Mutations in CCR3 render it missing in action

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Allergic diseases such as asthma, atopic dermatitis, and allergic rhinitis have become a serious cause of morbidity in the Western world. Therefore, there is an ongoing search for molecular and cellular mechanisms that contribute to the pathogenesis of allergic disease.1 Human and animal studies have identified that the accumulation of eosinophils into inflamed tissues is a hallmark of allergic diseases. Eosinophils are recruited from the bloodstream in response to locally produced chemokines and other chemotactic factors and are perceived to be major contributors to airway hyperresponsiveness and tissue damage in asthma.2 Among the most potent chemotaxis that attract eosinophils to allergic tissues are members of the eotaxin family, which bind to the chemokine receptor CCR3.2,3

CCR3 is a 7-transmembrane–spanning G-protein–coupled receptor that is highly expressed on eosinophils. Notably, CCR3 potently orchestrates eosinophil migration into inflamed tissues. Consistent with this notion, mice lacking CCR3 display substantially reduced lung eosinophilia in response to allergen challenge, and CCR3 has been shown as a key factor mediating allergen-induced skin eosinophilia and lung airway hyperresponsiveness.4,5 Mechanistically, CCR3 binds eotaxins (namely, CC chemokine ligands [CCL11/eotaxin-1, CCL24/eotaxin-2, and CCL26/eotaxin-3]), and although these chemokines are considered eosinophil-specific, various other cells implicated in allergic diseases such as mast cells, basophils, and Th2 cells can also express CCR3.3,5 Therefore, targeting CCR3 may be a potent avenue for future antiallergic therapy.6 Indeed, various CCR3 antagonists have been examined in animal experiments. For example, a recent study using a low-molecular-weight CCR3 antagonist has been reported to inhibit airway eosinophilia and subepithelial/peribronchial fibrosis induced by repeated antigen challenge in mice.7

In this issue, Wise et al8 describe the functional consequences of previously described nonsynonymous single nucleotide polymorphisms (SNPs) within the CCR3 gene. In particular, the authors demonstrate that 2 SNPs caused the retention of mutant CCR3 proteins at intracellular sites, apparently rendering the receptor unable to traffic to the outer cell membrane. This includes the T971C-SNP, which corresponds to a L324P substitution in the C-terminus of CCR3, and the T652A-SNP, leading to a C218S substitution in the fifth transmembrane domain.

In their study, the authors constructed and transfected plasmids encoding CCR3 coding regions containing 6 known SNPs into the pre-B cell line L1.2, which lacks endogenous CCR3 expression. This cell line was chosen for its ability to express exogenously transfected CCR3 molecules and its mutants, whereas other cell lines had failed in this regard. Initial screening for CCR3 expression using a flow-cytometric approach revealed that 2 of the CCR3 constructs, C218S and L324P, displayed substantially reduced surface expression of CCR3. Further analysis revealed that the decreased expression was not likely a result of reduced mRNA transcription or protein translation, because the intracellular expression levels of C218S were found to be comparable to wild-type L324P expression. Not surprisingly, functional analysis of the chemotactic responses of the mutant CCR3-bearing cells to CCL11/eotaxin-1 revealed that both C218S and L324P CCR3-expressing cells displayed substantially reduced chemotaxis in response to CCL11/eotaxin-1. Interestingly, the role of leucine 324 in the regulation of cell surface expression seems to be specific to CCR3, because a similar point mutation in position 324 of the homologous chemokine receptor, CCR1 (a receptor for CCL3/macrophage inflammatory protein 1α and others; also expressed by eosinophils), did not cause significant changes in receptor expression and/or function in L1.2 cells. The latter finding is of particular interest because it suggests that a differential regulatory mechanism of cellular/vesicular transport exists for these 2 chemokine receptors.

Previous studies have primarily focused on the processes of CCR3 trafficking after CCR3-ligand binding. CCR3 has been shown to undergo prolonged receptor internalization after incubation with various CCR3 ligands (including CCL11/eotaxin-1 and CCL5/RANTES) via a mechanism that is not dependent on intracellular signaling through G protein coupling, calcium transients, or protein kinase C.9,10 Furthermore, CCR3 internalization was not dependent on chronic ligand exposure and was not accompanied by receptor degradation.9 Wise et al8 demonstrate that under steady-state conditions, an intricate balance between CCR3 synthesis and degradation regulates the surface expression of CCR3. In an attempt to define the consequences of L324P mutation in CCR3 cellular localization, the authors examined the expression of CCR3 in cells expressing wild-type CCR3 and L324P CCR3 by using subcellular fractionation and confocal immunofluorescence imaging. Wild-type CCR3 was expressed throughout the cell in various compartments, on the basis of colocalization with organelle–specific markers: (1) the cell membrane, (2) the endoplasmic reticulum, (3) the Golgi apparatus, and (4)
lysocmes. In contrast with wild-type CCR3, L324P CCR3 was trapped at intracellular locations, as indicated by its predominant expression in Golgi and lysosomes, but was absent from the endoplasmic reticulum or cell surface. This finding indicates that the L324P mutation leads to misfolded CCR3 that is subsequently targeted to the endoplasmic reticulum for proteolytic degradation. The degradation of mutant CCR3 molecules is proposed to occur through the endoplasmic reticulum-associated degradation (ERAD) pathway. Therefore, L324 is likely an important regulatory region of CCR3 maturation and folding, which may interact with chaperones that escort mutant CCR3 molecules to late endosomes and lysosomes for degradation.

The ERAD pathway is responsible for the premature removal and degradation of another important regulatory molecule, the cystic fibrosis transmembrane regulator containing the potentially fatal ΔF508 mutation. This mutation leads to improper cystic fibrosis transmembrane regulator protein folding, which then preferentially targets it to the ERAD pathway rather than allowing it to proceed to the cell membrane. Recent studies have shown that by using specific drugs to target enzymes in the protein-folding pathway, it is possible to reconstitute the traffic of ΔF508 cystic fibrosis transmembrane regulator (CFTR) molecules to the cell surface, where the mutant CFTR molecule can function, albeit to a lesser degree than wild-type CFTR. In the case of CCR3 mutants, it is beneficial to understand how their surface expression is regulated to provide insights into how this may alter eosinophil recruitment.

Although this study reveals a novel pathway for the regulation of CCR3 surface expression (and therefore function), some caution needs to be taken because the entire study was conducted by using a transfected cell line that does not normally express CCR3 or respond to CCR3 ligands. Although CCR3 constructs are not easily expressed in most cell expression systems, it will be important to confirm these findings in CCR3-expressing cells such as eosinophils, which may exhibit differences from the transfected cell line. Given the relatively low frequencies of L324P and C218S SNPs (<1% of allele frequencies and 1/12 patients, respectively), the significance of these findings is yet to be defined. Moreover, although this is well beyond the scope of the current study, it is not clear why reduced cell surface expression of CCR3 mutants could be associated with the manifestation of disease. Future studies that are aimed at linking expression patterns of mutant CCR3 receptors will likely complement this work.

Despite these limitations, this study reveals a novel pathway for CCR3 cell surface expression. The therapeutic implications of this study may yield new strategies that are aimed at blocking cellular transporting mechanism that are specific for molecular targets expressed by cells mediating allergic reactions. Increasing our understanding of the molecular regulation and trafficking of CCR3 to the cell surface may reveal potential new targets for manipulating or reducing CCR3 expression in eosinophils, and thereby decrease the tissue-damaging effects of eosinophilic inflammation. Because reducing eosinophilic inflammation is a major goal in asthma and atopy, rendering CCR3 missing in action could provide a powerful approach to achieve this goal.

REFERENCES