

The role of the mast cell in asthma: Induction of airway hyperresponsiveness by interaction with smooth muscle?

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In a recent study, the difference between asthma and eosinophilic bronchitis (a condition characterized by cough but not airway hyperresponsiveness or airflow obstruction) was infiltration of airway smooth muscle (ASM) by mast cells. Mast cells produce a variety of lipid mediators, chemokines, cytokines, and enzymes that may interact with ASM cells to cause hyperreactivity to constrictive stimuli and proliferation, and activated ASM can produce stem cell factor and other chemokines, cytokines, and growth factors that may act in recruitment, differentiation, and retention of mast cells. Mast cell infiltration of the airways in asthma is T-cell-dependent, and T_H2 cytokines from T cells and other sources act in mast cell expansion from circulating and tissue precursors. The recent data on interactions of mast cells and ASM suggest that this could be an important contributor to airway hyperresponsiveness in asthma. Why this occurs in asthma and how it is sustained remain to be established. (*J Allergy Clin Immunol* 2004;114:58-65.)

Key words: Mast cells, asthma, chemokines, airway smooth muscle

Although the mast cell has long been associated with asthma, recent studies have added to our knowledge of the biology of this intriguing cell type and raised questions about how mast cells contribute to asthma pathophysiology. In particular, Brightling et al¹ conducted a study comparing bronchial biopsy specimens from asthmatic volunteers with biopsy specimens obtained from patients with eosinophilic bronchitis (EB). EB is characterized by chronic cough and sputum eosinophilia in the absence of the airway hyperresponsiveness (AHR) and reversible airflow obstruction that characterizes asthma.² Interestingly, both groups of patients shared many of the immu-

Abbreviations used

AHR: Airway hyperresponsiveness
ASM: Airway smooth muscle
EB: Eosinophilic bronchitis
HASM: Human airway smooth muscle
MC_T: Tryptase-only mast cell
MC_{TC}: Tryptase-chymase mast cell (human)
PAR-2: Protease-activated receptor
SCF: Stem cell factor
TLR: Toll-like receptor
VCAM-1: Vascular cell adhesion molecule 1

nopathologic features considered important in asthma, namely mucosal infiltration with eosinophils, T_H2 cytokine expression, and subepithelial collagen deposition. The only difference found between the 2 conditions was increased mast cell infiltration of the airway smooth muscle (ASM) layer in asthma, which was not seen in EB.¹

MAST CELLS AND IgE IN ALLERGIC ASTHMA

Mast cells were first described as tissue resident cells with prominent granules that were characteristic of allergic diseases. The description of IgE and its association with mast cell histamine release in the Gel and Coombs type I hypersensitivity reaction formed the basis for the initial understanding of the role of mast cells in asthma and acute allergic reactions. Indeed, in the 1970s, the cross-linking of IgE on high-affinity receptors on mast cells was regarded as the mechanism for the variable airflow obstruction that occurs in asthma and was the major target for therapeutic development of drugs such as cromoglycate.³ However, asthma treatment with antihistamines and cromones was disappointing, particularly compared with responses to inhaled corticosteroids. The emergence of the eosinophil and later T_H2 hypotheses together with results from animal models then eclipsed the mast cell's role in asthma, which was considered relevant to acute symptoms and the early asthmatic response to allergen challenge, but less involved in chronic asthma.⁴ Recent data must call for a re-evaluation of such a view. Effector functions of mast cell mediators have been extensively reviewed.

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This article focuses on mast cell function in the context of interaction with ASM.

MAST CELL DEVELOPMENT AND DIVERSITY

In common with other leukocytes, mast cells develop from CD34⁺ bone marrow progenitors.⁵⁻⁷ However, unlike other leukocytes, these cells mature in the tissue and circulate as committed immature precursor forms. An obligate factor in development of the mast cell lineage is stem cell factor (SCF) acting on its receptor, Kit.⁶⁻¹¹ This is confirmed by mast cell deficiency in mouse strains deficient in Kit (W/Wv) or the membrane-bound form of SCF: the Sl/Sl^d strain.^{8,9} Mouse committed mast cell precursors were isolated as Kit^{hi}, Thy-1^{lo} from fetal blood.¹² These cells lacked the high-affinity IgE receptor (FcεR1) but expressed mRNA for mast cell proteases. They could reconstitute mast cells in W/Wv mice and gave rise to immature mast cells when cultured in the presence of SCF. Human mast cell precursors circulate at the CD34⁺Kit⁺FcεR1⁻ promastocyte stage and express CD13 but not CD14, which distinguishes them from monocytes.^{13,14}

It is suggested that there is a constitutive population of mast cells found in connective tissues, which are controlled by SCF.⁵ This constitutive mast cell population appears to have an important role in innate immunity, and can be activated via Toll-like receptor (TLR) 2 and TLR4 (mouse mast cells) or TLR1, TLR2, and TLR6 (human cells) and CD48.^{7,15,16} Although IFN-γ reduces mast cell growth, it increases expression of the high-affinity IgG receptor FcγR1, which, together with TLR signals, acts to promote TNF and β-tryptase production, both of which play roles in antibacterial defense.⁷ This is most graphically demonstrated by the rapid demise of W/Wv mice after cecal puncture.^{17,18}

The increased numbers of mast cells seen during inflammation at mucosal sites (such as the respiratory mucosa in asthma and allergic diseases) are expanded in response to SCF together with T-cell-dependent cytokines including IL-3, IL-5, IL-6, IL-9, IL-10, IL-13, and, in some situations, IL-4.⁵⁻⁷

There is now evidence of considerable diversity of mast cells in terms of protease and surface receptor expression, and this is likely controlled by the local tissue milieu including cytokines, growth factors, chemokines, adhesion molecules, and extracellular matrix proteins.

Human mast cells have been subdivided on the basis of their content of either tryptase and chymase (MC_{TC}) or tryptase only (MC_T).¹⁹ The predominant mast cell type in the lung is MC_T, although MC_{TC} is also present, and the mast cells localized to the smooth muscle layer in asthma were of the MC_{TC} phenotype.¹ Although this division is a useful concept and correlates with other functional differences, it is likely an oversimplification, because mouse mast cells show great diversity of protease expression depending on *in vitro* culture conditions or *in vivo*

location, and multiple human mast cell tryptases are described.^{20,21} The crystal structure of human β-tryptase reveals that it is a tetramer²² that depends on heparin for stabilization and activity.²³ The tetrameric structure explains relative resistance to serine protease inhibitors but also limited substrate specificity. Tryptases activate protease-activated receptors (PAR-2),²⁴ which may be important for activity on smooth muscle. In addition, they have differential activity on neuropeptides, which may favor bronchoconstriction in asthma.²⁵ Mast cell phenotype in terms of tryptase expression appears plastic, varying with the tissue localization, as most elegantly shown in studies of mast cells in the villi of the mouse intestine.²⁶ TGF-β regulates expression of the mouse tryptase mouse mast cell protease 1,²⁷ but how relative expression of tryptases and chymase in human mast cells is regulated is unknown.

Much has been learned from *in vitro* studies of human mast cell development from CD34⁺ precursor cells by using SCF with IL-6 and IL-10, which results in approximately 60% mature mast cells by 10 weeks of culture.⁵ Similar culture systems have been used to study mouse mast cells. However, there is still much to learn about the control of migration and phenotypic differentiation of mast cells in different tissue environments.

REGULATION OF MAST CELL PHENOTYPE AND TISSUE SURVIVAL IN ASTHMA

Both mouse and human studies implicate T_H2 cytokines in control of the mucosal and epithelial mast cell infiltration that characterizes asthma.⁵ Interestingly, co-culture of human cord blood-derived mast cells with SCF and IL-4 increased FcεR1 expression, IgE-mediated histamine release, and cytokine mRNA transcripts for TNF, IL-5, IL-13, CCL3 (MIP-1α), and GM-SCF (compared with culture in SCF alone), whereas IL-5 did not alter FcεR1 or IgE-mediated histamine release and modestly increased transcripts for TNF, IL-5, CCL3, and GM-SCF but not IL-13.²⁸ This work has recently been extended by gene array analysis to describe the patterns (signatures) of gene activation in response to IgE cross-linking after culture in SCF alone or with IL-4, IL-5, or IL-9.²⁹ These cultured human mast cells, like dispersed gut mast cells, did not express transcripts for IL-4. This result contrasts with the mast cells described by Brightling et al¹ infiltrating ASM in asthma: these MC_{TC} mast cells expressed IL-4.

What, then, are the specific factors regulating the recruitment of precursors from the bone marrow, expansion and survival in the tissues, and specific tissue-dependent mast cell phenotype in asthma? Chemokines may play an important role in each of these steps. Cultured human mast cell precursors expressed functional CXCR2, CXCR4, CCR3, and CCR5 at 4 weeks of culture, but only CCR3 at the mature 9-week stage.³⁰ Thus, ligands for these receptors may act in recruitment of mast cell progenitors to specific tissue sites: whether receptors are

selectively expressed by differentiating subtypes of mast cells that are thus selectively recruited or differentiation occurs from a common precursor in the tissues remains uncertain, though the latter is perhaps more likely. CCR3 expression by both cultured human mast cells and nasal polyp mast cells was further upregulated from intracellular stores by FcεRI cross-linking.³¹

Data from the CCR3 knockout mouse are complex: when animals were sensitized by intraperitoneal ovalbumin with alum adjuvant followed by inhaled challenge, there was an increase in intraepithelial mast cell numbers in the trachea of the CCR3^{-/-} mice compared with the wild-type litter mates, and AHR was increased.³² However, when the animals were sensitized by epicutaneous exposure to ovalbumin, inhaled challenge did not result in mast cell recruitment to the airway epithelium in knockout or wild-type mice, and the AHR seen in the wild-type mice did not occur in the CCR3^{-/-} mice.³³ These findings likely result from differential activation of T-cell cytokines acting on mast cell precursors, but the reasons for the observed difference require further elucidation.

Coculture of human mast cell precursors with fibroblasts results in mature mast cells of MC_{TC} phenotype, and because fibroblasts are a rich source of the CCR3 ligand CCL11 (eotaxin), it is possible that CCR3 plays a role in differentiation of the MC_{TC} phenotype.³⁴ ASM can also produce chemokines, including CCL11.³⁵ Whether this is involved in mast cell infiltration of smooth muscle in asthma and why it should differ from EB remains to be determined.

Adhesion molecules will clearly play an important role in recruitment of mast cell precursors to the airway, and possibly in selective localization and retention of mast cells with the tissues. Cultured human mast cells expressed α4β1, β7, α1β2, and αMβ2 integrins and P-selectin ligand, and bound to E-selectin, P-selectin, and vascular cell adhesion molecule 1 (VCAM-1).³⁵ Interestingly, binding to IL-4-activated human umbilical vein endothelial cells was completely blocked by antibodies to α4β1, although binding to TNF-activated human umbilical vein endothelial cells was not.³⁵ Thus, α4β1 integrin may play an important role in mast cell progenitor recruitment to the allergic asthmatic airway, where IL-4 (and IL-13) upregulate VCAM-1 expression.

Mast cell survival is dependent on SCF; however, additional factors may be involved, including binding of IgE itself to the high-affinity IgE receptor. Recent data suggest that monomeric IgE/FcεRI interactions are important for several mast cell functions, including inhibition of BCL_{xL} expression and, hence, survival.³⁶ In addition, T_H2 cytokines act in mast cell survival,⁵⁻⁷ which may thus be promoted by T_H2 cells, but also by other cells producing T_H2 cytokines in the asthmatic airway, including basophils, eosinophils, epithelial cells, fibroblasts, and smooth muscle and mast cells.

One important question for understanding regulation of tissue mast cell numbers is whether the increase seen in the airway mucosa in asthma or rhinitis results from increased

differentiation and recruitment from bone marrow, increased local development and expansion of tissue precursors, or increased survival or proliferation of an existing mast cell pool. These may not be separate processes, but, for example, factors acting in traffic of precursors from bone marrow to tissues may differ from those driving expansion and survival in the mucosa. There is remarkably little information on this subject, but 1 article does suggest an increase in circulating SCF-responsive CD34⁺ cells in peripheral blood from asthmatic children compared with normal donors.³⁷ It is of note that mast cells cultured *in vitro* from cord blood precursors are IL-5-responsive and express IL-5Rα.⁷ It has been assumed in many studies (including our own) that CD34⁺IL-5Rα⁺ cells in bone marrow, blood, or tissue represent potential eosinophil precursors,^{38,39} but it is also possible that these could be mast cell precursors, and further characterization of such cells in terms of receptor expression and plasticity of differentiation potential is required.

MAST CELL EFFECTOR FUNCTIONS IN ASTHMA

Mast cells release preformed, stored mediators and synthesize other factors *de novo* on activation. The classical type 1 hypersensitivity reaction in acute asthma or the early response to allergen challenge results from IgE cross-linking by allergen and, thus, FcεRI signaling. It is now clear that this results in release of histamine, tryptases and chymase, and heparin, and synthesis of lipid mediators including leukotriene C4 and prostaglandin D2, which have roles in bronchoconstriction, edema, and recruitment of inflammatory cells.⁶ Studies have defined additional roles for histamine in activation of inflammatory cells, dendritic cells, and T cells.⁴⁰ Further, leukotriene and prostaglandin receptors are also described: CysLT1 and CysLT2 receptors for cysteinyl leukotrienes, BLT for leukotriene B4 (LTB4), and CrT_H2 for PGD2.⁴¹⁻⁴³ Both CysLT1 and CysLT2 are expressed by mast cells, and the balance between these is differentially regulated by IL-4 and IL-5.⁴⁴ Recently, BLT receptors (for LTB4) have been implicated in mast cell-mediated CD8⁺ T-cell recruitment,⁴⁵ and the PGD2 receptor CrT_H2 is selectively expressed by T_H2-type T cells⁴³; mast cells may thus interact with T cells in asthma through histamine, leukotrienes, and PGD2. In addition, mast cell activation by IgE cross-linking results in release of preformed cytokines, chemokines, and growth factors and *de novo* synthesis of others.

It is now clear that mast cell effector function can also be stimulated by stimuli that do not cross-link IgE, including IgE binding to FcεR1, IgG receptors (FcγR1 and III activate, whereas FcγR2 is inhibitory), complement receptors, histamine-releasing factors, and TLRs.⁷ Because different mast cells express different patterns of cytokines and other mediators, it will be important to clarify whether different signals result in differential

effector functions, and which signaling cascades are involved.

MAST CELL—ASM INTERACTIONS

If localization of mast cells to the ASM is important for the asthma phenotype, how might these 2 cell types interact, and how does this contribute to the features of asthma?

First, how might ASM cells recruit, retain, and activate mast cells in asthma? It is now clear the smooth muscle cells have the capacity to produce a wide variety of proinflammatory cytokines and chemokines. For example, IL-1 β or TNF-activated human ASM (HASM) produces CCL11, CCL5 (RANTES), and CCL2 (monocyte chemoattractant protein 1) as well as GM-CSF, IL-6, CXCL8 (IL-8), and IL-11.⁴⁶⁻⁴⁹ IL-4 and IL-13 can activate HASM CCL11 production.⁵⁰ Thus, in the inflammatory setting of asthma, HASM chemokine release may act in recruitment and retention of mast cells. This may then be amplified by mast cell products (such as IL-4 or TNF). In addition, activated HASM produces both soluble and membrane-bound SCF, which may thus play an important role in recruitment, survival, and differentiation of mast cell precursors and mature mast cells: to some extent, this is constitutive and may contribute to localization of mast cells in smooth muscle reported in normal airways.^{51,52} Recent data suggest that HASM may attract mast cells through release of TGF- β in addition to SCF.⁵³ In a culture system, the HMC-1 cell line showed chemotaxis to HASM culture supernatants after these had been activated with tryptase.⁵³ Both factors have been localized to smooth muscle in asthmatic airways.

Human ASM expresses a variety of adhesion molecules that could act in recruitment and retention of mast cells and their precursors, including VCAM-1 and intercellular adhesion molecule 1.⁴⁸ More recently, a novel mast cell adhesion molecule, spermatogenic immunoglobulin superfamily (SgIGSF), was described that acts in adhesion of mouse mast cells to fibroblast cell lines and was controlled by the transcription factor MITF. Whether this or other specific mast cell adhesion pathways act during mast cell/HASM interaction will be of interest.⁵⁴

Clearly, mast cell infiltration of the ASM in asthma could lead to direct activation of airway narrowing via release of histamine, leukotrienes, or other mediators. In addition, leukotrienes may play a role in HASM proliferation and other changes in remodeling.⁵⁵ Mast cells also interact with HASM through the effects of chemokines, cytokines, proteases, and growth factors. Tryptase and TNF can both increase HASM responsiveness to histamine and other constrictors; tryptases may do this partly through activation of PAR-2 receptors.^{56,57} Transmembrane tryptase but not β -tryptase could induce AHR when administered by aerosol to mice.⁵⁸ This effect was dependent on signal transduction and activator of transcription 6 activation by IL-4 and/or IL-13. Whether this was via an effect on smooth muscle or other cell types

remains to be determined. Mast cells may also play a role in HASM proliferation through tryptases⁵⁹ and growth factors such as platelet-derived growth factor.⁶⁰ In contrast, other mast cell-derived factors such as chymase,⁶¹ heparin,⁶² and IL-4 inhibit HASM proliferation,⁶³ whereas TGF- β inhibits proliferation to some growth factors, such as EGF, but enhances responses to leukotriene D4.^{64,65} TGF- β induces HASM production of extracellular matrix proteins.^{64,66} Activin A is also released from mast cells and was recently shown to induce smooth muscle proliferation *in vitro*.⁶⁷

The interaction of mast cells and HASM may also influence a wide variety of other inflammatory and structural cells in the asthmatic airway. For example, IL-6 induced from HASM will potentiate IgE synthesis and T_H2 cell development, whereas GM-SCF and CCL11 will amplify eosinophilic inflammation. Putative interactions between mast cells and HASM are summarized in Fig 1.

MAST CELL INVOLVEMENT IN ASTHMA

Numerous human studies have demonstrated increased mast cell numbers in the airway in asthma and have detected histamine, PGD₂, and tryptase in bronchoalveolar lavage fluid both in symptomatic asthma and after allergen inhalation challenge, suggesting mast cell degranulation.⁶⁸⁻⁷⁰ Mast cell numbers in bronchoalveolar lavage and airway biopsies could be related to AHR in some studies.⁶⁸ However, it is of note that other studies did not find increased mast cell numbers in either the airway epithelium or submucosa in asthma.⁷¹ Mast cell infiltration of ASM was reported in postmortem studies of both fatal and nonfatal asthma and in isolated tracheal rings from asthmatics,^{52,72} and was the major difference between asthma and EB in the study by Brightling et al.¹

Results from a mouse model of asthma showed that AHR and tissue eosinophils could still be evoked if the mast cell-deficient W/W^v mice underwent sensitization with intraperitoneal ovalbumin with aluminium hydroxide adjuvant (which favors a T_H2 response), but not if sensitization was without adjuvant.⁷³ Thus, mast cells can cause AHR, but it can also be elicited without these cells.

Mast cell infiltration of the airway mucosa is also a feature of nonatopic asthma.⁷⁴ How mast cells might be activated without a role for allergens is uncertain, but 1 possibility is that IgE is in fact involved, because local airway IgE synthesis was detected in nonatopic as well as atopic asthma,⁷⁵ and it has been suggested that an autoallergen termed Hom s 1 might activate IgE in atopic dermatitis.⁷⁶ Alternatively, mast cells might be activated by mechanisms that do not involve IgE cross-linking. It is of note that serum total IgE concentration in nonatopic asthma is often higher than in nonasthmatic, nonatopic subjects. This raises a potential role for monomeric IgE in sensitizing mast cells for activation in nonatopic asthma.

Mast cells are well established as profibrotic cells and release a variety of growth factors.⁷⁷ These cells are thus

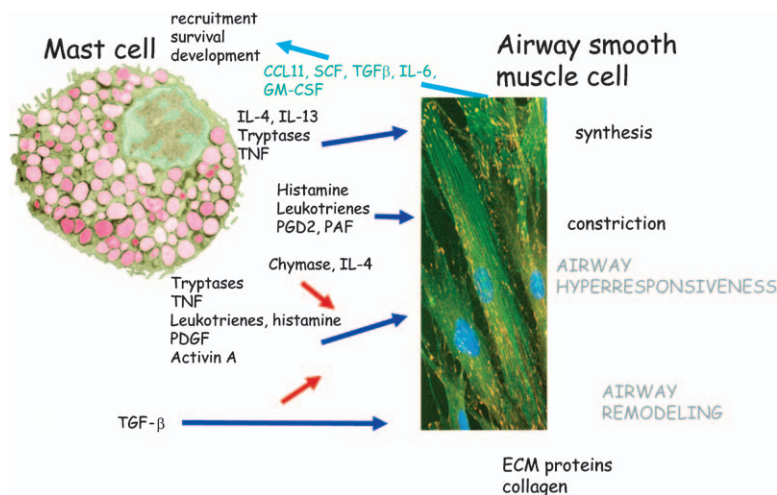


FIG 1. Mast cell/ASM cell interactions in asthma. ASM cells can produce SCF, cytokines, and chemokines active in recruitment and survival of mast cells, whereas mast cells produce mediators that directly constrict ASM, potentiate their constrictor response, cause ASM proliferation, and alter ASM to a synthetic phenotype that may contribute extracellular matrix proteins and collagens in airway remodeling. *PDGF*, Platelet-derived growth factor. Tania Vitalis and Linda Sharp/Wellcome Photo Library.

implicated in airway remodeling in asthma. The effect of airway remodeling on airway function and hyper-responsiveness remains uncertain. Subepithelial basement membrane deposition of extracellular matrix proteins occurs in both asthma and EB, and recent evidence suggests that this is at least partly dependent on IL-5 and eosinophils via TGF-β production.⁷⁸ However, patients with EB do not have AHR. Whether these patients have the smooth muscle accumulation or epithelial changes seen in asthma will be an important future question.

Clearly, other cell types play important roles in asthma pathogenesis, and much debate has occurred about the relative importance of mast cells and others cell types such as eosinophils and T cells. This partly resulted from work on the model of inhaled allergen challenge and dissection of the early and late asthmatic response. Although there is wide agreement that the early asthmatic reaction (EAR) is IgE-dependent and mast cells play a pivotal role,⁶ the late asthmatic reaction (LAR) can be IgE-dependent or IgE-independent, as seen by isolated LAR induced by intradermal injection or inhalation of allergen-derived peptide fragments that activate T cells but not IgE; there was no evidence of mast cell activation in such reactions.⁷⁹⁻⁸¹

EFFECTS OF ASTHMA THERAPY ON MAST CELLS

Steroid treatment of asthma reduces airway mast cells numbers, presumably in part through reduction in T_H2 cytokine expression. Other treatments have specific effects on mast cell function, including β₂ agonists, leukotriene antagonists, antihistamines, and cromones.⁸² More specifically, anti-IgE treatment is shown to inhibit both the early- and late-phase response to allergen challenge and reduce symptoms and steroid requirement in moderate to severe asthma.^{83,84} These effects may be

through reduction in mast cell activation (and presumably mast cell numbers in the airway, although this has yet to be confirmed), although other cells are activated through IgE. The effects of asthma treatment on mast cell infiltration of ASM will be of great interest.

REMAINING QUESTIONS

The study by Brightling et al¹ refocuses on the mast cell as an important determinant of the asthma phenotype. It is now clear that these cells are pleiotropic, multipotential, and complex. Further understanding of airway mast cell biology should open novel avenues for therapy. However, specific and pressing research questions remain:

The findings of Brightling et al¹ need to be confirmed and extended, preferably with more reliable ways of sampling ASM.

What is the situation in nonatopic asthma?

It remains to be shown why mast cells behave differently in these 2 related conditions: is this an effect of differences in mast cell or smooth muscle biology? Further analysis of airway samples is required.

Are there genetic determinants of airway mast cell-ASM interactions in asthma as opposed to other conditions?

What is the role of mast cell infiltration of HASM in airway remodeling, and what are its consequences?

What signals in and from ASM activate mast cells?

How do these changes respond to treatment?

CONCLUSION

The differences between EB and asthma in terms of HASM infiltration by mast cells, together with many mechanisms by which mast cells can stimulate and potentiate smooth muscle contraction and proliferation,

suggest that this interaction is an important determinant of AHR in asthma. It is of note that cough and other shared features of asthma and EB may be more associated with eosinophil activation. These different cell types may contribute to different aspects of airway remodeling in asthma, with HASM accumulation more dependent on mast cells, whereas extracellular matrix protein deposition may result more from eosinophil and myofibroblast activation. Future development of specific therapy to target these cell types will be of interest.

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