located more than 92 nucleotides upstream to the 3′-most exon–exon junction (Müller *et al.*, 2006). K10 transcript levels in the normal skin of the heterozygous father were ~50% compared with the wild type (Figure 2b). Heterozygous family members are clinically unaffected, which indicates that one K10 allele is sufficient to maintain a normal phenotype.

In the previously reported homozygous EH patients (Müller *et al.*, 2006) and in the present proband, K6, K16, and K17 are upregulated in the absence of human K10 protein and show a maximal expression at the sites of cytolysis (data not shown). In addition, suprabasal persistence of the basal keratin, K14, was found in our patient and in the recessive EH patient reported by Tsubota *et al.* (2008). In the K10 knockout mouse, decreased proteolysis and suprabasal persistence of the basal keratins, K5, K14, and K15, were shown, and keratin aggregates were found to consist of residual K1 that formed atypical heterodimers with K14 (Reichelt *et al.*, 2001). Formation of instable atypical heterodimers between residual K1 and another type I keratin, for example, K16 or K17, which form relatively poor intermediate filaments compared with those built up from keratins constitutively expressed in the epidermis (Takahashi *et al.*, 1994; Paladini *et al.*, 1996), could additionally contribute to skin fragility and cytolysis in recessive EH.

In conclusion, the characteristic ultrastructural picture consisting of sparse keratin filaments and keratin clumps that show a nearly homogenous, amorphous structure should prompt a detailed analysis of the pedigree to search for parental consanguinity and a recessive inheritance. All reported mutations in recessive EH to date are located in close proximity in the 2B domain of K10 (Figure 2c), suggesting a genetic hotspot in recessive EH. Expanding the catalog of known mutations in this disorder is important with respect to molecular diagnosis and genetic counseling.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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See related commentary on pg 2549

Desloratadine Inhibits Human Skin Mast Cell Activation and Histamine Release

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TO THE EDITOR

Most symptoms of allergic disease are caused by mast cell (MC) activation and subsequent release of mediators, particularly histamine (Metz and Maurer, 2007; Metz *et al.*, 2008). Research shows that preformed histamine is stored in large amounts in cutaneous MC granules (Metz and Maurer, 2007). Currently, the most common way to treat MC-driven diseases, such as allergic

Abbreviations: Ca, calcium; MC, mast cell

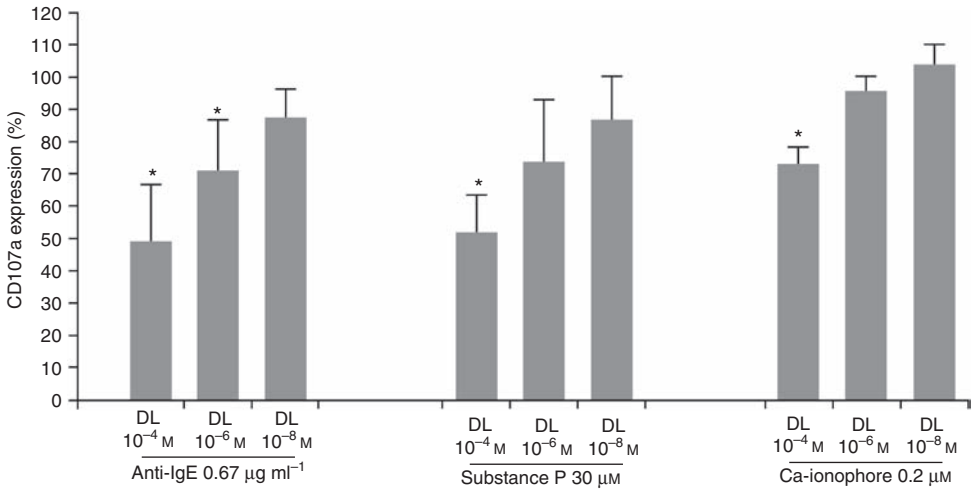
rhinitis or urticaria, is by administering H_1-antihistamines (Młynek *et al.*, 2008). Agents that inhibit activation of MCs and release of inflammatory mediators, such as histamine, that is, MC stabilizers, may provide additional benefits in these conditions (Wang *et al.*, 2005).

For the skin, agents for long-term treatment with specific MC-stabilizing properties remain to be elucidated. Of interest are recent reports, suggesting that some of the anti-allergic effects of non-sedating second-generation antihistamines, such as desloratadine, may be a result of MC-stabilizing properties (Zhao *et al.*, 2004; Kowalski *et al.*, 2005; Wang *et al.*, 2005). Desloratadine has been shown to inhibit histaminic, allergic, and inflammatory responses in murine and *in vitro* studies (Baena-Cagnani, 2001). However, because the inhibitory effects of desloratadine on human skin MC activation and mediator release have not been studied in detail, convincing data on a possible MC-stabilizing effect on allergic MC activation are scarce (Genovese *et al.*, 1997), and information on its effects on non-allergic activation of normal human MCs is lacking. Therefore, a study was conducted to explore whether desloratadine inhibits activation of human skin MCs and subsequent mediator release, whether such properties (if present) are dose dependent, and whether inhibition is limited to specific stimulators.

Mast cells were obtained by human skin collection during cosmetic surgery for which donors provided written informed consent. This study was conducted according to the Declaration of Helsinki and approved by the Institutional Review Board of the Charité–Universitätsmedizin Berlin in Germany. Purification of cells was achieved by incubation with CD117 magnetic beads and subsequent separation of labeled from unlabeled cells by passing them over the AutoMACS System (Miltenyi Biotech, Bergisch Gladbach, Germany). The isolated and purified human skin MCs were preincubated for 60 minutes with 200 µl of vehicle (PIPES albumin glucose with calcium and magnesium; Sigma-Aldrich, Munich, Germany) or desloratadine in concentrations of 1×10^{-8} to 1×10^{-4} M. All MCs were subsequently stimulated for 30 minutes with 400 µl of desloratadine or vehicle containing (final concentrations) anti-IgE (0.67 µg ml^{-1}), calcium (Ca)-ionophore (0.2 µM), or substance P (30 µM), after which expression of CD107a was detected and histamine release was quantified. An earlier study has confirmed that CD107a, also known as lysosome-associated membrane protein 1, is a previously unknown marker of MC activation and degranulation (Grützkau *et al.*, 2004). On flow cytometry of normal, resting human skin MCs, CD107a is only expressed at low levels on the cell surface. After stimulation with anti-IgE, Ca-ionophore, or substance P, intracellular CD107a is rapidly translocated to the cell membrane, and its expression can be well detected by fluorescent-activated cell sorter analysis employing anti-CD107a antibodies.

The statistical analysis for this study was based on a two-tailed Student's *t*-test for unpaired samples. The results of this analysis showed that desloratadine significantly inhibited CD107a expression on human skin MCs after stimulation with each of the three test substances (Figure 1). At the highest desloratadine concentration, the inhibitory effect was strongest in response to stimulation with anti-IgE (50.7% inhibition; $P<0.05$), followed by substance P (48.0% inhibition; $P<0.005$), and Ca-ionophore (26.7% inhibition; $P<0.01$). The absolute number of CD107a-positive cells in percentage of total cells after stimulation (set as 100%) were 50.1%, 61.3, and 59.8% for anti-IgE, substance P, and Ca-ionophore, respectively. The inhibitory action of desloratadine was dose dependent with all three test substances.

Desloratadine also significantly inhibited histamine release by human skin MCs after stimulation with each of the three test substances (Figure 2). At the highest desloratadine concentration, the inhibitory effect was strongest



*$P<0.05$ vs 100% stimulation for each test substance in the absence of desloratadine.

Figure 1. Effect of desloratadine (DL) on CD107a expression on human skin mast cells after stimulation with anti-IgE, substance P, or calcium (Ca)-ionophore. CD107a expression after stimulation of pretreated cells with each individual test substance is calculated as a percentage (mean ± SE) of release without desloratadine pretreatment (set to 100%). The results are pooled from three or four independent experiments.

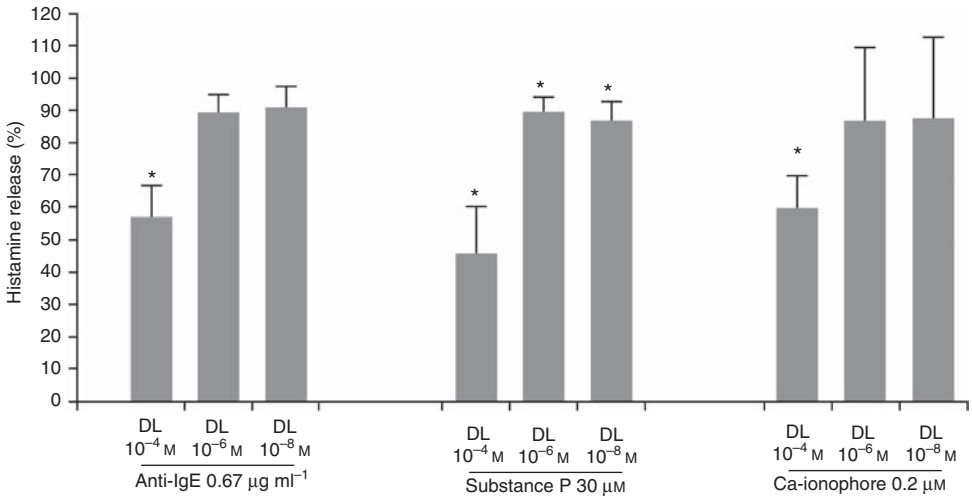


Figure 2. Effect of desloratadine (DL) on histamine release by human skin mast cells after stimulation with anti-IgE, substance P, or calcium (Ca)-ionophore. Histamine release after stimulation of pretreated cells with each individual test substance is calculated as a percentage (mean ± SE) of release without desloratadine pretreatment (set to 100%). The results are pooled from three or four independent experiments.

in response to stimulation with substance P (53.7% inhibition; $P<0.01$), followed by anti-IgE (42.6% inhibition; $P<0.005$), and Ca-ionophore (39.9% inhibition; $P<0.005$). Substance P-induced histamine release was also significantly inhibited at desloratadine concentrations of 10^{-6} M and 10^{-8} M (9.8 and 13.0% inhibition, respectively; $P<0.005$ for both). The absolute histamine release in percentage of the total cell histamine content after stimulation (set as 100%) was 21.8, 49.2, and 60.9% for anti-IgE, substance P, and Ca-ionophore, respectively.

No cytotoxicity was observed with desloratadine incubation of MCs at any of the drug concentrations (data not shown).

On the basis of these findings, we conclude that preincubation with desloratadine significantly inhibits expression of the MC-activation marker, CD107a, as well as subsequent histamine release, in primary human skin MCs. Moreover, the effects of desloratadine are not limited to IgE-dependent stimulation, but also extend to IgE-independent pathways, such as Ca-ionophore and substance P. Desloratadine can therefore be considered as an effective MC stabilizer *in vitro.* We also determined that this effect is generally dose dependent, with higher doses of desloratadine showing more potent inhibitory effects. One possible mechanism, by which desloratadine might exert its inhibitory effect on MCs is an interference either with intracellular calcium accumulation or with activation of intracellular calcium-dependent enzymes, for example, calmodulin.

Our results are in line with the earlier reports showing that the release of tumor necrosis factor by phorbol myristate acetate- or Ca-ionophore-stimulated human leukemic MCs is inhibited in a dose-dependent fashion by the antihistamines, azelastine, cetirizine, or loratadine, at concentrations that the authors concluded could be easily achieved therapeutically in the tissue (Lippert *et al.*, 2000). Although little data are available on skin concentrations of desloratadine at recommended dosing levels, one study found that skin concentrations 24 hours after a single 5-mg oral dose of desloratadine approached those that achieved significant MC stabilization in this study (Frossard *et al.*, 2008). Further investigation is warranted to clarify the cellular pathways involved in desloratadine-induced inhibition of CD107a expression and histamine release, and to assess the clinical effects of this inhibition on MC-mediated diseases of the skin.

CONFLICT OF INTEREST

Professor Marcus Maurer is or recently was an investigator, speaker, and/or advisor for Almirall Hermal, Bayer Schering Pharma, Biofrontera, Essex Pharma, Genetech, JADO Technologies, Jerini, Merckle Recordati, Novartis, Schering-Plough, Shire, Symbiopharm, UCB, and Uriach.

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The Role of Polymorphic Protein Tyrosine Phosphatase Non-Receptor Type 22 in Leprosy

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TO THE EDITOR

Leprosy, caused by *Mycobacterium leprae*, presents a spectrum of findings, with tuberculoid leprosy at one end and lepromatous leprosy at the other. Although humoral immune responses are observed throughout the spectrum, cell-mediated immunity is observed in the tuberculoid leprosy form of the disease (Ridley and Jopling, 1966). We previously showed a much stronger association of the *HLA* allele, *DRB1* 1501*, with multibacillary leprosy than with tuberculoid leprosy and a significant decrease of *DRB1*0701* in multibacillary leprosy as compared with the tuberculoid form and controls (Rani *et al.*, 1993). This suggested that *HLA* alleles may play a role in differentiating the manifestations of leprosy. However, an integrated role for genes involved in immune responses cannot be ruled out. Given that the T cells are anergic to *M. leprae* antigens in leprosy patients, we explored the role of protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) in that disease.

PTPN22 encodes for an 807 amino acid residue protein called LYP (lymphoid tyrosine phosphatase), which has been shown to negatively regulate T-cell signaling (Hasegawa *et al.*, 2004). A single-nucleotide polymorphism in the *PTPN22* gene at nucleotide position 1858 C>T (codon 620), resulting in an arginine-to-tryptophan (CGG to TGG) transition, has been shown to be a gain-of-function mutation, with a more potent negative regulation of T-cell signaling through reduced Lck (leukocyte-specific protein tyrosine kinase)-mediated phosphorylation of the TCRξ chain, reduced tyrosine phosphorylation of LAT (linker for activation of T cells), and reduced activation of Erk2 (Vang *et al.*, 2005). The mutant, LYP-Trp620, has been associated with several autoimmune diseases (Begovich *et al.*, 2004; Bottini *et al.*, 2004; Kyogoku *et al.*, 2004) Recently, Chapman *et al.* (2006) have shown its involvement in invasive pneumococcal disease and Gram-positive bacterial disease. Owing to its involvement in the downregulation of T-cell function and in invasive bacterial disease, one could hypothesize that LYP-Trp620 has a role in the manifestation of mycobacterial diseases as well.

To study the role of the *PTPN22* C1858T single-nucleotide polymorphism, 153 leprosy patients from North India—103 lepromatous patients, including borderline lepromatous patients (79 men and 24 women with a mean (±SD) age of 32.26±11.84 years), and 50 tuberculoid patients, including borderline tuberculoid patients (38 men and 12 women with a mean (±SD) age of 31.35±9.16 years)—diagnosed on the basis of immunological, histopathological, and bacteriological status, were compared with 365 ethnically matched healthy controls (191 men and 174 women with a mean (±SD) age of 36.02±10.31 years), using PCR followed by restriction digestion with the enzyme *Rsa*1, as described by Zheng and She (2005). The study was approved by the Human Ethics Committees of Ram Manohar Lohia Hospital and National Institute of Immunology. The restriction endonuclease, *Rsa*1, cleaves the DNA strand that contains the C nucleotide at the 1858th position and exhibits two bands of 176 and 42 bp. The mutant, 1858T, however, is not digested by *Rsa*1 and shows a single band of 218 bp (Supplementary Figure 1). All samples showing CT heterozygosity were repeated for both amplification and restriction digestion by another individual to confirm the genotyping. A total of 20% of the CC homozygous samples were repeated for both amplification and restriction digestion randomly, and the restriction digestion clearly showed the 176 bp band.

The frequency of the 1858T allele was significantly higher in both lepromatous ($P<0.00006$) and tuberculoid ($P<0.001$) leprosy patients than in normal healthy controls (Table 1). Although homozygous 1858TT was absent from both groups of patients, as well as from the control samples studied, heterozygous CT was significantly increased in both groups of patients (Table 1). All genotype frequencies in patients as well as in controls were in Hardy–Weinberg equilibrium. The allele frequency of the

Abbreviations: PTPN22, protein tyrosine phosphatase non-receptor type 22