Functional Chemokine Receptors in Allergic Diseases: Is CCR8 a Novel Therapeutic Target?

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Abstract: CC chemokine receptor (CCR) 8, which is expressed on Th2 cells and eosinophils, has been implicated in allergic diseases. This review represents an overview of the functional roles of CCR8 in the pathogenesis of eosinophilic inflammation and debates the potential of recently developed CCR8 antagonists to treat allergic disorders.

Keywords: Allergy, atopic dermatitis, bronchial asthma, chemokine, eosinophil, knockout mouse, Th2 cell.

PATHOGENESIS OF ALLERGIC INFLAMMATION

Eosinophilic inflammation has been recognized as a prominent pathological feature of allergic diseases such as bronchial asthma [1-3]. Accumulating evidence suggests that activated $CD4^+$ (helper) T cells are involved in the local infiltration and activation of eosinophils [1]. $CD4^+$ T cells develop into at least two distinct subsets, Th1 and Th2, based on their different functional capabilities and cytokine profiles [4, 5]. Th1 cells are characterized by secretion of IFN-γ and are adept at macrophage activation. Such cells have been demonstrated in numerous infectious disease models to activate appropriate host defenses against intracellular pathogens. Th2 cells produce IL-4, IL-5 and IL-13, and are involved in the development of humoral immunity protecting against extracellular pathogens. Th2 cells as well as Th2 cytokines are crucially implicated in the pathogenesis of allergic inflammation. $CD4^+$ T cells infiltrating inflamed tissues of allergic patients appear to have a Th2 phenotype [6]. IL-5 promotes the terminal differentiation of committed eosinophil precursors, eosinophil activation and chemotaxis, and prolongs the survival of eosinophils [7]. IL-4 is an essential factor for IgE production as it stimulates transcription at the Cε locus in B cells. This locus contains the exons encoding the constant-region domains of the IgE ε-heavy chain [8]. In addition to its IL-4-like function due to receptor overlapping, IL-13 induces mucus hypersecretion from airway epithelial cells and impacts on the contractile properties of bronchial smooth muscle [9].

Mast cells also play an important role in the development of eosinophilic inflammation. Upon triggering through antigen-captured IgE, mast cells release numerous chemical mediators that induce an immediate allergic reaction. Furthermore, they synthesize eosinophil-acting mediators including Th2 cytokines [10]. The involvement of IgE/mast cells in peripheral tissue eosinophilia has been demonstrated using animal models of allergic inflammation [11]. Taken these findings together with the fact that Th2 cytokines facilitate IgE synthesis [8], Th2 cells seem to orchestrate the development of eosinophilic inflammation by activating eosinophils directly as well as through an IgE/mast cell-dependent cascade.

CHEMOKINES AND CHEMOKINE RECEPTORS

The trafficking cascades of leukocytes including $CD4^+$ T cells throughout their life stages are tightly controlled by chemokines - structurally related chemotactic cytokines that signal through 7-transmembrane G-protein-coupled receptors. Chemokines are 70-80-amino acid proteins with well-characterized three-dimensional structures usually stabilized by two disulfide bridges, and can be divided into four subclasses: CXC, CC, C, and CX3C, depending on the arrangement of the N-terminal cysteine residues [12-14]. A redundant and promiscuous relationship is observed between the approximately 50 chemokines and 20 chemokine receptors identified thus far. Thus, a single chemokine may bind to several receptors, whereas a single chemokine receptor may bind several chemokines. The relationship is further complicated by the facts that chemokine receptors are constitutively expressed in some cell types while they are inducible in others, and that some chemokines display agonistic and antagonistic activity against different receptors [15, 16]. According to this complex relationship, chemokines specifically regulate physiological trafficking cascades of numerous leukocyte subpopulations by binding to and activating their receptors expressed on target cells. In addition, they also participate in pathological settings by recruiting leukocytes into the sites of inflammation.

CCR8 IS RELATED TO ALLERGIC INFLAMMATION

In the pathogenesis of allergic diseases, several chemokine receptors are differentially expressed in $CD4^+$ T cell subsets; CCR1, CCR5 and CXCR3 are preferentially expressed in Th1 cells. In contrast, it has been reported that Th2 cells specifically express CCR3, CCR4 and CCR8 [17-

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21]. Among these Th2 cell-specific chemokine receptors, functional roles of CCR3 and CCR4 in allergic eosinophilic inflammation have been well characterized by studies using knockout mice, blocking antibodies and specific antagonists [14, 22, 23]. On the other hand, the essential contribution of CCR8 to the development of allergic diseases has not been fully evaluated. CCR8, which was originally identified as a receptor for the CC chemokine ligand (CCL) 1 (human I-309, murine T cell-activated gene 3 (TCA-3)) [24, 25], is expressed in a variety of cell types - helper and regulatory T cells, monocyte/macrophages, NK cells and endothelial cells [26-30]. CCL1 is abundantly produced by mast cells, dendritic cells and endothelial cells *in vivo* [31]. Stimulation with CCL1 induces Ca^{2+} influx and accordingly, chemotaxis in CCR8-expressing cells [24, 25]. CCR8 also has the potential to associate with CCL16 and CCL17 [29, 32], although the reliability and physiological significance of these interactions have not been confirmed. Importantly, CCR8 is selectively induced in antigen-activated eosinophils and can regulate migration and activation of these cells [33], suggesting a crucial role of this receptor in eosinophilic inflammation.

CCR8 IN MOUSE ASTHMA MODELS

Nevertheless, the contribution of CCR8 to the pathogenesis of allergic inflammation, especially in bronchial asthma is controversial. Expression of CCR8 as well as CCL1 is elevated in inflamed lung tissue in murine models of asthma [34-36]. However, studies using CCR8-deficient mice demonstrated either an important or dispensable role of CCR8 in allergic airway inflammation. The first study reported by Chensue *et al*. showed that *Schistosoma mansoni* soluble egg antigen-induced granuloma formation as well as ovalbumin and cockroach antigen-induced airway inflammation were defective, whereas a prototypical Th1 response elicited by *Mycobacteria bovis* purified protein derivative was intact in $CCR8^{-/-}$ mice [37]. In contrast, no impairment of allergic inflammation, including lung, peripheral blood and bone marrow eosinophilia, and Th2 cytokine levels produced in the lung and serum was detectable in $CCR8^{-/-}$ mice generated by Chung *et al*. and Goya *et al* [35, 38]. Goya *et al*. also demonstrated that administration of blocking antibodies against CCR8 and CCL1 failed to affect the eosinophilic inflammation [38], whereas modest downregulation of eosinophil recruitment in the lung by blockade of CCL1 was observed by Bishop and Lloyd [36]. It is hard to explain the discrepancy in these results, though it might be due, at least in part, to differences in protocols and the genetic background of the mice used. As CCL1/CCL8 interaction may participate in the development of eosinophilic inflammation in some murine models of asthma, a critical role of CCR8 in human allergic disease cannot be completely expected from this evidence.

CLINICAL INVESTIGATION OF CCR8

Several clinical studies have investigated the functional role of CCR8 in human allergic diseases. As the expression of CCR8 in Th2 cells in human peripheral blood has not been identified, analysis of endobronchial biopsies from asthmatic patients demonstrated that the expression of CCR8 in T cells was upregulated upon segmental antigen provocation [39]. In addition, the number of $CCR8^{+}$ T cells in the airway mucosa correlated with the degree of airflow limitation during the late-phase reaction. Human mast cells produced CCL1 upon triggering though IgE [31, 40], even though expression of CCL1 was not identified in the antigen-challenged airway mucosa or epithelial cells of asthmatic patients [39]. In contrast to bronchial asthma, the levels of CCL1 in skin and blood are significantly upregulated in atopic dermatitis [31]. The majority of T cells in human skin express CCR8 and respond to CCL1 [41, 42]. These findings, taken together with data from mouse studies, imply that the CCL1/CCR8 axis might play a more important role in the pathogenesis of human atopic dermatitis, rather than bronchial asthma.

CCR8 ANTAGONISTS

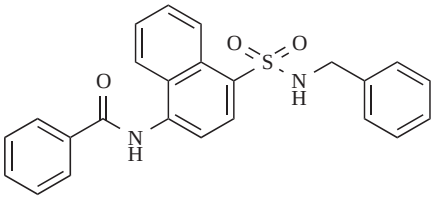
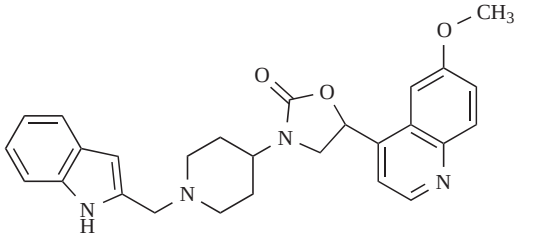
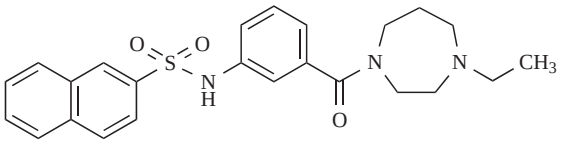
The viral CC chemokine, MC148, encoded by the poxvirus molluscum contagiosum (MCV) was first identified as a natural antagonist against human CCR8 [43]. MC148 blocked CCL1/CCR8 binding (IC50 = 0.27 nM) and inhibited CCL1-induced Ca^{2+} mobilization and chemotaxis of CCR8-transfected HEK293 cells. High specificity of MC148 for CCR8 was confirmed by comparative analysis employing a panel of 16 chemokine receptors [43].

Only a few low-molecular weight chemical CCR8 antagonists have been developed to date. Millennium Pharmaceuticals, Inc. and GlaxoSmithKline, Inc. have disclosed patents for CCR8 antagonists (Table **1**). Compounds of Millennium, naphthylsulfonamide derivatives, have been claimed to have efficacy for the treatment of asthma, atopic dermatitis, allergic rhinitis, systemic anaphylaxis, allergies, urticaria, rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, myasthenia gravis, juvenile onset diabetes, glomerulonephritis, autoimmune thyroiditis, graft rejection and Behçet's disease. They displayed inhibitory activity against CCL1 binding to CCR8 expressed on L1.2 cells (Ki = 0.5 to 30 μM). The selectivity of these compounds against other chemokine receptors has not been described. Compounds of GlaxoSmithKline, oxazolidin-2-one derivatives and bicyclicaryl-benzensulfonamide derivatives, have been claimed to treat asthma and other respiratory disorders including bronchitis, bronchiectasis, chronic obstructive pulmonary disease, cystic fibrosis, sinusitis and rhinitis, although the biological activity of these compounds has not been published. Considering that these patents were disclosed in 2004, it is most likely that many other CCR8 antagonists are currently under development and will be released hereafter.

DIFFERENCES AMONG SPECIES

Investigation of the *in vivo* effects of CCR8 antagonists on allergic inflammation is currently awaited. However, as with many therapeutic drugs, differences among animal species may become an obstacle to evaluate the potency of CCR8 antagonists to treat human allergic diseases. In the case of CCR3, more than 10 pharmaceutical companies have developed CCR3 antagonists and most of them displayed

Table 1. CCR8 Antagonists

Name	Company	Representative Compound	Effect	Reference
MC148	ICOS	MCV-derived chemokine	Ki = 0.27 μM CCL1/CCR8 binding	[42]
CCR8 inhibitors	Millennium		Ki = 0.5-30 μM CCL1/CCR8 binding	WO 0458709 WO 0458736
Compounds	GlaxoSmithKline	 	No description	WO 0432856 WO 0473619 WO 0474438

potent inhibitory activity against CCL11/CCR3 binding, CCL17-mediated Ca^{2+} influx and chemotaxis [23]. However, only a few compounds were shown to suppress allergic inflammation in animal studies. The compounds A-122057 and A-122058 of Abbott Laboratories reduced CCL11-induced eosinophil infiltration into the peritoneal cavity upon oral administration at a dose of 10 mg/kg,, though the effective dose used appears much higher than that estimated from their *in vitro* activity [23]. Potent and selective antagonistic activity against human CCR3 was demonstrated with compounds SB-297006 and SB-328437 of GlaxoSmithKline [44]. However, they displayed less than 1/10,000-fold inhibitory activity against binding of mouse and guinea pig CCL11 to CCR3. Also, in regard to CCR8, MC148 was unable to bind and block responses through murine CCR8 [45].

Differences in the effects or potency of CCR antagonists among animal species are mainly due to their inflexible structure and low sequence homology. The three-dimensional structure of CCRs is highly restricted, as they pass through the plasma membrane 7 times. In addition, amino acid sequences of mouse and guinea pig CCR3 are only 70% and 68% identical to that of human CCR3, respectively. Considering the case of CCR3, the effects of CCR8 antagonists on murine models of allergic inflammation may prove to be disappointing, since the homology between human and mouse CCR8 is 71% (Fig. **1**). On the other hand, CCR8 of rhesus monkey is highly identical (94%) to

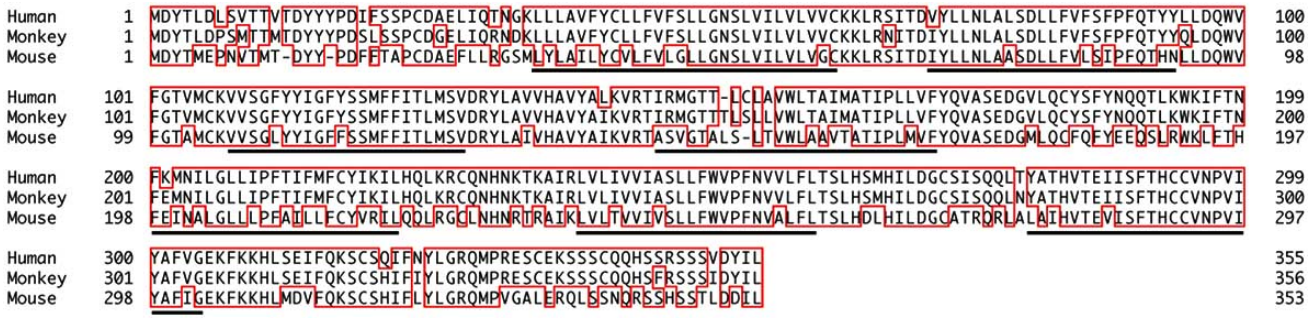


Fig. (1). Homology of CCR8.

Amino acid sequences of human, rhesus monkey and mouse CCR8 are aligned. Identical residues are enclosed. Predicted transmembrane domains are underlined.

that of humans, suggesting that *in vivo* studies using this species will be useful to evaluate the efficacy of inhibitors against CCR8, and potentially other CCRs, for the treatment of human allergic diseases.

CONCLUDING REMARKS

Eosinophilic inflammation is a characteristic feature of allergic diseases and is associated with the differentiation of helper T cells to a Th2 phenotype. CCR8, expressed in Th2 cells and eosinophils, seems to have a functional role in eosinophilic inflammation. However, studies using CCR8-deficient mice have confounded the contribution of this receptor to the pathogenesis of bronchial asthma. On the other hand, recent studies have implied that CCL1/CCR8 interaction may be more important for the development of atopic dermatitis. To date, a few CCR8 antagonists have been developed as potential anti-allergic drugs. Investigation of the *in vivo* effects of these compounds on allergic inflammation, in consideration of the homology of this receptor among animal species, is required in order to elucidate the ability of CCR8 inhibitors to treat allergic diseases.

REFERENCES

[1] Corrigan, C.J.; Kay, A.B. *Immunol. Today*, **1992**, *13*, 501.
[2] Azzawi, M.; Bradley, B.; Jeffery, P.K.; Frew, A.J.; Wardlaw, A.J.; Knowles, G.; Assoufi, B.; Collins, J.V.; Durham, S.; Kay, A.B. *Am. Rev. Respir. Dis.*, **1990**, *142*, 1407.
[3] Bentley, A.M.; Maestrelli, P.; Saetta, M.; Fabbri, L.M.; Robinson, D.S.; Bradley, B.L.; Jeffery, P.K.; Durham, S.R.; Kay, A.B. *J. Allergy Clin. Immunol.*, **1992**, *89*, 821.
[4] Mosmann, T.R.; Cherwinski, H.; Bond, M.W.; Giedlin, M.A.; Coffman, R.L. *J. Immunol.*, **1986**, *136*, 2348.
[5] Mosmann, T.R.; Coffman, R.L. *Annu. Rev. Immunol.*, **1989**, *7*, 145.
[6] Romagnani, S. *Immunol. Today*, **1991**, *12*, 256.
[7] Hamelmann, E.; Gelfand, E.W. *Immunol. Rev.*, **2001**, *179*, 182.
[8] Oettgen, H.C.; Geha, R.S. *J. Clin. Invest.*, **1999**, *104*, 829.
[9] Wills-Karp, M. *Immunol. Rev.*, **2004**, *202*, 175.
[10] Gordon, J.R.; Burd, P.R.; Galli, S.J. *Immunol. Today*, **1990**, *11*, 458.
[11] Coyle, A.J.; Wagner, K.; Bertrand, C.; Tsuyuki, S.; Bews, J.; Heusser, C. *J. Exp. Med.*, **1996**, *183*, 1303.
[12] Rossi, D.; Zlotnik, A. *Annu. Rev. Immunol.*, **2000**, *18*.
[13] Rot, A.; von Andrian, U.H. *Annu. Rev. Immunol.*, **2004**, *22*, 891.
[14] Garcia, G.; Godot, V.; Humbert, M. *Curr. Allergy Asthma Rep.*, **2005**, *5*, 155.
[15] Loetscher, P.; Pellegrino, A.; Gong, J.H.; Mattioli, I.; Loetscher, M.; Bardi, G.; Baggiolini, M.; Clark-Lewis, I. *J. Biol. Chem.*, **2001**, *276*, 2986.
[16] Loetscher, P.; Clark-Lewis, I. *J. Leukoc. Biol.*, **2001**, *69*, 881.
[17] Sallusto, F.; Mackay, C.R.; Lanzavecchia, A. *Science*, **1997**, *277*, 2005.
[18] Zingoni, A.; Soto, H.; Hedrick, J.A.; Stoppacciaro, A.; Storlazzi, C.T.; Sinigaglia, F.; D'Ambrosio, D.; O'Garra, A.; Robinson, D.; Rocchi, M.; Santoni, A.; Zlotnik, A.; Napolitano, M. *J. Immunol.*, **1998**, *161*, 547.
[19] D'Ambrosio, D.; Iellem, A.; Bonecchi, R.; Mazzeo, D.; Sozzani, S.; Mantovani, A.; Sinigaglia, F. *J. Immunol.*, **1998**, *161*, 5111.
[20] Bonecchi, R.; Bianchi, G.; Bordignon, P.P.; D'Ambrosio, D.; Lang, R.; Borsatti, A.; Sozzani, S.; Allavena, P.; Gray, P.A.; Mantovani, A.; Sinigaglia, F. *J. Exp. Med.*, **1998**, *187*, 129.
[21] Loetscher, P.; Uguccioni, M.; Bordoli, L.; Baggiolini, M.; Moser, B.; Chizzolini, C.; Dayer, J.M. *Nature*, **1998**, *391*, 344.
[22] Lloyd, C.M.; Rankin, S.M. *Curr. Opin. Pharmacol.*, **2003**, *3*, 443.
[23] Elsner, J.; Escher, S.E.; Forssmann, U. *Allergy*, **2004**, *59*, 1243.
[24] Roos, R.S.; Loetscher, M.; Legler, D.F.; Clark-Lewis, I.; Baggiolini, M.; Moser, B. *J. Biol. Chem.*, **1997**, *272*, 17251.
[25] Tiffany, H.L.; Lautens, L.L.; Gao, J.-L.; Pease, J.; Locati, M.; Combadiere, C.; Modi, W.; Bonner, T.I.; Murphy, P.M. *J. Exp. Med.*, **1997**, *186*, 165.
[26] Haque, N.S.; Fallon, J.T.; Taubman, M.B.; Harpel, P.C. *Blood*, **2001**, *97*, 39.
[27] Haque, N.S.; Zhang, X.; French, D.L.; Li, J.; Poon, M.; Fallon, J.T.; Garbel, B.R.; Taubman, M.B.; Koschinsky, M.; Harpel, P.C. *Circulation*, **2000**, *15*, 786.
[28] Iellem, A.; Mariani, M.; Lang, R.; Recalde, H.; Panina-Bordignon, P.; Sinigaglia, F.; D'Ambrosio, D. *J. Exp. Med.*, ***2001***, *194*, 847.
[29] Inngjerdingen, M.; Damaj, B.; Maghazachi, A.A. *J. Immunol.*, **2000**, *164*, 4048.
[30] Bernardini, G.; Spinetti, G.; Ribatti, D.; Camarda, G.; Morbidelli, L.; Ziche, M.; Santoni, A.; Capogrossi, M.C.; Napolitano, M. *Blood*, **2000**, *96*, 4039.
[31] Gombert, M.; Dieu-Nosjean, M.C.; Winterberg, F.; Bunemann, E.; Kubitza, R.C.; Da Cunha, L.; Haahtela, A.; Lehtimaki, S.; Muller, A.; Rieker, J.; Meller, S.; Pivarcsi, A.; Koreck, A.; Fridman, W.H.; Zentgraf, H.W.; Pavenstadt, H.; Amara, A.; Caux, C.; Kemeny, L.; Alenius, H.; Lauerma, A.; Ruzicka, T.; Zlotnik, A.; Homey, B. *J. Immunol.*, **2005**, *174*, 5082.
[32] Nomiyama, H.; Hieshima, K.; T., N.; Sakaguchi, T.; Fujisawa, R.; Tanase, S.; Nishiura, H.; Matsuno, K.; Takemori, H.; Tabira, Y.; Yamamoto, T.; Miura, R.; Yoshie, O. *Int. Immunol.*, **2001**, *13*, 1021.
[33] Oliveira, S.H.; Lisa, S.; Martines, A.C.; Wiekowski, M.; Sullivan, L.; Lukacs, N.W. *J. Leukoc. Biol.*, **2002**, *71*, 1019.
[34] Mathew, A.; MacLean, J.A.; DeHaan, E.; Tager, A.M.; Green, F.H.; Luster, A.D. *J. Exp. Med.*, **2001**, *193*, 1087.
[35] Chung, C.D.; Kuo, F.; Kumer, J.; Motani, A.S.; Lawrence, C.E.; Henderson Jr, W.R.; Venkataraman, C. *J. Immunol.*, **2003**, *170*, 581.
[36] Bishop, B.; Lloyd, C.M. *J. Immunol.*, **2003**, *170*, 4810.
[37] Chensue, S.W.; Lukas, N.W.; Yang, T.Y.; Shang, X.; Frait, K.A.; Kunkel, S.L.; Kung, T.; Wiekowski, M.T.; Hedrick, J.A.; Cook, D.N.; Zingoni, A.; Narula, S.K.; Zlotnik, A.; Barrat, F.J.; O'Garra, A.; Napolitano, M.; Lira, S.A. *J. Exp. Med.*, **2001**, *193*, 573.
[38] Goya, I.; Villares, R.; Zaballos, A.; Gutierrez, J.; Kremer, L.; Gonzalo, J.-A.; Varona, R.; Carramolino, L.; Serrano, A.; Pallares, P.; Criado, L.M.; Kolbeck, R.; Torres, M.; Coyle, A.J.; Gutierrez-Ramos, J.-C.; Martinez-A, C.; Marques, G. *J. Immunol.*, **2003**, *170*, 2138.
[39] Pania-Bordignon, P.; Papi, A.; Mariani, M.; Di Lucia, P.; Casoni, G.; Bellettato, C.; Buonsanti, C.; Miotto, D.; Mapp, C.; Villa, A.; Arrigoni, G.; Fabbri, L.M.; Singaglia, F. *J. Clin. Invest.*, **2001**, *107*, 1357.
[40] Gilchrest, H.; Cheewatrakoolpong, B.; Billah, M.; Egan, R.W.; Anthes, J.C.; Greenfeder, S. *Life Sci.*, **2003**, *73*, 2571.
[41] Schaerli, P.; Ebert, L.; Williamann, K.; Blaser, A.; Roos, R.S.; Loetscher, P.; Moser, B. *J. Exp. Med.*, **2004**, *199*, 1265.
[42] Colantonio, L.; Iellem, A.; Singaglia, F.; D'Ambrosio, D. *Eur. J. Immunol.*, **2002**, *32*, 3506.
[43] Luttichau, H.R.; Stine, J.; Boesen, T.P.; Johnsen, A.H.; Chantry, D.; Gerstoft, J.; Schwartz, T.W. *J. Exp. Med.*, **2000**, *191*, 171.
[44] White, J.R.; Lee, J.M.; Dede, K.; Imburgia, C.S.; Jurewicz, A.J.; Chan, G.; Fornwald, J.A.; Dhanak, D.; Christmann, L.T.; Darcy, M.G.; Widdowson, K.L.; Foley, J.J.; Schmiidt, D.B.; Sarau, H.M. *J. Biol. Chem.*, **2000**, *275*, 36626.
[45] Luttichau, H.R.; Gerstoft, J.; Schwartz, T.W. *J. Leukoc. Biol.*, **2001**, *70*, 277.

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