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Mast Cells and GI Motility Disease

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Many patients with gastrointestinal motility diseases often present with various extra-intestinal disorders that are seemingly unrelated to the problems in the gastrointestinal tract (GIT). Particularly, patients with Chronic Idiopathic Intestinal Pseudo-Obstruction (CIP) have reported incidences of Raynaud's phenomena, leukopenia, pain in the lower back or upper right quadrant, pulmonary distress, hepatitis, chronic fatigue, arthritis, headaches, recurrent septicemia, neurological deficits, parasthesias, dysesthesias, frequent candidiasis, and various autoimmune disorders. They often have disturbances of the autonomic nervous system function, which can produce orthostatic hypotension; heart palpitations; disturbances of vision, perspiration, and motility; and venous pooling. Various allergies have been reported with itching, hives, edema, rashes, and flushing. These last symptoms are also normally the result of histamine release from mast cells.

Mast cells have long been known to contribute to the discomforts of mankind by releasing histamine and producing the miseries of allergies. However, patients with mast cell disease may also present with a multitude of disparate symptoms such as arthritis, GERD, constipation, malabsorption, cramping, severe abdominal bloating, mitral valve prolapse, short term memory problems, headache, nausea, dizziness, interstitial cystitis, vasculitis, spontaneous bruising, diarrhea, flushing, heart palpitations, sudden blood pressure rises or drops, osteoporosis, bone pain, changes in cognitive function and mood, and even severe anaphylactoid reactions with shock (Wolff). Is it possible that with such a significant overlap of symptomotology, that a diagnosis of illness might depend on the route of medical investigation?

The mast cell was first named by Paul Erlich in 1887 because of its appearance. All the clustered metachromatic granules in the cell reminded him of a “well fed cell”, and thus the name Mastzellen from the German. While mast cells are generally ubiquitous and present throughout the body, all body tissues that come in direct contact with the outside world have mast cells and they are most concentrated in the skin, gut, nasal passages, lung, urinary tract, and other mucus membranes. Each “well fed” mast cell can contain up to 500 granules that store a variety of molecules including arachidonic acid products, biogenic amines, cytokines, neuropeptides, chemoattractants, proteoglycans, and proteolytic enzymes (Theoharides). However the principle chemical mediator of mast cells is histamine which can cause tissue swelling, itching, flushing and other noxious skin responses. It is what causes the itching and swelling of the mosquito bite and the itch of healing wounds with the rapid growth of new tissue. But it can also cause systemic responses such as headache, nausea, dizziness, and diarrhea, and can be

involved in other GI disease such as gastric ulcer and IBS.

In fact histamine is the most important paracrine secretion in the stomach and it is thought that paracrine secretions may also function as neurotransmitters in the gut. Histamine, gastrin and acetylcholine are the most important local controls of acid secretion in the stomach where it binds to specific H2 receptors, and H2 receptors in the stomach are10 times more sensitive to histamine than those in blood vessels. Histamine has a steady basal release in the gut but is increased when Gastrin or Acetylcholine secretion is increased (Rang). It is also a "neuroactive" mediator and can affect the blood pressure directly. Of the four types of histamine receptors that have been recognized pharmacologically, three are located in the gut and a series of experiments with dispersed colon mast cells has shown that at least two pathways could exist for mast cells to amplify their own activation-degranulation signals in an autocrine or paracrine manner (Xie).

Although mast cells derive from stem cells in the bone marrow, they are not found in the peripheral blood, but their precursors may be found in the bone marrow, blood, mucosal and connective tissues. When the precursors are appropriately stimulated they proliferate and differentiate into mast cells and then can reside in the tissues for months or years. The wide distribution throughout the tissues and their ability to increase their numbers dramatically during various pathological conditions have led to many conjectures about their function. It has been postulated that they may have a role in regulation of gastric acid, the microvasculature, and even the repair of connective tissue, but their role in allergic inflammation and atopic illness have remained the focus of attention (Stenton).

Historically mast cells have been thought of as the "cry wolf" cells of the body, the "alarm bells" that overreacted to allergens and seemed to have no other function. However, more recent understandings of the function of mast cells indicate that many of these responses are only the side effects of their real role as potent regulators of the immune system. Researchers at Duke University Medical Center reported in the July 6, 1999 issue of the Proceedings of the National Academy of Science that they had discovered that mast cells recognize harmful bacteria and trigger the body's innate immune system by releasing tumor necrosis factor (TNF) and recruiting neutrophils, the infection-clearing cells. Mast cells can selectively produce different classes of mediators in response to specific pathogens, thereby allowing the selective recruitment of specific cell types, such as neutrophils, eosinophils, dendritic cells, and T cells (Marshall).

The human immune system has two parts, the innate, which yields the first line of defense against illness, always with the identical immediate response triggered by any invading pathogen, and the adaptive, which tailors a response to a specific pathogen. The innate system attacks the pathogen for the first few days until the adaptive response is primed. When the adaptive immune system is triggered, lymph nodes recruit T-cells, which proliferate and sensitize B-cells to produce very specific antibodies targeting the particular pathogen. The swelling of the lymph nodes is an indication that the adaptive

immune system is active.

When Duke University scientists introduced bacteria into mutant mast cell-deficient mice, their lymph nodes did not swell, but these same animals' nodes did swell when they were injected with mast cells. Also, activation of skin mast cells produced a rapid rise in TNF in the lymph nodes along with the recruitment of T cells indicating that mast cells are a part of both the innate and adaptive immune systems.

The Duke team's discovery also explained the function of the CD48 protein. Discovered over ten years ago on the surface of mast cells, the Duke team showed that antibodies to CD48 will block mast cells from recognizing bacteria and releasing the signaling molecule TNF-a. This indicates that the CD48 receptors on mast cells are specific to recognizing bacteria and alerting the immune system to attack them.

Dr. Salvatore Pizzo, a member of the Duke University Research team, has said, "When you pick up a textbook two years from now that shows how the immune system functions and the way a node responds to an infectious agent, you are going to see a whole new pathway," and that it may very well be, " . . . a major shift in the understanding of the immune system."

So, with this current understanding of the role of mast cells in the immune system, what constitutes pathology?

Mastocytosis (MC) is a disease characterized by abnormal growth and accumulation of mast cells. This can occur in the skin, in internal organs, or in both. The disorder produces too many mast cells and so activation can release a much greater concentration of chemical mediators with the resultant systemic reactions. In cutaneous mastocytosis (CM) accumulation is only in the skin, whereas in systemic mastocytosis (SM), accumulation is found in internal organs. In contrast to a reactive mast cell increase during an inflammation, mast cells in systemic mastocytosis (SM) are monoclonal in nature, i.e. derived from a single abnormal cell clone. While the cutaneous form of mastocytosis (CU) usually appears in early childhood, most patients that are diagnosed as adults have systemic mastocytosis (SM), and most, but not all, will have the skin lesions known as Urticaria Pigmentosa (UP). For a diagnosis of CM, a biopsy of the skin may be sufficient and in one study skin biopsies taken from UP lesions contained roughly 600 mast cells per square millimeter, compared to about 40 mast cells in the same area in normal skin. However, some patients have a lower mast cell load and there is really no reliable cut-off point that can indicate if a mast cell count is within the limits of normal skin, or whether it is increased (Wolff).

A diagnosis of SM in adults can best be established by bone marrow biopsy. However, many false negatives have been reported from first biopsies and the presence of excess mast cell progenitors may be only a sufficient but not necessary condition. In other words, a positive bone marrow biopsy will definitely indicate SM, but a negative biopsy

does not rule it out. While not as reliable, another test is the alpha pro-tryptase test, performed by Dr Lawrence Schwartz in Virginia, or the 24-hour N-methyl histamine test (not the regular urine histamine test). It would seem reasonable that if chronic GI dysmotility was accompanied by extra-intestinal symptoms that at least a tryptase test would be valuable in evaluating the root of the illness. Dr. Theoharides at Tufts University School of Medicine has indicated an interest in working with researchers in our field by doing tryptase and IL6 testing in his laboratory. From his experience, the IL6 level is a better index of disease involvement. All that would be required would be spinning down the serum from a blood draw in a red-top vial and sending it frozen to his address after the patient has spent several days without taking any antihistamines. This would seem to be an excellent project for a grant proposal.

Many patients with urticaria and mast cell disease have tested positive with the autologous serum skin test (ASST). In this test, the patient's own blood is spun down and separated into plasma that is then injected back under the skin. A wheal at the injection site usually indicates an autoimmune disease. And in the book, "Urticaria and Angioedema," by Drs. Greaves and Kaplan, the basophil histamine release test is described to confirm a positive ASST.

There is also the mast cell activation disorder. In this case either a greater or even a normal number of mast cells may be “twitchy” or too easily activated by stimuli and may even be activated by autoantibodies. For these patients, symptoms may appear from the release of mediators when a histamine threshold has been reached. If one thinks of a “histamine bucket” representing some critical level at which symptoms appear, then any addition to this “bucket” is a burden that commands attention. When some cumulative load from stress, environmental activating stimuli, endogenous histamine, and ingested histamine cause the bucket to “overflow”, then the appearance of symptoms can manifest. Patients whose symptoms wax and wane over time may fall into this category as their histamine “load” varies with circumstance.

Histamine is released by mast cells when some trigger stimulates them, but it can also be found in all food as part of the natural spoiling process and can reach a reactive level in human beings long before any signs of spoilage occurs. Bacteria convert the amino acid histidine, found in all proteins, to histamine. Some foods, such as cheese and other fermented foods like alcoholic beverages and vinegars have high histamine levels due to fermentation during the manufacturing process. Fin fish can develop high levels of histamine in the flesh from bacteria in the gut of the fish with levels rising with the time from catch to dressing of the fish. And then some foods have high levels of histamine that occur naturally, such as eggplant and spinach. And it appears that the inhibition of intestinal histamine-metabolizing enzymes by various mediators can cause a decrease in histamine detoxification in the intestinal mucosa resulting in increased intestinal uptake and urinary excretion of unmetabolized histamine (Taylor). Also, quite a few food additives such as dyes and preservatives can trigger the release of histamine. The excellent book entitled Dietary Management of Food Allergies and Intolerances by

Janice Vickerstaff Joneja, Ph.D, RDN, lists two histamine restricting diets that may be of value to those with this symptomatology since limiting any additional source of histamine to the trigger threshold could help to control the disease. A list of foods that contain histamine has been supplied to AGMD as a resource for patients that might have MC.

That food allergy can produce symptoms at other physical sites has been a puzzle to the medical community. A food allergen often produces GI symptoms such as diarrhea, abdominal pain, vomiting, or bloating, but some patients also rapidly develop such symptoms as asthma, rhinitis, urticaria, arthritis, or migraine. It has been postulated that histamine from gut mast cells might bind to sensory nerves and produce an afferent signal that the CNS could route to another site—a response known as neurogenic switching. Sick building syndrome and multiple chemical sensitivity are other maladies that might be explained by neurogenic switching shunting inflammatory stimuli to a remote flare at the diseased site. The time of onset from neurogenic switching depends only on nerve conduction velocity while immunogenic switching would depend on circulating times in the bloodstream and diffusion time in the tissues, thus explaining the rapid onset of symptoms. And it has been shown that vagotomy will protect rats from lethal anaphylaxis without changing the production of either antibody or histamine release, indicating a neuronal pathway as a mechanism of action; but the role of the mast cell is again paramount (Meggs).

Since mast cells have such a profound involvement in the normal immune system response, an involvement in autoimmune disorders might be expected, and in fact, in the March 2000 issue of the Journal of Experimental Medicine, scientists at the Emory University School of Medicine reported the discovery of a connection between mast cells and the development of Multiple Sclerosis (MS). MS is a well-known autoimmune disease that damages the central nervous system by attacking the protective myelin sheath that lines the nerve cells, leading to impairment of nerve function. Previously, most research into MS has focused on the idea that T cells attack the myelin sheath, but the Emory researchers took note of the recent appreciation that mast cells are prevalent in the central nervous system and produce many of the same cytokines and proteases/enzymes that are known to contribute to myelin sheath degradation. Then by using mutant mast cell-deficient mice, they attempted to induce the mouse equivalent of MS, a mouse disease called experimental allergic encephalomylitis (EAE), by injected myelin proteins known to cause EAE. The mast cell-deficient mice had a greatly reduced EAE incidence, but again, when mast cells were injected into the mice, the disease severity was restored to levels seen in normal mice. Dr Melissa Brown, an Emory University pathologist on the team speculates that since mast cells are in close proximity to blood vessels in the CNS, the release of histamine, known to cause vasodilation and leakage of blood vessels, could open the blood brain barrier and allow the access to other damaging cells such as the T cells, as well as releasing other directly damaging proteases directly from the mast cells themselves (http://wwwsciencedaily.com/releases/2000/03/000308081524.htm), and it has been

shown that breakdown of the blood-brain barrier has preceded any clinical or radiographic signs of MS (Theoharides). This new appreciation of the likely role of mast cells in MS also raises the question as to whether drugs such as cromolyn sodium antihistamines used to treat mast cell disease would be effective in treating MS.

The gut biopsy specimens of patients with inflammatory bowel diseases have been examined for histamine release. When specimens were compared to control subjects, the mast cell count in patients with ulcerative colitis was increased and the mast cell count from inflamed tissue was greater than that of normal tissue, and at anti-IgG4 challenge, histamine release was generally confined to patients with inflammatory bowel disease suggesting that mast cells may play a part in inflammatory bowel disease (Nolte).

Researchers at the University of Sydney Department of Medicine have reported that intestinal mast cell degranulation from a prior enteric infection or allergy may play a part in gut hypersensitivity in Irritable Bowel Syndrome both in motor response and visceral perception and that psychological stress may trigger this via the brain-gut axis (Gui). Furthermore, investigators at a medical institute in Amsterdam have proposed that microscopic inflammation of neurogenic origin (invisible at endoscopic investigation) is responsible for the altered motility and sensitivity to pain in the GIT as the result of mast cell activation with the chemoattraction of inflammatory cells from the bloodstream as the most important step in the pathogenic cascade that can alter motility and sensitivity along the entire GIT. When the Amsterdam group set up experimental animal models for stress-induced IBS and post-operative ileus, they observed that motility changes were indeed preceded by the occurrence of local inflammatory sites and that prevention of this inflammation normalized motility (Boeckxstaens).

So, we have seen that similar symptoms may present with idiopathic GI motility disease and with mast cell disease. We should also appreciate that many of the drugs used for nausea, vomiting, and abdominal cramping, such as elavil are also potent antihistamines. Doxepin is often prescribed for depression associated with chronic illness and it is also a potent blocker of H1 and H2 receptors. Periactin is often prescribed to promote weight gain and is used as a sedative for psychiatric patients and is also an H1 antagonist. Research on rodent mast cells have shown that benzodiazepines, including valium, can bind directly to mast cells and thereby inhibit the mast cells from releasing excessive mediators. Often patients may be prescribed drugs for stress when exotic illnesses confound the treating physicians, so it could be that in some cases the right drug is being given for perhaps the wrong reasons.

When Dr. Thomas Abell was at UTBowld Hospital in Memphis, his research team examined many full-thickness intestinal biopsies of patients with motility diseases and he has reported edema in the intestinal wall, an infiltration of lymphocytes, and often a high level of circulating non-specific auto-antibodies (Abell). While the lymphocytic infiltration may be indicative of some as yet unknown pathogen (Smalley), could it also be the result of the chemoattractant recruitment by mast cell mediators? Could the

edema found in the intestinal wall be the result of histamine release and the subsequent tissue swelling that it is known to produce from blood vessel leakage? Are the circulating autoantibodies somehow related to autoimmune urticaria, which can be triggered by autoantibodies? I have attached in the appendix color photographs taken from the website of The Wasa Workgroup on Intestinal Disorders in Vasa, Finland (http://www.gastrolab.net/ku27.htm). These photographs show patchy white lesions on the wall of the throat and esophagus that the researchers think may be urticaria in the GIT (Bjorknas). And one wonders what results would be obtained if future intestinal biopsies were examined for evidence for the proliferation of mast cells.

Patients with urticaria and mast cell disease are typically treated with various combinations of antihistamines which block histamine receptor sites on cells, with a fine-tuning of both dosage levels and drug choice often being very specific to the patient. A very successful choice is the well-known "ZZ" combo of zyrtec and zantac since this combination blocks both the H1 and H1 receptors. Other antihistamines include ChlorTrimeton, Benadryl, Dramamine, Claritin, and Tavist. The anti-leukotrienes such as Singulaire have also been of value in the successful drug cocktails. Compounds that both block the release of histamine from mast cells and antagonize H1 receptors include azelastine, ketotifen, permirolast and others. It should be noted that since many other mediators are released from mast cells in addition to histamine, those strategies that prevent mast cell degranulation are of greater value.

In this light, there is a published account of the remediation of ileus due to mast cell involvement that warrants attention. US patent number 5,958,407 was issued in 1999 to the University of California at San Francisco and the details released through their Office of Technology Management. The patent was issued for the "Methods for the Treatment of Post-operative Ileus". More specifically, the description is for treating post-operative ileus by preventing mast cell degranulation via administration of specific compounds. From the title this would seem very specific to surgical situations, however, investigating the patent description in depth suggests that the mechanism of action that induces ileus in healthy individuals following surgical manipulation might also apply to persons with idiopathic GI motility issues that might involve mast cell degranulation.

The loss of intestinal motility, termed post-operative ileus, is a major complication of abdominal surgery often resulting in long hospital stays. The trauma of abdominal surgery and the consequential intestinal manipulation causes infiltration of mast cells and their degranulation, resulting in the release of the enzymes tryptase and chymase. Both of these enzymes can cleave proteinase-activated receptor 2 (PAR-2) on colonic cells releasing the N-terminal SLIGRL peptide agonist of PAR-2 resulting in bowel stasis. There are pro-inflammatory mast cells present in the intestinal wall and the manipulation of the intestines results in inflammation and further influx and degranulation of mast cells. It is the infiltration and degranulation of mast cells in the colon that release tryptase and chymase, which activates the PAR-2 receptor.

Earlier this author has postulated that circulating endotoxins from a leaky gut could contribute to the extra-intestinal symptoms of CIP (Alford). The increased intestinal permeability leads to the leaking of endotoxin into the peripheral circulation, which can cause various disease processes throughout the body. Injection of laboratory mice with endotoxin has been shown to produce auto-antibodies and soluble immune complexes in the blood, with individual mice showing marked differences in circulating immune complex responses (Bloembergen). Since up to 50% of patients with chronic urticaria (CU) have circulating auto-antibodies that are thought to provoke the CU, it may be that a positive feedback loop is maintained with the activating of mast cells by the circulating auto-antibodies perpetuating the release of endotoxin, which produces more circulating auto-antibodies with mast cell activation.

Prior to this patent claim by the University of San Francisco group, extreme cases of post-operative ileus were treated with surgical intervention or with drugs to increase the colon's motility. The patent claims that these approaches have historically been ineffective in dealing with the problem of ileus and its complications and that their described method based on the discovery that proteinase-activated receptor 2 is expressed in colonic muscle and that activation of PAR-2 inhibits GI motility has great efficacy for preventing or resolving the ileus. It is claimed that their method of treatment accelerates recovery by preventing mast cell degranulation, inhibiting tryptase and chymase, and antagonizing PAR-2 (US Gov. Patent Office Website).

The author has included an abridged form of the patent description in an appendix and the entire patent description can be read in detail on the US Government Patent Website at – http://patft.uspto.gov/netahtml/srchnum.htm—which also describes the evaluation of appropriate compounds as well as their treatment method.

The new appreciation of the role of mast cells in the immune system and the consideration of a role for mast cell involvement in the GI motility diseases is engaging and could provide an interesting direction of investigation and treatment, particularly in the subset of patients that also exhibit allergies and have evidence of flushing, hives, unusual solitary bumps anywhere on the body, or have autonomic dysfunction or GI symptoms that wax and wane. A closer look at the treatment for mast cell disorders may yield new strategies for GI motility dysfunction.

A short list of principal investigators in the MC arena is provided in appendix ii for further information and dialog.

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APPENDIX i Selection from the US Government Patent # 5,958,407 http://patft.uspto.gov/netahtml/srchnum.htm

BACKGROUND OF THE INVENTION

Inhibition of intestinal motility, especially colonic motility, is a major complication of abdominal surgery. The condition, termed post-operative ileus, delays the normal resumption of food intake after surgery and often leads to prolonged hospitalization.

Mast cells are pro-inflammatory cells that are normally present in the wall of the intestine. Manipulation of intestine and intestinal inflammation are accompanied by influx and degranulation of mast cells in the wall of the intestine (Vermillion). Mast cell tryptase and chymase are proteases that account for 25% of the total protein of mast cells (Caughey). They are released from mast cells upon degranulation within the wall of the colon.

Heretofore, postoperative ileus has been treated, in extreme cases, with surgical intervention to unblock the colon. Ileus may also be treated with drugs that act to increase colonic motility, such as Leul3-motilin and prostaglandin F2 alpha. However,

these approaches have generally been ineffective in significantly reducing the period of postoperative ileus and its complications. It would therefore be useful to provide a more effective method of treating post-operative ileus, in particular, to accelerate recovery time following colonic surgery.

SUMMARY OF THE INVENTION

The present invention is directed to a method of treating or preventing post-operative ileus in a mammalian subject. The method includes administering to the subject, a pharmaceutically effective amount of a compound that is effective in (i) preventing mast cell degranulation, (ii) inhibiting tryptase and chymase, or (iii) antagonizing PAR-2.

The treatment is based on the discoveries that proteinase-activated receptor 2 (PAR-2) is expressed in colonic muscle cells, and that activation of PAR-2 inhibits colonic motility. The PAR-2 receptor is activated, at least in part, by tryptase and chymase, produced by infiltration and degranulation of mast cells.

For preventing mast cell degranulation, the compound is preferably cromolyn, doxoantrazole, quercetin, tranilast, ketotifen, tiacrilast, azelastine, lodoxamide, mepyramine, picumast, or water-soluble constituents of the Ginkgo biloba episperm.

For inhibiting tryptase, the compound is preferably leech-derived tryptase inhibitor, APC-366, and BABIM and related amidines, TLCK, GMCHA-Ophbut, or a dipeptide tryptase inhibitor.

For inhibiting chymase, the compound is preferably chymostatin, chymostatin analogues, .alpha.-1-antichymotrypsin.

For antagonizing PAR-2, the compound is one capable of inhibiting the mobilization of Ca.sup.+2 in cells transfected with the PAR-2 gene, and stimulated by trypsin or activating peptide (SLIGKVD-NH.sub.2 and SLIGRL-NH.sub.2, for human and murine PAR-2, respectively).

Where the treatment compound is a polypeptide, such as leech-derived tryptase inhibitor, chymostatin, chymostatin analogues, and .alpha.-1-antichymotrypsin, the compound may be delivered by orally administering a DNA construct capable of transfecting colonic cells, and expressing the polypeptide in the colonic cells.

For orally active compounds, such as cromolyn, doxoantrazole, quercetin, tranilast, ketotifen, tiacrilast, azelastine, lodoxamide, mepyramine, picumast, water-soluble constituents of the Ginkgo biloba episperm, APC-366, BABIM and related amidines, TLCK, and GMCHA-Ophbut, the compound is preferably administered orally. Alternatively, the compound may be administered by parenteral route, such as

intraperitoneally or intravenously.

In another aspect, the invention includes a method of identifying compound candidates for use in treating post-operative ileus. The method includes screening test compounds for their ability to inhibit the mobilization of Ca.sup.+2 in cells transfected with the PAR-2 gene, when the cells are stimulated by trypsin or activating peptide ((SLIGKVD-NH.sub.2 or SLIGRL-NH.sub.2), and selecting the compound as a candidate for the treatment if significant inhibition of Ca.sup.+2 mobilization in the cells, when compared with activated cells in the absence of the compound, is observed. ILEUS MAST CELL PATENT REFERENCES Armstrong, R. W., PCT Intl. Appn. Pubn. No. WO 9502566 (January 1995).

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APPENDIX ii BIO OF SELECT MASTOCYTOSIS RESEARCHERS IN USA

Dr. Cem Akin, MD., Ph.D.

Dr. Cem Akin is an Assistant Professor at University of Michigan's Department of Medicine - Division of Allergy and Clinical Immunology, where he heads a new research facility, one of the few in the country devoted to the research of Mast Cell

Disease. Here he leads a team which conducts studies of cellular and molecular pathologic mechanisms in mast cell disorders. Formerly of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Dr. Akin established himself there as a leading mast cell researcher (receiving the Staff Recognition Award for 4 consecutive years of 2000-2003) and developed novel techniques for identifying mast cell disease and determining the most promising treatment options for patients. Dr. Akin is the recipient of the TMS (The Mastocytosis Society) research grant of $44,000. Dr. Akin's new research facility at University of Michigan will continue his critical studies as he establishes a Center for both Mastocytosis patient care and for research in collaboration with faculty from the University of Michigan Comprehensive Cancer Center and the Division of Allergy and Clinical Immunology. In his clinic, Dr. Akin diagnoses and treats mast cell diseases in people referred by doctors throughout the country, including patients with unexplained symptoms who need specialized testing. Dr. Akin serves on the American Academy of Allergy, Asthma, & Immunology Mast Cell DisordersTask force and the TMS Medical Advisory Board. He is also a consultant for The Mastocytosis Society, Canada Mastocytosis Support, and the European Competence Network on Mastocytosis.

Dr. Mariana Castells MD, Ph.D.

Dr. Castells is a clinician/teacher at the Brigham and Women's Allergy Services, the Co-Director of the Allergy and Clinical Immunology Training Program at the Brigham and Women's Hospital, a researcher in the mast cell biology group, and an Assistant Professor of Medicine at Harvard Medical School. As a researcher Dr. Castells has her own independent research laboratory which studies mast cell inhibitory receptors and desensitizations. Taking particular interest in drug adverse reactions, anaphylaxis, Mastocytosis, physical allergies including exercise induced anaphylaxis, food allergies, urticaria, and immunodeficiencies, Dr. Castells receives nationwide and international patient referrals. Dr. Castells specific interest in Mastocytosis has prompted the establishment of the Mastocytosis Registry (a registry for patients), of which she is Director. Dr. Castells also donates her time as Advisor to TMS.

Dr. Joseph H. Butterfield, MD.

Dr. Butterfield is a Consultant at the Mayo Clinic's Department of Immunology in Rochester, Minnesota and a Professor of Medicine at the Mayo Clinic College of Medicine. In 1985 Dr. Butterfield successfully cloned an immature mast cell line from the peripheral blood of a patient with Mast Cell Leukemia, and thus holds the prestige of being the inventor of the Human Mast Cell Line HMC-1. Since then he has seen more and more Systemic Mastocytosis patients along with patients of disorders of the release of mast cell mediators. Due to the great volume of these patients seen by Dr. Butterfield, Systemic Mastocytosis now plays a primary role in his clinical practice, and new patients appear on a weekly basis both nationally and internationally. Dr. Butterfield is an accomplished speaker, presenting his research worldwide on international and

national levels. Dr. Butterfield serves as Advisor to TMS. Over the past 20 years he has devoted himself to improving the treatment of patients with Systemic Mastocytosis.

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Dr. Theoharides has ongoing research interests in four related areas: (a) molecular events involved in mast cell stimulus-response coupling in allergic reactions and in the pathophysiology of inflammatory disorders: atopic dermatitis, arthritis, coronary artery disease, as well as in interstitial cystitis/chronic prostatitis, migraine headaches and multiple sclerosis for which Dr. Theoharides' group has developed in vivo and in vitro models; (b) the ability of mast cells to release some of their mediators, especially cytokines, selectively that may explain how they participate in inflammation; moreover, selective release of angiogenesis factors may help explain the high number of mast cells around tumors, such as breast carcinoma and melanoma; (c) the role of mast cells in mediating the effect of stress by responding to corticotropin-releasing hormone (CRH), or its analogue urocortin (Ucn), with cytokine release, as well as secretion of CRH and Ucn; (d) the regulation of the expression, identification of the phosphorylated sites, as well as molecules that increase and sustain the phosphorylated state of a 78 kDa mast cell phosphoprotein he and his associates have cloned. In its phosphorylated state, this protein inhibits mast cell secretion and proliferation; select compounds could serve as anti-allergic/anti-inflammatory drugs, and possibly anticancer agents. Dr. Theoharides has numerous patents and is interested in using natural molecules in food supplements for the treatment of allergic/inflammatory and malignant conditions (Related web site: Algonot.com). He is also interested in drug and biomedical research policy. He has served as the Clinical Pharmacologist of the Massachusetts Drug Formulary Commission continuously since 1986; he has also served on the Supreme Health Council, of the Ministry of Health, the HealthCare Board of the Ministry of Labor and Human Resources and the National Drug Organization of the Hellenic Republic.

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