Selective Expression of Potassium Channels during Mast Cell Differentiation*

Michael A. McCloskey and Yi-xin Qian†
From the Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011-3223

In rodents, mast cell progenitors differentiate into distinct mucosal and serosal phenotypes which differ markedly in their functional responses to antigenic and peptidergic stimulation. Although the molecular basis of mast cell differentiation or functional specialization is unknown, it is possible that regulation of calcium entry contributes to one or both processes. The prolonged secretory response of mucosal mast cells (MMC) and the antigen-elicted synthesis of interleukin-3 by immature MMC both require a rise of cytoplasmic calcium sustaining K+ conductance was constitutively active and a latent outwardly rectifying K+ conductance was elicited upon ligation of cell surface adenosine or P2 purinergic receptors linked to G proteins of the G, family. Stimulation of P2 receptors dramatically potentiated antigen-triggered secretion in a pertussis toxin-sensitive manner, suggesting that activation of the outwardly rectifying K+ channel may regulate antigen-dependent functions of MMC.

Mast cells are multifunctional leukocytes which contribute to the specific immune response against helminth parasites and initiate anaphylactic reactions to allergens. They originate from hematopoietic progenitors present in the bone marrow and fetal liver. During normal ontogeny or as a consequence of parasitic infection these progenitors are recruited by various peripheral tissues, wherein they differentiate further under the influence of microenvironmental factors which remain to be fully characterized. In many and perhaps most species, multiple mast cell phenotypes are generated, often in a tissue-specific manner (1). These phenotypes differ in several important functional attributes as well as in their content of inflammatory mediators, granule ultrastructure, life span, and IgE receptor expression.

Mast cell heterogeneity is best defined in rodents, where two phenotypes are currently recognized. The well studied rat peritoneal mast cell (RPMC) is the prototype serosal mast cell, also known as the connective tissue mast cell. Rat mucosal mast cells are typified by the population that expands in the intestinal mucosa in a Th-1 and IL-3-dependent manner during parasitic infections, releasing rat mast cell chymase II systemically (2, 3). Rat BMMC cultured in vitro with IL-3 also resemble intestinal MMC in terms of granule protease expression and several other properties (4–6). Mast cells of both phenotypes secrete allergic mediators upon cross-linkage of their IgE receptors, but unlike RPMC, rat intestinal MMC do not secrete histamine in response to several neuropeptide secretagogues (7). Nor do the anti-asthmatic drugs cromolyn and theophylline block antigen-stimulated secretion of histamine by rat intestinal MMC (8, 9). In vitro, cross-linkage of IgE receptors on rodent IL-3-dependent mast cells stimulates them to proliferate and to synthesize and release IL-8, IL-4, and other cytokines implicated in the mucosal immune response against helminths (10, 11).

The molecular differences between mast cell subsets that may contribute to these distinct functional profiles are unknown. MMC secrete much more slowly than do RPMC (12) and this prolonged secretory response as well as the antigen-induced synthesis of IL-3 by immature murine MMC depend upon an elevation of cytoplasmic calcium (13, 14) which is sustained by Ca2+ influx through the plasma membrane. In many electrically inexcitable cells, including tumor MMC (15–17), receptor-initiated Ca2+ entry is inhibited by membrane depolarization. This raises the possibility that receptor-coupled ion channels could modulate the Ca2+-dependent functional responses of MMC via control of membrane potential.

Transformed RBL-2H3 cells, related to rat mucosal mast cells (18), express a receptor-linked K+ channel, the activity of which appears to support the normal secretory response to antigen (19). This outwardly rectifying K+ (Ko,R) channel has not been detected in numerous studies of RPMC (20–22), suggesting a possible difference in K+ channel expression between serosal and mucosal mast cells. This interpretation remains tenuous, however, because transformed cells can express ion channels not present in cells of the corresponding normal phenotype (23). Moreover, RBL-1 cells (24), derived from an earlier passage of the same tumor that RBL-2H3 cells were derived from, do not express the Ko,R channel (25). Genetic differences between RBL sublines are well documented (26), and variability between RBL cells of the same 2H3 subline grown in different laboratories is a common occurrence. Hence, to assess the physiological significance of the Ko,R channel in genuine mast cells, it is crucial to determine whether nontransformed MMC of defined origin also express this channel and its functionally coupled receptors.

* This work was supported by Biotechnology and University Research Grants from the Iowa State University and by NIH Grant GM48144. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1724 solely to indicate this fact.

† Current address: Amgen Inc., Thousand Oaks, CA 91320-1789.

‡ The abbreviations used are: RPMC, rat peritoneal mast cell; BMMC, bone marrow-derived mast cells; IL, interleukin, MMC, mucosal mast cells; IR, inwardly rectifying; OR, outwardly rectifying; RBL, rat basophil leukemia; ADP･S, adenosine 5'-O-(2-thiodiphosphate); ATP･S, adenosine 5'-O-(3-thiotriphosphate); GDP･S, guanosine 5'-O-(2-thiodiphosphate); GTP･S, guanosine 5'-O-(3-thiotriphosphate); NECA, 5'-[O-ethyl carboxamido]adenosine; pF, picofarad; nS, nanosecond.
Here we used patch-clamp recording to study the expression of ion channels in rat IL-3-dependent BMMC. Two K+ channels were expressed by these cells: a constitutive inwardly rectifying K+ (K+IR) channel and a latent outwardly rectifying K+ (K+oR) channel activated through receptors coupled to proteins of the G class. As shown in previous work and confirmed again here, neither K+ channel is present in RPMC. Ligation of purinergic receptors linked to the K+IR channel markedly enhanced antigen-stimulated secretion in a pertussis toxin-sensitive manner, consistent with the idea that activation of the K+oR channel modulates antigen-triggered secretion. Identification of the K+IR and K+oR channels in rat BMMC could provide a useful handle to explore the molecular basis of mast cell functional heterogeneity. This work was presented in abstract form at the 1993 meeting of American Association of Immunologists (27).

EXPERIMENTAL PROCEDURES

Reagents—Chemicals were obtained as follows: 5'-N-ethylcarboxyamido-adenosine was obtained from Sigma. ADPβS, guanosine 5'-triphosphate, GDPβS, and GTPγS were from Boehringer Mannheim. [3H]-Hydroxytryptamine was from DuPont NEN. Purified BC4 monoclonal anti-rat FceRI was a gift of Dr. Reuben Siraganian, and the pLIR1 plasmid containing a genomic clone of rat interleukin-3 was supplied by Dr. Ian G. Young. Trinitrophenylated bovine serum albumin was synthesized by reaction of trinitrobenzenesulfonic acid with the protein at room temperature in borate-buffered saline (pH 9.0).

Cell Preparation—Bone marrow cells from 6-12-week-old Fisher 344 rats were cultured at 37 °C in the presence of rat recombinant IL-3 (28) in liquid culture. Growth medium consisted of 70% RPMI 1640 (containing 20% horse serum and 50 μM 2-mercaptoethanol) plus 30% COS-1 supernatant containing rat IL-3. COS-1 monkey kidney cells (5 × 10⁶) in 10-cm dishes were transfected with 100 μg of the pLIR1 plasmid using standard calcium phosphate precipitation, the cells were washed 12–18 h after transfection and supernatants harvested 3 days later. COS-1 cells were cultured in Dulbecco’s minimal essential medium containing 10% fetal bovine serum. No growth of mast cells occurred with sham-transfected COS-1 supernatants. For the patch-clamp experiments, nine independent rat BMMC cultures were studied (average age 19 days; range 12–29 days).

Adult Fisher 344 or Sprague-Dawley rats were used as a source of RPMC. Rats were anesthetized with ethyl ether, decapitated with a guillotine, and a mixture of cells obtained by lavage of the peritoneal cavity. Total cells were harvested by centrifugation at 900 rpm for 10
Note a positive shift in reversal potential between reversal potential \( E_r \) and logarithm of external K⁺ concentration (mM). *Squares* indicate average ± S.E. for 8-12 cells; line is least squares fit to data. The slope of 52 mV is close to that predicted by the Nernst equation for a K⁺-selective membrane.

Whole cell current-voltage curves of a cell dialyzed with standard potassium aspartate pipette solution at four different concentrations of extracellular K⁺. Note positive shift in reversal potential and increase in inward current upon increase in extracellular [K⁺] from 4.5 to 163 mM. *Inset* shows linear relationship between reversal potential \( E_r \) and logarithm of external K⁺ concentration (mM). *Squares* indicate average ± S.E. for 8-12 cells; line is least squares fit to data. The slope of 52 mV is close to that predicted by the Nernst equation for a K⁺-selective membrane.

**RESULTS AND DISCUSSION**

Previous studies have established that IL-3-dependent rat BMMC resemble intestinal MMC in terms of protease expression, glycosaminoglycan composition, life span, and secretory function (4, 6). In the present experiments, after 1–3 weeks in culture, 75–85% of the cells expressed the high-affinity IgE receptor (FceRI), as determined from flow cytometric analyses using the BC4 anti-FceRI antibody (33). Essentially all the contaminating cells in 2–3 week cultures were macrophages. Mast cells were easily identified for patch-clamping by their prominent cytoplasmic granules, made evident upon spreading of the cells on polylysine-coated coverslips (Fig. 1). This immobilization procedure did not trigger secretion, as the spontaneous release of cell-associated \(^{3}H\)serotonin during a 60-min assay was not larger for polylysine-adherent cells (14.9 ± 4.9%, \( n = 3 \)) than it was for suspended cells (18.8 ± 0.8%, \( n = 3 \)). Cells also were immobilized on coverslips coated with extracellular...
Fig. 4. a, induction of outwardly rectifying $K^+$ conductance, $G_{KOR}$, and decay of $G_{KR}$, in cell dialyzed with potassium aspartate containing 100 μM GTPγS. Indicated traces were recorded from 12 to 379 s after break-in to the whole cell recording configuration. $G_{KOR}$ and development of $G_{KR}$ normal Ringer (NR) was replaced with potassium Ringer (KR) in bathing solution. The large positive shift in reversal potential of $G_{KOR}$ (arrows) indicates that the induced conductance is specific for $K^+$ ions. c, extracellular ADP (10 μM) activates $G_{KOR}$ selectively.
matrix from murine 3T3 fibroblasts (30).

Thin section electron micrographs of mast cells in a 4-week culture are shown in Fig. 2. Although many secretory granules are filled completely with an electron-dense core, others are not. Primary intestinal MMC exhibit a similar distribution of granule morphologies (2), whereas RPMC granules are essentially all filled with an electron-opaque core. The origin of vesicle heterogeneity in MMC is unclear; some consider that the electron-lucent vesicles are “immature” and others that copy, the diameter of rat BMMC used here was half-time for induction of this K+ current in different cells was obtained from measurements of membrane capacitance (Fig. 3), the reversal potential (E_r) and magnitude of this current increased systematically with increase in the concentration of extracellular potassium ([K^+]_o). The linear relation of E_r versus log ([K^+]_o), with a slope of 52 mV, indicates that the constitutively present inward current is highly selective for K^+. The slope conductance, G_{KIR}, was measured by a least squares fit to the current-voltage curve between -90 and -100 mV. In mammalian Ringer (4.5 mM K^+), G_{KIR} averaged 1.85 ± 0.12 nS (n = 184). Substitution of K^+ for Na^+ in the bath (164.5 mM [Na^+]_o) increased G_{KIR} by 4.5-fold to 8.19 ± 1.14 nS (n = 16).

A second conductance was elicited in 41 out of 42 cells dialyzed with 100 µM GDPβS, an irreversible activator of G proteins (Fig. 4a). GDPβS also caused the disappearance of the constitutive G_{KIR} in some cells, as shown in Fig. 4a. This revealed that the induced current was outwardly rectifying above ~80 mV in normal Ringer, and it simplified determination of the ionic selectivity of the induced current. Fig. 4b shows that replacement of Na^+ in the bath with K^+ caused a large positive shift of E_r (arrows) from -78.1 ± 2.5 mV (n = 6) to 5.9 ± 1.2 mV (n = 4) ([K^+]_o/[Na^+]_o = 0.99), indicating a K^+-selective current. The half-time for induction of this K^+ current in different cells was quite variable (t_1/2 = 49–361 s), averaging 140 ± 18 s (n = 20).

The RBL-2H3 cell line expresses A_2 adenosine receptors (36, 37) and P_2y purinergic receptors (19, 38), both coupled to pertussis toxin-sensitive G proteins (19, 36). We tested ligands active at these receptors and found that they rapidly induced G_{KORB} in rat BMMC without affecting currents through the inwardly rectifying K^+ channel (Fig. 4c). In 32 out of 52 cells, extracellular application of 10 µM ADP induced G_{KORB}, averaging 1.87 ± 0.18 nS, a 7.7-fold increase over the basal conductance. This is significantly different from G_{KORB} elicited by GTPγS even at 90% confidence limits. Ionic substitution experiments showed that the induced current is K^+-selective. The adenosine analog 5′-N-ethylcarboxamido)-adenosine (50 µM) activated G_{KORB} in 5 of 7 cells, with a peak conductance of 1.23 ± 0.17 nS.

Induction of G_{KORB} by both agonists was much faster than that caused by GTPγS (t_1/2 < 10 s). The half-time for induction could be much less than 10 s, given that the half-time for bath exchange was ~10 s. The rapid increase in conductance was not due to incorporation of channels into the plasma membrane from secretory vesicle membrane, as neither agonist caused an increase in membrane capacitance during the induction period. Conversion of ADP to adenosine by surface ectoenzymes was not required for induction of G_{KORB}, as the poorly hydrolyzable ATP analog ADPβS was much more potent than ADP. Activation of G_{KORB} was maximal at 1 µM ADPβS, but 1 µM ADP elicited only 53 ± 13% (n = 3) of the maximal response obtained at ≥10 µM ADP. That ADP and ADPβS can induce G_{KORB} indicates that the relevant receptor is not the P_2x receptor for ATP, which was first identified in RPMC (39).

Two findings suggest that induction of G_{KORB} by ADP was mediated by heterotrimeric G proteins. First, pretreatment with pertussis holotoxin (100 ng/ml, 12–18 h) blocked induction of G_{KORB} by ADP in 9 of 9 trials. In these cells the mean increase in conductance over basal caused by 10 µM ADP was 0.01 ± 0.01 nS, compared to 1.63 nS in control cells. Second, the GDP analog GDPβS prevented induction of G_{KORB} by ADP in 4 out of 4 trials. After dialysis for 1 min with a pipette solution containing 3 mM GDPβS, 0.3 mM GTP, addition of 10 µM ADP increased the basal conductance by only 0.04 ± 0.01 nS. GDPβS competes effectively with GTP for binding to heterotrimeric G proteins (40), but it does not activate the G protein. That G_{KORB} is elicited by stimulation of G proteins with intracellular GTPβS also supports the idea that extracellular purines could act by a similar pathway.

Although adenine nucleotides are known to cause a transient increase in cytoplasmic [Ca^{2+}] ([(Ca^{2+})_i]) in mast cells (38), elevation of [Ca^{2+}]_i apparently was not required for the activation of G_{KORB} by ADP. Our standard pipette solution contained 1.1 mM EGTA, had an estimated free [Ca^{2+}]_i ~10 nM, and it supported activation of G_{KORB}, averaging 1.22 ± 0.17 nS (n = 14). To more rigorously eliminate the possibility that [Ca^{2+}]_i, transients induced by ADP were responsible for channel activation, cells were dialyzed with a pipette solution containing 11 mM EGTA ([Ca^{2+}]_i ~10 nM). Dialysis of similarly-sized RPMC with 11 mM EGTA prevents the increase of intracellular Ca^{2+} caused by GTPβS or inositol-1,4,5-trisphosphate (41). Rather than decrease, the magnitude of G_{KORB} induced by ADP in cells so treated was significantly larger (2.53 ± 0.20 nS, n = 15) than in cells dialyzed with standard pipette solution (p < 0.01). Apparently, ADP can activate the K^+ channel through a Ca^{2+}-independent pathway.

Currents through the K^+ and K^+IR channels were distinguished with the channel antagonists Ba^{2+} and nitrendipine. Barium at 50 µM completely blocked G_{KORB} in normal Ringer without affecting G_{KORB} (not shown). In contrast, although 15 µM nitrendipine had no effect on G_{KORB}, 1.5 µM nitrendipine
hypothesize that one function of the K\textsubscript{\textit{IR}} channel is to provide voltage curves, and by the selective activation of the K\textsubscript{\textit{IR}} channel via membrane potential. Although the constitutive K\textsubscript{\textit{IR}} channel activation to Ca\textsuperscript{2+}-dependent functions, we examined of the K\textsubscript{\textit{IR}} channel (42). Hence, the K\textsubscript{\textit{IR}} and K\textsubscript{\textit{IR}} channels can be separated based upon their different pharmacology, current-voltage curves, and by the selective activation of the K\textsubscript{\textit{IR}} channel via surface receptors for purines.

Maintenance of a sufficiently negative membrane potential is necessary for receptor-mediated Ca\textsuperscript{2+} influx into many electrically excitable cells, including tumor MMC (15–17). We hypothesize that one function of the K\textsubscript{\textit{IR}} channel is to provide a switching mechanism to control antigen-stimulated Ca\textsuperscript{2+} entry via membrane potential. Although the constitutive K\textsubscript{\textit{IR}} channel should keep the membrane hyperpolarized at rest, examination of the \textit{I-V} curve indicates that this channel is inoperative following modest depolarization, as occurs upon antigenic stimulation of tumor MMC (43–45). Moreover, there is some indication that elevation of [Ca\textsuperscript{2+}], to levels similar to those achieved following antigenic stimulation may inhibit the K\textsubscript{\textit{IR}} channel (46). In this circumstance the relative activity of G\textsubscript{KOR} may exert a marked influence on Ca\textsuperscript{2+} entry via its control of membrane potential. To study the possible contribution of K\textsubscript{\textit{IR}} channel activation to Ca\textsuperscript{2+}-dependent functions, we examined the effect of extracellular ADP on antigen-triggered secretion of 5-hydroxytryptamine. Fig. 5 shows that ADP caused a striking enhancement of 5-hydroxytryptamine release, \simi~60–70\% of which was blocked by pretreatment of the cells with pertussis toxin. Although the effects of ADP are likely to be pleiotropic, the present results are consistent with a role for G\textsubscript{KOR} in regulation of the secretary response. Further experiments are required to delineate the exact contribution of K\textsubscript{\textit{IR}} and K\textsubscript{\textit{IR}} channels to the control of calcium-dependent functions of MMC.

In numerous studies of RPMC there is no mention of the existence of either the K\textsubscript{\textit{IR}} or K\textsubscript{\textit{IR}} channel (see, e.g. Refs. 20–22). Expression of the constitutive K\textsubscript{\textit{IR}} channel is high enough in both RBL-2H3 cells and rat MMC such that it is readily detected in normal Ringer solution (4.5 mM external K\textsuperscript{+}) or mast cell Ringer (2.5 mM K\textsuperscript{+}), so one expects that if it were expressed at similar levels in RPMC it should have been detected readily. In the present experiments, we failed to detect G\textsubscript{KOR} in any of 38 RPMC bathed either in normal Ringer or mast cell Ringer. A minuscule inwardly rectifying K\textsuperscript{+} conductance was present in 1 of 25 RPMC bathed in K Ringer; the density of G\textsubscript{KOR} in this cell (0.06 nS/pF) was much lower than the average G\textsubscript{KOR} of either rat BMMC or RBL-2H3 cells (see below). That many hundreds of RPMC have been dialyzed with GTP\textsubscript{Y}S in other laboratories without a single report of the induction of G\textsubscript{KOR}, indicates that G\textsubscript{KOR} is not present in RPMC. In the present work, dialysis of RPMC with 100 \mu M GTP\textsubscript{Y}S also failed to activate G\textsubscript{KOR} (n = 4). Furthermore, extracellular application of 10 \mu M ADP to RPMC dialyzed with 0.3 \mu M GTP failed to elicit G\textsubscript{KOR} in each of the 11 cells tested. We conclude that RPMC do not express significant levels of either potassium channel found in rat BMMC and RBL-2H3 cells.

Rat BMMC previously have been shown to possess hallmark of primary intestinal MMC, including expression of mast cell chymase II but not chymase I, lack of responsiveness to compound 48/80 and neuropeptides, a shorter life span than RPMC, and much lower histamine content than RPMC. There is a general conception, however, based primarily on studies with murine BMMC, that all cultured mast cells are immature and can be forced along either the mucosal or serosal differentiation pathway by appropriate cytokines. Whether this is true for rat BMMC is an open issue, because in vitro studies of reversible granule protease expression (47) and in vivo studies of “phenotype switching” of cultured mast cells introduced into mast cell-deficient hosts (48) have not been performed with the rat system. The fidelity with which the ion channel repertoire of rat BMMC mimics that of rat intestinal MMC remains an important question for future studies of this difficult-to-isolate primary cell population (2, 49).

The rat BMMC used here clearly were more mature than RBL-2H3 cells, based upon granule ultrastructure, microscopically observable exocytosis and granule release upon stimulation with antigen, and expression of certain surface markers. Qualitatively, both BMMC and RBL-2H3 cells both possessed K\textsubscript{\textit{IR}} and K\textsubscript{\textit{IR}} conductances as well as the same two receptor-G protein signal transduction pathways leading to activation of G\textsubscript{KOR}. Quantitatively, however, the current density of the constitutively active inward rectifier was substantially smaller in rat BMMC than in RBL-2H3 cells (0.26 versus 0.46 nS/pF), whereas the GTP\textsubscript{Y}S-induced outward rectifier was present at rat BMMC than in RBL-2H3 cells (0.32 versus 0.22 nS/pF). It will be interesting to see if this trend is reflected in even higher expression of G\textsubscript{KOR} and lower expression of G\textsubscript{KIR} in the intestinal MMC than in rat BMMC.

Previous electrophysiological studies of nontransformed rat mast cells have utilized rat peritoneal mast cells, and the results of these studies often are assumed to apply to mast cells in general. Yet RPMC typify just one of two distinct rat mast cell subsets that arise when mast cell progenitors recruited to different tissues differentiate and mature along alternate pathways. The present results combined with extensive previous studies of RPMC indicate that during differentiation of rat mast cells from their progenitors, two K\textsuperscript{+} channels are differentially expressed. Whether these channels are expressed in the fully mature intestinal MMC or are restricted to the immature or intermediate stages of rat mast cell differentiation remains to be determined. In either case, linkage of the K\textsubscript{\textit{IR}} channel to multiple surface receptors for inflammatory mediators suggests that it may contribute to the functional activities of immature mast cells, such as their recruitment by helminth-infected tissue, or to functions unique to mucosal mast cells per se. The possible role of K\textsuperscript{+} channel expression in the differentiation process itself also remains to be explored.

Acknowledgments—We are grateful to Dr. Ian G. Young of the Australian National University (Canberra) for the generous gift of the pRBL1 plasmid and to Dr. Richard Lewis (Stanford, CA) for the software used in patch-clamp recording. We also are indebted to Dr. Reuben

\textsuperscript{2} Y.X. Qian and M. A. McCloskey, unpublished.
REFERENCES


