking gp49B1 were hyperresponsive to activation revealed that the mast cells of the normal littermate were constitutively attenuated establishing a paradigm for tissue-based control.

In 1998, the Division of Rheumatology, Immunology and Allergy moved again to the newly constructed Smith Building of the Dana-Farber Cancer Institute. We had collaborated with this group in studying the differential regulation of LTC₄S and hematopoietic PGD₂ synthase in mouse bone marrow-derived mast cells. One of their researchers led the targeted disruption of LTC₄S and each of the cysLT receptors, CysLT₁ and CysLT₂. That a strain lacking LTC₄ biosynthetic function was markedly attenuated for the PCA reaction revealed a role for the cysLTs equivalent to the secretory granule constituents in increasing the plasma leak in the PCA reaction of the BALB/c strain. Importantly, the 57B/6 strain was markedly protected against tracheal bleomycin instillation-induced pulmonary septal fibrosis, revealing a role for the cysLTs in chronic inflammatory host responses. These findings are confirmed in the receptor null strains in that the CysLT₁ mediates the vascular leak and the CysLT₂ receptor mediates the septal thickening with macrophages, fibroblasts and extracellular matrix proteins.

Joshua A. Boyce, MD, FAAAAI, modified the technique for growing human cord blood-derived mast cells and found that the LTC₄S gene was profoundly regulated by interleukin-4 (IL-4) at the transcript, protein, and functional level. That IL-3 or GM-CSF translocated 5-lipoxygenase to the outer nuclear membrane wherein LTC₄S is expressed resulted in further increase in cysLT generation of culture-derived human mast cells primed with IL-4.

These studies, as well as all that I have noted, involve many other trainees who are still developing their programs elsewhere.