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Cutting Edge: Genetic Variation Influences FcεRI-Induced Mast Cell Activation and Allergic Responses

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Mast cell responses are influenced by a diverse array of environmental factors, but little is known about the effect of genetic background. In this study, we report that 129/Sv mice had high levels of circulating IgE, increased expression of the high-affinity receptor for IgE (FcεRI), and greater sensitivity to anaphylaxis when compared with C57BL/6 mice. Bone marrow-derived mast cells (BMMCs) from 129/Sv mice showed more robust degranulation upon the engagement of FcεRI. Deficiency of the Src family kinase Lyn enhanced degranulation in 129/Sv BMMCs but inhibited this response in C57BL/6 cells. C57BL/6 lyn−/− BMMCs had reduced expression of the Src family kinase Lyn, and increasing its expression markedly enhanced degranulation. In human mast cells the silencing of Lyn or Fyn expression resulted in hyperdegranulation or hypodegranulation, respectively. The findings demonstrate a genetic influence on the extent of a mast cell’s response and identify Fyn kinase as a contributory determinant. The Journal of Immunology, 2007, 179: 740–743.

The plasticity of a mast cell (MC) has long been recognized and the environmental milieu of the tissue in which the MC resides is a known contributory factor. MCs mature in the peripheral tissue, and the organs they inhabit have considerably different environments. It is thought that this adaptability provides the MC with the needed flexibility to respond appropriately to an immune challenge in the context of the particular tissue where it resides.

Although the granule protein composition (tryptases) of MCs was previously demonstrated to be different in 129/Sv versus C57BL/6 mice, one less well-studied facet is how MC responsiveness is influenced by genetics. It is well recognized that both genes and environment are key in the development of allergy and asthma. It is thought that the skewing of immune responses toward a Th2 phenotype is requisite. However, in a Th2 setting individuals with similar levels of high affinity, allergen-specific IgE Abs that react to the same allergen can, nonetheless, respond differently to a challenge. These genetically determined divergent responses are also manifest in mouse models of MC function. In this respect, we and others have reported that deficiency in the Src family protein tyrosine kinase Lyn results in increased MC responsiveness upon engagement of FcεRI, whereas other studies demonstrate no change in responsiveness or decreased responsiveness in the absence of Lyn. We hypothesized that these differences might be determined by a difference in the MC responsiveness of the two commonly used mouse strains (129/Sv and C57BL/6).

Our findings demonstrate that in vivo and in vitro responses of MCs from the 129/Sv or C57BL/6 backgrounds differed markedly. The findings may well explain the conflicting observations of the various aforementioned studies on the effects of Lyn deficiency in MC degranulation.

Materials and Methods

Abs and reagents

Abs and reagents used in this study have been described elsewhere (7, 12, 13). Biotinylated human IgE was prepared as described (14). Streptavidin was purchased from Sigma-Aldrich. Secondary Abs were previously described (13).

MCs, cell cultures, activation, lysates, immunoprecipitation, and immunoblotting

Congenic wild-type (WT) 129/SvJ and C57BL/6J mice (8–12 wk of age) and their Lyn-deficient counterparts (129/Sv-Lyn−/− and B6.129S4-Lyn−/−) were obtained from The Jackson Laboratory and used in accordance with National Institutes of Health guidelines and a National Institute of Arthritis and Musculoskeletal and Skin Diseases-approved animal study proposal. The bone marrow-derived MC (BMMC) cultures, lysate preparations, immunoprecipitations, and immunoblotts used have been described elsewhere (7, 12, 13). Human MCs (HuMCs) were developed from CD34+ cells as described (15). FcεRI stimulation of HuMCs (sensitized with biotinylated IgE) was done with the indicated concentrations of streptavidin. Lysates were prepared and proteins identified as described (16).

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3 Abbreviations used in this paper: MC, mast cell; BMMC, bone marrow-derived MC; HuMC, human MC; PLC, phospholipase; siRNA, short hairpin RNA; WT, wild type.
**Lentivirus expression and short hairpin RNA (shRNA) vector construction and gene transduction**

The construction of the lentiviral vectors carrying active and catalytically inactive forms (K296R) of Fyn kinase and shRNA for human Fyn and Lyn silencing as well as the gene transduction strategy was essentially as previously described (16). The sense and antisense oligonucleotide sequence for the targeting of Lyn (GenBank accession nos. M16038 and M79321) and Fyn (GenBank accession nos. M14333, S74774, and BC032496) were performed as described (7) with a minor modification. Rat mononuclear cells were performed essentially as previously described (17). Passive systemic anaphylaxis was determined by one-way ANOVA (129/Sv and 129/Sv mice).

**Measurement of cytotoxic calcium, degranulation, cytokine production, eosinophil migration, and anaphylaxis**

Calcium measurements on fura-2-loaded cells were previously described (16). Release (degranulation) of the granule marker β-hexosaminidase was assayed as previously described (7, 12). For cytokine secretion, a multiplex array was performed essentially as previously described (17). Passive systemic anaphylaxis was performed as described (7) with a minor modification. Rat monoclonal anti-IgE (100 μg; BD Biosciences) was used to challenge the mice to circumvent the possible inhibition of the binding of Ag-specific IgE used to sensitise the mice because of the high levels of circulating IgE in 129/Sv mice.

**Results and Discussion**

Infectious challenge of C57BL/6 or 129/Sv mice has revealed a skewing of immune responses toward Th1 or Th2, respectively (18). To assess whether this skewing was evident under non-challenge conditions, we evaluated the amount of circulating IgE and peritoneal MC FcεRI expression for both, as these are known to increase in a Th2 environment. 129/Sv mice had markedly increased serum IgE levels and increased FcεRI expression for both, as these are known to increase in a Th2 environment. 129/Sv mice had markedly increased serum IgE levels and increased FcεRI expression for both, as these are known to increase in a Th2 environment. 129/Sv mice had markedly increased serum IgE levels and increased FcεRI expression for both, as these are known to increase in a Th2 environment.
showed an increased capacity to recruit eosinophils when Lyn expression (129/Sv lyn−/−) was lost (Fig. 1E).

The difference between the in vivo responses suggested that the increased expression of FceRI, because of high IgE levels in 129/Sv vs C57BL/6, was a dominant factor in the enhanced response of 129/Sv mice. To test whether other cell-intrinsic factors might play a role, we assessed the degranulation response of in vitro cultured WT and Lyn-deficient (lyn−/−) BMMCs. No significant differences were observed, although reduced, in 129/Sv lyn−/− but not in C57BL/6 lyn−/− BMMCs (Fig. 3B). Analysis of the calcium response revealed a 2-fold difference (from 200 to ~400 nM) in the FceRI-induced intracellular calcium concentrations of C57BL/6 and 129/Sv WT BMMCs, respectively (Fig. 3C). Lyn-deficiency caused the loss of calcium responses in both backgrounds, with a slow rise in calcium as previously noted (9).

In the course of this work we discovered that C57BL/6 BMMCs expressed considerably less Fyn kinase than BMMCs derived from 129/Sv mice (Fig. 4A). Thus, we explored the possibility that this difference might contribute to the loss of degranulation in C57BL/6 lyn−/− BMMCs vs the hyperdegranulation observed in 129/Sv lyn−/− cells. Fig. 4B shows that ectopic expression of Fyn in C57BL/6 lyn−/− BMMCs restored degranulation. Three independent experiments using individual cultures showed a marked restoration of degranulation with a trend toward enhanced degranulation when compared with C57BL/6 WT BMMCs achieving significance (p < 0.05) at high Ag concentration. The restored degranulation was associated with restoration of the calcium response (Fig. 4C) to the level of the intracellular calcium concentrations observed in 129/Sv WT BMMCs (Fig. 3C). Thus, these experiments demonstrated that differences in the expression of Fyn kinase contributed to the enhanced degranulation response of 129/Sv vs C57BL/6 BMMCs. This suggests that both Lyn and Fyn are required for normal calcium responses and that the ratio of Lyn:Fyn expression may be important in determining the action of Fyn kinase and its role in degranulation.

The relevance of these conflicting findings on the degranulation in the two Lyn-deficient mouse strains was explored in cultured CD34−-derived HuMCs. Fig. 4D shows that the selective silencing of Fyn and Lyn expression in HuMCs caused suppressed and enhanced degranulation, respectively.

![FIGURE 3. Differences in the signaling and calcium responses of WT and Lyn-deficient BMMCs from C57BL/6 and 129/Sv mice. A, Phosphorylation of signaling proteins in BMMCs from WT or Lyn-deficient BMMCs. Immunoblots were performed using the indicated phospho (P)-specific Abs on proteins from unstimulated cells (0 min) or cells stimulated with Ag (50 ng/ml). Equal protein loading was determined with an Ab to actin or to the respective protein. One representative of four individual experiments is shown. Numbers are the mean fold increase in phosphorylation (for all experiments, n = 4) as determined by densitometric quantification (using NIH Image J software). B, Phosphorylation (P)-of PLCγ and PLCγ2 was determined by a phosphotyrosine immunoblot of immunoprecipitated (IP) protein. PLCγ-associated phospho-LAT was also detected. Numbers represent mean fold increase in phosphorylation (for all experiments, n = 3) as determined by densitometry. C, Calcium response of the indicated genotypes using fura 2 fluorometry. IgE-sensitized cells were stimulated with Ag (20 ng/ml) as indicated by the arrow and calcium responses were monitored for the indicated time. One representative of three experiments is shown. Ctrl, Control.](http://www.jimmunol.org/)

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FIGURE 4. Fyn kinase expression is reduced in C57BL/6 BMMCs and Fyn contributes to degranulation in mouse and human MCs. A, Cell lysates from the indicated genotypes were probed with Abs to Fyn and Lyn. Equal protein loading is indicated by the actin immunoblot. One representative of three experiments is shown. Numbers are the relative quantitation of Fyn with respect to WT cells (⁎, p < 0.05; **, p < 0.005). B, Degranulation of WT and Lyn-deficient C57BL/6 BMMCs from control lacZ-transduced, Fyn-transduced, or Lyn catalytically inactive (Fyn CI)-transduced cells. Inset shows percent inhibition of Fyn expression levels for one representative experiment. Degranulation was measured by β-hexosaminidase release using the indicated Ag doses. Data are mean ± SD from three individual experiments. Significance was determined by a Student t test relative to WT (lacZ) responses (⁎, p < 0.05; **, p < 0.005). C, Calcium response of the indicated transduced genotypes using fura 2 fluorometry. IgE-sensitized cells were stimulated or not with Ag (20 ng/ml) and monitored for the indicated time. One representative of four experiments is shown. D, CD34+–derived HuMCs were transduced with shRNA to silence Lyn (Lyn<sup>sh</sup>) and Fyn (Fyn<sup>sh</sup>) expression or with control (Ctrl) shRNA (lacZ). Immunoblots with Abs to Fyn, Lyn, and actin reveal the relative expression of these proteins in the transductants. Transduced HuMCs were sensitized with biotinylated IgE and stimulated with the indicated concentrations of streptavidin (Ag) for 30 min. Degranulation was measured by hexosaminidase release. Data are means ± SD from five individual experiments. Significance is relative to WT cells (⁎, p < 0.05; **, p < 0.001).

findings demonstrate that Fyn has a positive role in degranulation whereas Lyn is a negative regulator of degranulation in HuMCs. Our findings provide several important lessons. First, MC responses are clearly altered by the genetic makeup of the cells. We find that while the in vivo environment (high IgE levels and FcεRI expression) influences MC responsiveness (anaphylaxis), the level of Fyn expression is also a likely contributor. Second, mouse neonates of various genetic backgrounds (including C57BL/6) were shown to have a Th2 skewing of immune responses (19), suggesting that C57BL/6 mice, unlike 129/Sv or BALB/c mice, develop a Th1 skewing in later life that may result from genetic and environmental influences. This brings into question what is an appropriate genetic background for in vivo allergic models. Third, the findings suggest that the apparent controversy over whether Lyn kinase plays a negative regulatory role in MC degranulation likely results from the varying genetic makeup of the mice or cells under study. Importantly, in HuMCs we find that Fyn and Lyn have a positive and negative influence, respectively, on this response. Finally, our findings further warn that a phenotype observed in only one mouse strain may have relevance to humans.

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Disclosures

The authors have no financial conflict of interest.

References