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Human Skin Mast Cells Express Complement Factors C3 and C5

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We examine whether complement factor C3 or C5 is synthesized by human skin–derived mast cells and whether their synthesis is regulated by cytokines. C3 and C5 mRNAs were assessed by RT-PCR, and proteins by flow cytometry, confocal microscopy, Western blotting, and ELISA. C3 and C5 mRNAs were each expressed, and baseline protein levels/10^6 cultured mast cells were 0.9 and 0.8 ng, respectively, and located in the cytoplasm outside of secretory granules. C3 accumulated in mast cell culture medium over time and by 3 d reached a concentration of 9.4 ± 8.0 ng/ml, whereas C5 levels were not detectable (<0.15 ng/ml). Three-day incubations of mast cells with IL-1α, IL-1β, IL-17, IFN-γ, IL-6, or anti-FcεRI did not affect C3 protein levels in culture medium, whereas incubations with PMA, TNF-α, IL-13, or IL-4 enhanced levels of C3 1.7- to 3.3-fold. In contrast with C3, levels of C5 remained undetectable. Importantly, treatment with TNF-α together with either IL-4 or IL-13 synergistically enhanced C3 (but not C5) production in culture medium by 9.8- or 7.1-fold, respectively. This synergy was blocked by attenuating the TNF-α pathway with neutralizing anti-TNF-α Ab, soluble TNFR, or an inhibitor of NF-κB, or by attenuating the IL-4/13 pathway with Jak family or Erk antagonists. Inhibitors of PI3K, Jnk, and p38 MAPK did not affect this synergy. Thus, human mast cells can produce and secrete C3, whereas β-tryptase can act on C3 to generate C3a and C3b, raising the likelihood that mast cells engage complement to modulate immunity and inflammation in vivo.


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Abbreviations used in this article: MC_T, mast cell containing tryptase but not chymase; MC_TC, mast cell coexpressing chymase along with tryptase; PFTo, pifithrin-$\alpha$; qRT-PCR, quantitative real-time PCR; SCF, stem cell factor; VCU, Virginia Commonwealth University.

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Materials and Methods

Reagents

Mouse IgG anti-human C3/C5 mAb and biotin-rabbit IgG anti-human C3 (Abcam, Cambridge, MA); mouse IgG anti-human C5/C5b mAb (Cell Sciences, Canton, MA); sheep IgG anti-human C3c Ab, sheep IgG anti-human C5 Ab, and rabbit anti-C3 Ab (GeneTex, Irvine, CA); goat IgG anti-human C5 (Complement Technology, Tylex, TX); goat IgG anti-human TNFR-α Ab, goat IgG anti-human TNFR-β Ab, and goat IgG anti-human TNFR-ε Ab were obtained as SECONDARY ABs. Soluble TNFR-α purified human C3, C5, C3a, recombiant human C5a, Jak 1 inhibitor, 1 cyclic-piphthiran-α (PPTra), tyrosphostin AG9, U0126, SB203580 (EMD Millipore, Billerica, MA); human C5a (Complement Technology, Tylex, TX); and IRDye 800CW-conjugated secondary Ab (LI-COR Biosciences). Labeling was analyzed using an Odyssey infrared imaging system (LI-COR Biosciences). For immunoprecipitation of C3, rabbit anti-C3 Ab was incubated with Protein A/G PLUS-Agarose (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h, washed, and added to 30 ml skin mast cell culture medium with Protease Inhibitor Cocktail. Set 1 for 1 h at room temperature followed by incubations with alkaline phosphatase-conjugated secondary Ab (1:1000 dilution; BD Biosciences), dilution at 1:10 000 and 1:10 000. The amount of C3a was determined from the standard curve made by each value of threshold cycle and normalized by dividing the β-actin threshold cycle value. Primer pairs for C3, anti-actin, and anti-C3 were as follows: C3 (188 bp), sense 5'-TCACCTGTTCAACCAAAAGCTGAC-3' and anti-actin (127 bp), sense 5'-TGCTCCCAGTTGGTGACGAT-3' and antisense 5'-AGGCACCAGGG-3' and antisense 5'-TTTCTTAGTGGC TCGGATCTTCCA-3'. C5 (186 bp), sense 5'-GTTGAAACCGCCGAGAACAGC-3' and antisense 5'-AGGGAAGGGACFACCAGCAAGA-3'; β-actin (127 bp), sense 5'-AGGGCACCAGGCGTGTA-3' and antisense 5'-TGGCTCCAGCTTGTTGACGAT-3'.

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) were performed with the CFX96 Real-Time system using a C1000 Touch Thermal cycler (Bio-Rad, Hercules, CA) and QPCR SYBR Green Low ROX mixes (Thermo Fisher Scientific, Waltham, MA). Total RNA was isolated from mast cells by RNAeasy Mini kit (Qiagen, Valencia, CA), and 1 μg RNA was treated with RNase-free DNase (Promega, Madison, WI) at 37°C for 1 h. After dena- turation at 65°C for 10 min, cDNA was synthesized using the Ready-To-Go You-Prime First-Strand Beads for cDNA synthesis (GE Health Care Bio- sciences, Pittsburgh, PA) with oligo(dT) primer (Life Technologies) for 1 h at 37°C. Reaction samples include 10 μl SYBR green/ROX PCR Master Mix, 0.5 μl of primer, and 0.5 μl of cDNA. The mixture was incubated at 95°C for 1 min, followed by 40 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 20 s. For each sample, the threshold was calculated using the CFX Manager software. The expression level of each gene was normalized to the expression level of β-actin (1:10 000 dilution; BD Biosciences), and the fold change was calculated using the ΔΔCT method.

Results

C3 and C5 proteins are produced by skin mast cells

To detect C3 and C5 proteins in human skin mast cells, we fixed, permeabilized, labeled with anti-C3/C3b, anti-C5/C5b, or

Western blotting and immunoprecipitation

Skin mast cells were extracted using 10 mM Tris-HCl, pH 7.4, buffer containing 150 mM NaCl, 1 mM EDTA, 0.5% sodium deoxycholate, 1% Nonidet P-40, 0.1% SDS, and Protease Inhibitors Set (Roche Applied Science, Indianapolis, IN) for 1 h at 4°C and centrifuged. Supernatants were mixed with SDS-sample buffer containing 2% 2-ME, boiled for 5 min, and sub- jected to electrophoresis on a 8–16% polyacrylamide gradient gel contain- ing SDS. Proteins were transferred onto a nitrocellulose membrane using a Novex system (Life Technologies, Long Island, NY) for 1 h at 50 V. After applying blocking buffer (LI-COR Biosciences, Lincoln, NE) for 1 h at room temperature, membranes were labeled with each primary Ab for 1 h at room temperature, followed by 1-h incubation on the same membrane with IRDye 800CW-conjugated secondary Ab (LI-COR Biosciences). Labeling was analyzed using an Odyssey infrared imaging system (LI-COR Biosciences). For immunoprecipitation of C3, rabbit anti-C3 Ab was incubated with Protein A/G PLUS-Agarose (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h, washed, and added to 30 ml skin mast cell culture medium with Protease Inhibitor Cocktail. Set 1 for 1 h at room temperature followed by incubations with alkaline phosphatase-conjugated secondary Ab (1:10 000 dilution; BD Biosciences), and 1:10 000 dilution at 1:10 000. The amount of C3a was determined from the standard curve made by each value of threshold cycle and normalized by dividing the β-actin threshold cycle value. Primer pairs for C3, anti-actin, and anti-C3 were as follows: C3 (188 bp), sense 5'-TCACCTGTTCAACCAAAAGCTGAC-3' and anti-actin (127 bp), sense 5'-AGGGCACCAGGCGTGTA-3' and antisense 5'-TGGCTCCAGCTTGTTGACGAT-3'.

C3 and C5 ELISAs

C3 and C5 protein levels, respectively, were measured by ELISA using 1 μg/ml sheep anti-C3c and sheep anti-C5 Abs for capture and 1 μg/ml biotin-rabbit IgG anti-C3 and biotin-goat IgG anti-C5 (goat IgG anti- human C5 IgG was purified by protein G-Sepharose chromatography from antisera prepared by Comp Tech and was then conjugated with biotin in-house) Abs for detection. After incubation with avidin-peroxidase (1:1000 dilution; BD Biosciences), 2.2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammium salt solution (0.3 mg/ml; Sigma-Aldrich) was added and the absorbance at 405 nm was measured using a Spec- traMax 384 Plus plate reader (Molecular Devices, Sunnyvale, CA). The lower detection limit for each protein was 0.15 ng/ml.

Statistics

Statistical analyses were performed using SigmaStat (Systat Software, Chicago, IL). For parametric data, Student t test for two-group and ANOVA for more than two-group comparisons were used, whereas for nonpara- metric data, a Kruskal–Wallis ANOVA on ranks was used to compare more than two groups. Differences were considered statistically significant when p values were < 0.05.
isotype-matched negative control Ab, and assessed these cells by flow cytometry. As depicted in Fig. 1A, both anti-C3 and anti-C5 Ab showed a positive signal compared with isotype control. To confirm the specificity of the shift, we showed purified human C3 or C5 to compete with the corresponding Ab (Fig. 1B), consistent with C3 and C5 being present within these mast cells. Confocal analysis showed a diffuse cytoplasmic labeling pattern with both anti-C3 and anti-C5 Abs (Fig. 1C), unlike the granular pattern seen with Abs against tryptase, a well-recognized component of the mast cell secretory granule (56, 57). Mast cells stimulated with anti-FcεRI Ab for 15 min secreted β-hexosaminidase, another secretory granule marker in mast cells (58, 59), but negligible amounts of C3 or C5 (Fig. 1D), confirming the subcellular localization of cytoplasmic C3 and C5 being outside of the secretory granules. Importantly, having cultured these mast cells for 6–12 wk in serum-free medium containing no detectable C3 and C5 (<0.15 ng/ml), exogenous complement factors cannot account for the C3 and C5 detected, supporting their production by these mast cells.

To further examine the C3 and C5 protein produced by skin mast cells, we performed Western blotting with protein extracts, using sheep anti-human C3 Ab and biotin-goat anti-human C5 Ab (Fig. 1E). Both C3α- and β-chains and C5α- and β-chains were evident in skin mast cell extracts, with molecular masses comparable with those detected in extracts of A549 and HMC-1 cells and with those in purified preparations of C3 and C5. C3 and C5 proteins secreted into the mast cell culture medium also were examined. Immunoprecipitation from culture medium was performed using rabbit IgG anti-human C3 or anti-human C5 bound to protein A/G-agarose to analyze these proteins. As shown in Fig. 1F, eluates from the anti-C3:protein A/G-agarose step revealed C3α- and C3β-chains. Interestingly, the C3α-chain was partially cleaved. In contrast, C5 protein was not recovered from mast cell culture medium by immunoprecipitation. Levels of C3 and C5 produced by skin mast cells were more precisely quantified by ELISA. Medium alone contained 0.15 ng/ml at time 0 by ANOVA. The average of four independent experiments, each one normalized to the maximal amount of C3 for that experiment. *p < 0.05, comparing values on days 1–6 with medium at time 0 by ANOVA.

**FIGURE 1.** C3 and C5 protein expression in human skin mast cells. (A) Detection of C3 and C5 in fixed and permeabilized human skin mast cells labeled with anti-C3/C3b or anti-C5/C5b Ab by flow cytometry. (B) Purified C3 and C5 compete with labeling with anti-C3/C3b and anti-C5/C5b Abs, respectively, as in (A). (C) Confocal microscopy of human fixed and permeabilized mast cells were labeled first with nonimmune mouse IgG, IgG anti-C3/C3b (C3), IgG anti-C5/C5b (C5), anti-tryptase (tryptase) Ab, and then with FITC-conjugated goat anti-mouse IgG Ab (original magnification ×500). (D) Release of β-hexosaminidase, C3, and C5 by mast cells stimulated for 15 min with 22E7 (100 ng/ml) in three replicates from two different skin preparations. (E) Western blot analysis of C3 and C5 proteins expressed by human skin mast cells. Cell extracts (2 × 10⁶ skin mast cells and 1 × 10⁶ A569 and HMC-1 cell equivalents/lane) were run in two gels. (F) C3, spontaneously secreted into the culture medium, was immunoprecipitated with rabbit IgG anti-C3 and detected by Western blotting with goat IgG anti-C3 Ab (lane 1). The α (120 kDa) and β (75 kDa) subunits of commercial C3 are shown in lane 2. (G) Unstimulated skin mast cells in culture produce C3 in a time-dependent manner. Medium from cultured skin mast cells (10⁶ cells/ml) containing 100 µg/ml soybean trypsin inhibitor was collected at the times indicated and assessed for C3 content by ELISA. Medium alone contained <0.15 ng/ml C3. Data shown are the average of four independent experiments, each one normalized to the maximal amount of C3 for that experiment. *p < 0.05, comparing values on days 1–6 of culture with medium at time 0 by ANOVA.
The effect of cytokines reported to stimulate C3 synthesis in cells other than mast cells, including IL-1α, IL-1β, IL-4, IL-6, IL-13, IL-17, TNF-α, and IFN-γ, were examined with human skin mast cells. Although exposure to PMA for 3 d significantly increased levels of C3 in the culture medium, neither IL-1α, IL-1β, IFN-γ, nor aggregation of FcεRI (22E7 mAb) had such an effect (Fig. 3A). Levels of C3 mRNA also were unaffected by stimulation with 22E7 for 4 h (data not shown). IL-6, as well as the combination of IL-1β with IL-6, also failed to affect C3 levels (Fig. 3A). TNF-α, IL-4, and IL-13 stimulation each enhanced C3 levels 2- to 3-fold above those associated with unstimulated cells (Fig. 3B, 3C). Importantly, the combination of TNF-α with IL-4 and of TNF-α with IL-13 synergistically enhanced C3 levels 9.8 ± 6.6 and 7.1 ± 5.1-fold, respectively. IL-17 had no effect on C3 levels, either by itself or in combination with TNF-α. C5 protein again was not detected in culture medium after exposure to these cytokine combinations. To confirm that the synergistic effect of TNF-α on C3 was, in fact, due to the TNF-α in these commercial preparations, we blocked TNF-α protein by either anti–TNF-α Ab or recombinant soluble TNF-αR. In each case, the synergetic effect of TNF-α and IL-4 was completely blocked by these treatments (Fig. 3D).

To examine the signaling pathways involved in cytokine-stimulated C3 production, we examined the effects of inhibitors of PI3K and MAPK (Fig. 4A). The synergistic effect of TNF-α together with either IL-13 or IL-4 was significantly, but not completely, inhibited by an Erk (U0126) inhibitor, but not by a PI3K (wortmannin), a JNK (SP600125), or a p38 MAPK (SB203580) inhibitor. JNK reportedly is involved in TNF-α signaling and Erk in IL-4/IL-13 signaling.

Because the NF-κB pathway plays a major role in TNF-α signaling, the effect of an NF-κB inhibitor (CAY10470) was examined. As shown in Fig. 4B, the synergistic effect of TNF-α

**FIGURE 2.** C3 and C5 mRNA expression in human skin mast cells. (A) RT-PCR analysis of human skin mast cells. The sizes of the products are 186 (C3), 188 (C5), and 127 bp (β-actin). Extracts of human skin mast cells, HMC-1 cells, and U937 cells were obtained and subjected to RT-PCR using the same primers and cycling parameters as for qRT-PCR, except termination occurred after 35 cycles and Taq DNA polymerase was used. (B) Relative amount of C3 and C5 mRNA levels of lung mast cells (LMC) and human skin mast cells by qRT-PCR. Levels of C3 and C5 mRNAs were measured by qRT-PCR and normalized to β-actin. U937 cells served as a positive control. Bars indicate the median values of C3 and C5 levels; error bars indicate the 95% confidence intervals. Median values of C3 or C5 were compared between skin and lung mast cells using a Mann–Whitney rank sum test.

**FIGURE 3.** Effect of cytokines on C3 synthesis by human skin mast cells. Th1, Th2, and Th17 cytokines and PMA can increase C3 production by skin mast cells. Skin mast cells (10^6 cells/ml) were treated with each stimulant for 3 d with 100 μg/ml soybean trypsin inhibitor, and C3 concentrations in culture medium were measured by ELISA. (A) Medium alone, 22E7 (10 ng/ml), PMA (10 ng/ml), IL-1α (10 ng/ml), IL-1β (10 ng/ml), IFN-γ (100 ng/ml), and IL-6 (10 ng/ml) were used as indicated, with different mast cell preparations being used in the experiments depicted to the left and right of the dashed line. (B) IL-4 (5 ng/ml) and TNF-α (10 ng/ml) were used as indicated. (C) IL-13 (100 ng/ml), IL-17 (100 ng/ml), and TNF-α (10 ng/ml) were used as indicated. (D) Anti–TNF-α Ab (5 μg/ml; isotype-matched control IgG at 5 μg/ml) and recombinant soluble TNF-αR (2.5 μg/ml) each block the synergistic effect of TNF-α (10 ng/ml) on IL-4 (5 ng/ml)-stimulated production of C3 by skin mast cells. Data shown are the average of at least three independent experiments. *p < 0.05 in (A)–(C) for comparing various triggers with medium by ANOVA and in (D) for comparing the effects of anti–TNF-α IgG, control IgG, or rTNF-αR on C3 production by IL-4 and TNF-α (data to right of dashed line).
together with either IL-4 or IL-13 was completely inhibited. Because only the IL-4Rα/IL-13Rα1 receptor is stimulated by both IL-4 and IL-13, and this receptor signals through Tyk2 and Jak1, the effect of Jak inhibitor-1 was examined. As shown in Fig. 4C, the synergistic effect of IL-4 together with TNF-α was almost completely inhibited by Jak inhibitor-1. Because Jak inhibitor-1 is promiscuous among all Jak family members, including Tyk2, the effect of a Tyk2-specific inhibitor, tyrphostin AG9, was examined. Tyrphostin AG9 also inhibited the synergistic effect of IL-4, indicating the likely involvement of IL-13Rα1–associated Tyk2 signaling. Inhibition of STAT6, a downstream signaling molecule of the IL-4 response, by PFTα also inhibited the synergy of TNF-α and IL-4. To examine whether the Jak/STAT pathway is also involved in TNF-α signaling in human skin mast cells, we examined the effect of Jak inhibitor 1 on TNF-α–mediated IL-6 synthesis; IL-6 response was significantly inhibited by 26 ± 7 and 51 ± 8% at 0.5 and 1 μM Jak inhibitor 1, respectively, indicating that TNF-α signals, in part, throw a Jak/STAT pathway (data not shown). Fig. 4D shows that the small increment in C3 synthesis caused by TNF-α stimulation was also inhibited, by only 25 and 40% at 0.5 and 1 μM Jak inhibitor 1, respectively, and these levels of inhibition were not statistically significant.

Discussion
Complement proteins and mast cells cross-interact with one another and bridge both innate and adaptive immune responses. Human mast cells of the MC_TC type, essentially the exclusive type in skin, are distinguished from the MC_T type, the dominant type in lung parenchyma and small-bowel mucosa, by expressing both tryptase and chymase instead of tryptase alone, and by being responsive to the anaphylatoxins C5a and C3a, secreting both pre-formed and newly generated mediators when exposed to these anaphylatoxins, a functional phenotype that is maintained during the serum-free culture conditions used in this study. Moreover, mast cell tryptase can generate C3a and C5a from their respective precursors. This study extends this relationship by showing for the first time, to our knowledge, that both C3 and C5 are responsive to the anaphylatoxins C5a and C3a, secreting both pre-formed and newly generated mediators when exposed to these anaphylatoxins, a functional phenotype that is maintained during the serum-free culture conditions used in this study.
Support of their localization outside of secretory granules, degranulation of skin mast cells with anti-FcεRI Ab did not stimulate secretion of C3 or C5. Instead, C3 was constitutively secreted, its concentration in culture medium steadily increasing from <0.15 ng/10^6 cell equivalents in the initial medium to 8.6 ng/10^6 cell equivalents by day 3 and ~17 ng/10^6 cell-equivalents by day 6, indicating that more C3 was secreted within a week than remained inside these cells. In contrast, C5 was not detected in the culture medium at any time point through 6 d, remaining at <0.15 ng/10^6 cells. Whether C5 might have an intracellular role or simply has a yet to be identified secretion signal remains to be determined. Secreted C3, when Western blotted, exhibited a doublet near the 26 (and granzyme B (60), resulting in C3b (αβ). Mast cell proteases other than tryptase, including membrane-bound metalloproteases, also might have a role in activating C3 and C5. Proteases in mouse Kupffer cells (44), rat alveolar macrophages, and human neutrophils (61), and in a human kidney epithelial cell line (62) process C5 or C3 in a similar manner. Although skin mast cells are typically cultured in serum-free medium with SCF for 2–3 mo before being used experimentally, these culture conditions did not account for the production of C3 and C5, because comparable mRNA and protein levels were found in freshly dispersed and purified skin mast cells. Moreover, levels of C3 secretion from freshly isolated skin mast cells were similar to those that had been in culture for 2–3 mo.

Several inflammatory cytokynes, each by themselves, are known to enhance C3 or C5 protein levels, depending on the cell type and species, including IL-1α, IL-1β, IL-4, IL-6, IL-17, TNF-α, and IFN-γ (31–34, 36–39, 63–65). Whether cytokynes might influence the amounts of C3 or C5 secreted by human skin mast cells was experimentally addressed. Unlike reported for fibroblasts, neither C5 (Fig. 3A,C) nor C5 (data not shown) levels in skin mast cells were affected by IL-1α, IL-1β, IL-17, or IFN-γ. C3 is a type I acute-phase glycoprotein, induced by IL-6 and IL-1 in combination better than by either alone (66–68), for example, in human skin fibroblasts (69, 70) or rat hepatoma cells (64). Indeed, both IL-1– and IL-6–responsive elements have been identified in human and mouse C3 promoter genes (71, 72). However, neither IL-6 nor IL-1β, nor the combination of these cytokynes, induced significant C3 production by skin mast cells, indicating response differences among distinct cell types and/or species.

IL-4, IL-13, or TNF-α modestly enhanced secreted levels of C3 (but not those of C5). However, when combinations of IL-4 or IL-13 with TNF-α were assessed, a remarkable synergism became evident, dramatically increasing secretion of C3 by mast cells (but again not affecting that of C5). The combination of TNF-α with either IL-13 or IL-4 also enhanced C3 production by human fibroblasts (38), although the mechanism was not addressed. The synergy from combining IL-13 or IL-4 with TNF-α could be blocked with either neutralizing anti–TNF-α Ab, with recombinant TNFR, or with an inhibitor of NF-κB, indicating dependence on TNF-α activity and its downstream activation of NF-κB. Because activated mast cells can produce TNF-α and IL-13, such cells may enhance local C3 production by autocrine and paracrine pathways, with paracrine pathways potentially affecting both mast cells and nonmast cells. IL-13 acts primarily on the IL-4Ra/IL-13Rα1 heterodimer, whereas IL-4 can act on this receptor, as well as the IL-4Ra/γc heterodimer. Human mast cells express both IL-4Ra/γc and IL-4Ra/IL-13Rα1 (73), with IL-4 known to enhance mediator release by different types of human mast cells (74–76). Triggering either of these receptors activates Jak1/STAT6 and the PI3K and Ras/MAPK pathways (77–80). The human C3 promoter has STAT6 binding sites (81, 82), consistent with the observed upregulation of C3 expression. The IL-13Ra1 subunit, but not the IL-4Ra or γc subunits, reportedly signal through Tyk2 (79). The IL-4/13–TNF-α synergies were attenuated with the Tyk2-specific inhibitor Tyrophostin AG9 almost as well as with the Jak inhibitor-1 (which inhibits all four members of the Jak family, including Tyk2) (78–80), indicating that the IL-4Ra/IL-13Rα1 heterodimers account for most, if not all, of the synergy added by IL-4 or IL-13 to TNF-α. However, because TNF-α signaling also involves the Jak/STAT pathway (83, 84), TNF-α may act, in part, through this pathway. It is also possible TNF-α–induced Jak/STAT activation enhances IL-4R–mediated Jak/STAT signaling. The lack of an effect by Wortmannin, SP600125, or SB203580 argues that neither PI3K, Jnk, nor p38 MAPK, respectively, is involved in the synergistic production of C3, whereas partial inhibition by U0126 indicates Erk involvement. Because TNF-α signals in part through JNK (85, 86), whereas IL-4 and IL-13 signal in part through Erk (80, 87), it is not surprising that Erk clearly contributes to this synergism, whereas the lack of effect of a Jnk inhibitor suggests TNF-α involvement does not involve Jnk.

Mast cells certainly encounter IL-4/13 and TNF-α at sites of inflammation, such as the asthmatic airways and atopic dermatitis skin, themselves being an important source of IL-13. Increased production of C3 by mast cells and conversion of C3 to C3a and C3b at these sites, perhaps by mast cell tryptase, might further modulate inflammation and immunity. Local production of C3 by dendritic cells in mice, for example, promotes Th1 development (41, 47). C3a can amplify pathologic process by increasing va- sopermeability, contracting bronchial smooth muscle, stimulating dendritic cells to modulate T cell activation, or activating mast cells and basophils, of likely importance in allergic disorders such as asthma and in autoimmune urticaria (88–98). Furthermore, C3adesArg, a metabolite of C3a, serves as an adipokine associated with obesity, type II diabetes, and coronary artery disease (99). Indeed, mast cell numbers are increased in the white adipose tissues of obese subjects (100) and the bronchial smooth muscle of asthmatics (101), providing them with the opportunity to influence disease activity by both producing C3 and, through tryptase or other proteases, converting C3 to C3a and C3adesArg. When C3 is cleaved to yield C3a, C3b presumably is also generated, exposing its reactive thioester domain, thereby allowing covalent binding to nearby proteins or carbohydrates, opsonizing those targets, enhancing their immunogenicity and their susceptibility to phago- cytosis or endocytosis (11). Thus, this newfound ability of human mast cells to produce C3 has implications for their involvement in diseases ranging from asthma to obesity.

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Disclosures
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