The Toxicology of Chemokine Inhibition

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Abstract: The dividing line between essential physiological inflammatory processes and excessive pathological inflammation is often very thin – in some circumstances, indeed, it may be non-existent. Devising anti-inflammatory medications that effectively target only the pathological component therefore remains a central challenge for the pharmaceutical industry. At present, the general rule is that the more powerful the anti-inflammatory effect of a drug, the greater the side-effects that accompany it. Steroids, for example, are potent anti-inflammatory medications, but they have a diverse array of side effects that substantially limit their use. Since chemokines play a central role in regulating the immune system, and in particular, the trafficking of leukocytes, inhibiting their action may represent a powerful new therapeutic strategy for treating diseases with an inflammatory component. However, this potential will only be realized if it is possible to interfere with chemokine signaling networks, without inducing unacceptable side effects. Although very little, direct human toxicology has been carried out using chemokine inhibitors, there is now a sufficient body of indirect and circumstantial evidence (for example, from genetically modified mice and from animal model studies using chemokine inhibitors) to allow a tentative assessment of the biological impact of chemokine inhibition. The purpose of this review is to outline the available data and to speculate on the likely toxicological profile resulting from chemokine inhibition. The tentative conclusion is that anti-inflammatory therapy achieved through chemokine inhibition may have fewer side effects than originally expected, even when the actions of multiple chemokines are inhibited simultaneously.

INTRODUCTION

The immune system is a highly complex network of intercommunicating cell types. Numerous chemical and protein signaling molecules are required to tightly regulate the temporal and spatial distribution of the immune system cells, ensuring that they are able to perform their essential function of host defense, while minimizing the collateral damage to the host. Increasing the complexity still further, many immune cells have been co-opted to perform physiological duties unrelated to host defense. For example, monocyte-derived macrophages in bone, generally termed osteoclasts, have the specialist function of resorbing mineralized bone matrix [1]. Interfering with immune cell function is therefore likely to be a fairly dangerous business: not only will host defense likely be compromised, but a range of other unrelated physiological processes may be knocked out of balance.

Corticosteroids, which are perhaps the most powerful anti-inflammatory medication in the physician's toolbox, have such a diverse range of side-effects that their use (at least at higher doses or for an extended period) is limited to the treatment of severe, or even life-threatening, inflammatory conditions. Side-effects from chronic steroid use are not limited to impaired immune function (which results in increased opportunistic infection rates), but also includes behavioral changes, myopathy and other changes in body composition, disruption of fluid and electrolyte balance, dermatological problems, osteoporosis and increased risk of peptic ulcers [2]. Many of these effects are particularly severe in children, with growth retardation and failure to thrive being the major complications [3]. An important goal, therefore, in anti-inflammatory drug research is the identification of compounds with similar efficacy to steroids, but with a reduced side-effect profile.

The molecular mechanisms which result in the anti-inflammatory activity of steroids are complex, involving a plethora of transcriptional changes in a wide variety of cell types [2]. It is likely that these changes in transcription patterns are responsible for both the beneficial immunomodulatory effects, and also many of the undesirable side-effects associated with steroid use. Importantly, however, in contrast to many non-steroidal anti-inflammatory agents (such as cyclooxygenase inhibitors), steroids profoundly suppress leukocyte recruitment to sites of inflammation, rather than simply suppressing leukocyte function after recruitment has occurred. It is likely that this suppression of leukocyte recruitment contributes substantially to the broad benefits of steroid treatment in diseases such as asthma, psoriasis and autoimmune disorders [2]. Consequently, other strategies to inhibit leukocyte recruitment, but without the wide-ranging transcriptional modulation associated with steroids, offer the promise of powerful, yet safe, anti-inflammatory efficacy.

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1389-5575/05 $50.00+.00

Table 1. Phenotype of Chemokine Receptor Knockout Mice. The Effects of Chemokine Receptor Deficiency on Leukocyte Recruitment and Immune System Organization are Highlighted in Blue, the Effects on Models of Human Diseases in Green, and the Effects on Susceptibility to Infection in Red

CCR receptors		
CCR1	Reduced NK cell recruitment	9
	Impaired granulomatous inflammation, associated with Th1/Th2 cytokine imbalance	10
	Prolonged allograft survival in models of transplantation	11
	Resistance to experimental autoimmune encephalitis model of multiple sclerosis	12
	Decreased pancreatitis-associated lung injury	13
	Increased glomerular injury in nephrotoxic nephritis	37
	Increased susceptibility to infection with *Aspergillus fumigatus* and *Toxoplasma gondii*	14,15
CCR2	Reduced migration of Langerhans cells to draining lymph nodes	50
	Reduced atherosclerosis in apoE-deficient mice	16,17
	Reduced airway hypersensitivity after antigen challenge	51
	Resistance to experimental autoimmune encephalitis model of multiple sclerosis	18
	Increased glomerular injury in nephrotoxic nephritis	38
	Increased airway allergic inflammation in response to *Aspergillus* spp.	52
	Increased susceptibility to pulmonary *Cryptococcus* infections, and inability to clear *Listeria monocytogenes* infection	19,20
CCR3	Reduced number of eosinophils in small intestine under basal conditions, with corresponding increase in spleen	53
	Reduced eosinophil recruitment to lung following antigen challenge in models of asthma	53
CCR4	Resistance to LPS-induced endotoxic shock	54
CCR5	Enhanced delayed-type hypersensitivity reaction	21
	Increased susceptibility to pulmonary *Cryptococcus* infections, and inability to clear *Listeria monocytogenes* infection	21,22
CCR6	Absence of dendritic cells from sub-epithelial dome of Peyer's patches, associated with reduced humoral response to oral antigens (although response to sub-cutaenous antigen presentation is normal)	24
	Increase in specific T cell subsets (CD4+ CD8+ ab- TCR cells) in the mucosa	24
	Reduced eosinophil recruitment to lung, and reduced airway hypersensitivity, as well as reduced IL-5 amd serum IgE in cockroach antigen model of allergic pulomonary inflammation	27
CCR7	Altered secondary lymphoid organ structure, associated with reduced humoral response to antigens	25,26
	Reduced delayed-type hypersensitivity	26
CCR8	Defective Th2 immune response in antigen-induced granuloma formation and allergic lung inflammation	28
CCR9	Decreased Tcell populations in epithelial tissues	55
CXCR receptors		
CXCR1	Reduced neutrophil recruitment and granuloma formation	29
CXCR2	Lymphadenopathy, splenomegaly with increased B cell numbers amd reduced neutrophil recruitment	30,56
	Reduced atherosclerosis in LDL-receptor deficient mice	31
	Delayed re-epithelialisation following wounding	57
	Increased susceptibility to infection with *Toxoplasma gondii* and to urinary tract infections	32,33
CXCR3	No observable phenotype without infective challenge	23
	Prolonged allograft survival in models of transplantation	23
CXCR4	Non-viable embryos, with defects in vasculogenesis, haematopoiesis and neuronal migration	34, 35
CXCR5	Defects in B cell homing and secondary lymphoid structure – defective lymph nodes, abnormal germinal centre formation in spleen and absence of Peyer's patches	36
CX3CR receptors		
CX3CR1	Reduced NK cell recruitment	58

One such approach is to inhibit the function of the signaling molecules which are responsible for directing leukocyte trafficking. A wide range of molecules of various classes (including interleukins, interferons, chemokines, classical chemoattractants, prostanoids and other lipid mediators) provide the necessary complexity to control leukocyte movement in an exquisite, temporal and spatial dance to maintain the normal functioning of the immune system. Such complexity provides both an opportunity and a hurdle to anti-inflammatory drug design: precise control might be achievable through delicate, carefully chosen intervention, but understanding which signals to inhibit or augment to achieve a particular outcome might prove challenging.

Among these families of signaling molecules, the chemokines (a superfamily of 8-12 kDa proteins structurally related to interleukin-8) represent a particularly attractive target. The superfamily consists of more than 50 ligands signaling through more than 20 receptors, which are members of the G-protein coupled receptor class [4]. Genetic knockout studies and in vivo neutralization experiments suggest that this chemokine signaling network plays a central role in directing leukocyte migration, both during basal trafficking and in response to localized inflammatory stimulation. Appropriate modulation of chemokine signaling might, therefore, realize the goal of blocking spatially, temporally or quantitatively inappropriate leukocyte recruitment, while leaving basal trafficking largely in tact.

As a result, there have been a number of attempts to generate chemokine inhibitors, which can be divided broadly into two groups: receptor-specific antagonists which block the function of one or a small group of chemokines signaling through one or two closely-related receptors [5]; and broad-spectrum chemokine inhibitors (BSCIs) which can block the function of many, if not all, chemokines simultaneously [6]. The specific antagonists offer the prospect of delicate control of the recruitment of specific leukocyte subsets under particular conditions, but the inherent redundancy of the system means that the impact of specific receptor blockade may be subtle. In contrast, the BSCIs offer the prospect of greater impact on a redundant system of parallel signals, but with the attendant loss of fine control and the possibility of unwanted side-effects.

TOXICOLOGY OF CHEMOKINE RECEPTOR ANTAGONISTS

Although preclinical and early clinical studies are now well underway with a range of small molecule antagonists of chemokine receptors (most notably the CCR1 antagonist

Table 2. Phenotype of Chemokine Ligand Knockout Mice. The Effects of Chemokine Ligand Deficiency on Leukocyte Recruitment And Immune System Organization are Highlighted in Blue, the Effects on Models of Human Diseases in Green, and the Effects on Susceptibility to Infection in Red

CC Chemokines			
CCL2	MCP-1	Reduced atherosclerosis in apoE-deficient mice	59,60
		Reduced macrophage infilitration in the blood vessel wall	61
		Significant delay in re-epithelialisation following wounding	62
		Ateenuated monocyte influx in thioglycollate-induced peritonitis and in delayed-type hypersensitivity response	63
CCL3	MIP-1α	Reduced inflammation following viral infection	39
		Increased susceptibility to infection with paramyxovirus, *Aspergillus* spp. and *Klebsiella pneumoniae*	40-42
		No effect on re-epithelialisation following wounding	62
		No observable phenotype without infective challenge	64
CCL5	RANTES	Reduced delayed-type hypersensitivity response	65
CCL11	Eotaxin	No effect on eosinophil accumulation in lung inflammation	66
		No effect on eosinophil accumulation or airway hyper-reactivity in models of asthma	67
CXC Chemokines			
CXCL10	IP-10	Impaired T cell proliferation and IFN-γ secretion to antigenic stimulation	43
		Reduced delayed-type hypersensitivity response	43
		Increased susceptibility to neurotropic mouse hepatitis virus	43
CXCL12	SDF-1α	Perinatal mortality with ventricular septal defects	44,45
		Reduced B cell prog1nitors in fetal bone marrow and liver, but reduced myeloid precursors only in bone marrow	45
CX3C Chemokines			
CX3CL1	Fractalkine	Reduced circulating F4/80+ leukocyte subset, but no effect on acute inflammatory response	68
		Reduced infarct size and mortality in cerebral ischemia model of stroke	69
		Reduced atherosclerosis in apoE-deficient mice	70

BX471 and the CXCR4 antagonist AMD3100, both reviewed in detail elsewhere in this issue [7,8]) there is little toxicological information available. The extent to which these compounds provide anti-inflammatory benefit is becoming clear, but the likely side-effects to accompany such benefit remain largely unknown.

However, genetic deletion studies in mice of both the chemokine receptors and their ligands provide early indications of the likely toxicological consequences of specific chemokine receptor blockade in vivo. A wide range of such genetically modified mice have been generated and characterized, allowing patterns to be discerned. A summary of the mouse lines reported to date is presented in Table **1** (for receptor deletions) and Table **2** (for ligand deletions).

Deletion of many of the individual CC chemokine receptors (and in particular those receptors whose ligands are members of inducible classes of chemokines) results in a similar pattern of anti-inflammatory effects accompanied by increased susceptibility to opportunistic infection. Deletion of CCR1, for example, reduces NK-cell [9] and granulocyte [10] recruitment, which is presumably responsible for the prolongation of allograft survival [11], resistance to autoimmune encephalitis [12] and decreased lung injury in a pancreatitis model [13] which have been reported, as well as for the increased susceptibility to infection with *Aspergillus fumigatus* and *Toxoplasma gondii* [14,15] Similarly, CCR2 deletion reduces chronic inflammation in models of atherosclerosis [16,17], as well as acute autoimmune encephalitis [18], but increases susceptibility to pulmonary infection with *Cryptococcus* species [19] and impairs the ability to clear infections with *Lysteria monocytogenes* [20]. Similar patterns are seen with CCR5 [21,22], and probably with CCR3 and CCR8 as well, although fewer data have been published.

Taken together, these studies strongly suggest that while specific chemokine receptor antagonists may show therapeutically useful anti-inflammatory activity, chronic blockade of chemokine receptors will likely result in increased opportunistic infections, with a very similar pattern to that seen with chronic steroid use [2]. However, where careful histological evaluation has been reported [23], no other abnormalities have been detected suggesting that chemokine receptor blockade may be free from many of the other side-effects associated with glucocorticoid treatment.

The impact of deletion of CC chemokine receptors with basal ligands (such as CCR6 and CCR7) is somewhat different. Leukocyte recruitment to sites of peripheral inflammation is largely unaffected, but secondary lymphoid structure is altered [24-26]. As a result, humoral responses to antigens are somewhat impaired (probably as a result of dendritic cell recruitment), accompanied by reduced allergic responses [26-28]. To date, the impact of such impaired humoral responses on susceptibility to infection has not been reported, although it seems likely that such effects will be seen, at least in some infection models.

Deletion of CXC chemokine receptors has similar impact to the deletion of CC chemokine receptors. CXCR1, CXCR2 and CXCR3 deletions all reduce leukocyte recruitment to sites of peripheral inflammation, affecting both macrophages and granulocytes [23,29,30]. This results in protection from acute allograft rejection and reduces chronic inflammation in models of atherosclerosis [23,31], but again increases susceptibility to infection [32,33].

Uniquely, among chemokine receptors studied to date, deletion of CXCR4 shows embryonic lethality with defects in the vasculature, hematopoiesis and migration of neuronal precursors [34,35]. As a result, there is less direct evidence on the role of CXCR4 in the adult immune system, and the toxicological consequences of treatment with CXCR4 antagonists such as AMD3100 are less easy to predict.

Deletion of CXCR5, like the receptors for the basal CC chemokines, results in a profound alteration in secondary lymphoid structure with defects in B cell homing, defective lymph nodes and abnormal germinal center formation in the spleen. Although the detailed impact of CXCR5 deletion on the organogenesis of Peyer's patches and splenic germinal centers has been reported [36], there has been no extensive study of the consequences of such immune disregulation, either in terms of impact on clinically relevant inflammatory processes or susceptibility to infection. As with CCR6 and CCR7 deletion, however, it seems likely that the reduced humoral immunity that accompanies secondary lymphoid disorganization will result in at least some degree of susceptibility to infection.

For all the receptors that have been studied in sufficient detail, the basic pattern seems to be the same: reduced recruitment of multiple leukocyte subsets to various tissues resulting in both beneficial anti-inflammatory properties and increased susceptibility to infection. On top of this basic pattern, however, there are a number of paradoxical observations of pro-inflammatory effects of chemokine receptor deletion, suggesting that, at least under certain conditions, chemokines can exert anti-inflammatory activity. For example, both deletion of CCR2 and CCR1 led to increased susceptibility to experimental glomerulonephritis [37,38]. These findings suggest that selecting the right chemokine receptor to inhibit to treat a particular disease with an inflammatory component may be difficult.

There have been fewer reports of mouse lines with deletions of the chemokine ligands, and those that have been reported often display only very mild phenotypes. However, in common with the observations from the chemokine receptor knockout mice, the general pattern of effects is very similar: mild impairment of stimulated immune responses, associated with increased susceptibility to infection. For example, CCL3 deficient mice show increased susceptibility to disease following exposure to paramyxovirus, *Aspergillus fumigatus* and *Klebsiella pneumoniae* [39-42]. Similarly, CXCL10 deficient mice showed an impaired ability to control replication of a neurotropic mouse hepatitis virus [43].

In almost every case where susceptibility to infection has been assayed in mice deficient in either a single chemokine receptor or ligand, a mild increase in susceptibility has been reported. This is the only consistent finding of any side effect associated with chemokine antagonism, and with the exception of the embryonic lethality of the deletion in CXCL12 [44,45] and its cognate receptor CXCR4 [34,35], there is no indication of any other major histopathological defects likely to be associated with blockade of a single

chemokine or its receptor. These findings provide considerable hope that the chemokine receptor specific antagonists under development may provide at least some anti-inflammatory benefit with a tolerable side-effect profile.

TOXICOLOGY OF BSCIs

When the first BSCIs suitable for use *in vivo* were described [46,47], it was assumed that blocking signals from multiple chemokines simultaneously, might provide a more powerful anti-inflammatory effect than the receptor-specific antagonists, but at the cost of a much more severe side-effect profile. If chemokines were responsible for much of the direction of leukocyte trafficking [4], then it seemed logical to assume that broad spectrum chemokine blockade might lead to complete disruption of immune system organization and a profound immunosuppression.

It was interesting to note, therefore, that even chronic administration of NR58-3.14.3, a peptide BSCI, at doses which suppressed LPS-induced leukocyte recruitment into skin [46] was associated with remarkably little systemic perturbation of immune system organization. Mice treated with NR58-3.14.3 from implantable osmotic minipumps for 6 months showed no alterations in peripheral blood leukocyte counts, or in the basal levels of mucosal macrophages. Although a detailed histological examination of the secondary lymphoid organs of mice after chronic treatment with a BSCI has not been reported, no gross abnormality was detected in a wide range of tissues. Despite a profound inability to mount a pathological inflammatory response, the impact of broad-spectrum chemokine blockade on the unchallenged immune system was remarkably mild [6].

Both the peptide and non-peptide families of BSCIs have now been extensively studied in a wide range of animal models of both acute and chronic inflammation, and in each case, attenuation of leukocyte recruitment to the site of inflammation has been reported, with an associated amelioration of symptoms [6,46,48,49]. However, crucially, in none of these studies to date, has the impact of BSCI administration on susceptibility to infection been measured. Given the consistent defects in host defense associated with complete deficiency of most of the individual chemokine receptors or ligands, it seemed likely but not certain that pharmacological broad-spectrum chemokine blockade might also lead to increased susceptibility to infection. It is already clear, however, that any such increased susceptibility is not significantly more severe in the BSCI-treated mice, than in the individual chemokine receptor knockout mice. Mice treated with NR58-3.14.3 for 6 months were kept in normal animal house conditions without contracting any obvious infections, consistent with their normal peripheral blood and tissue leukocyte counts.

The unexpected lack of side effects associated with chronic BSCI treatment might have its origins in the

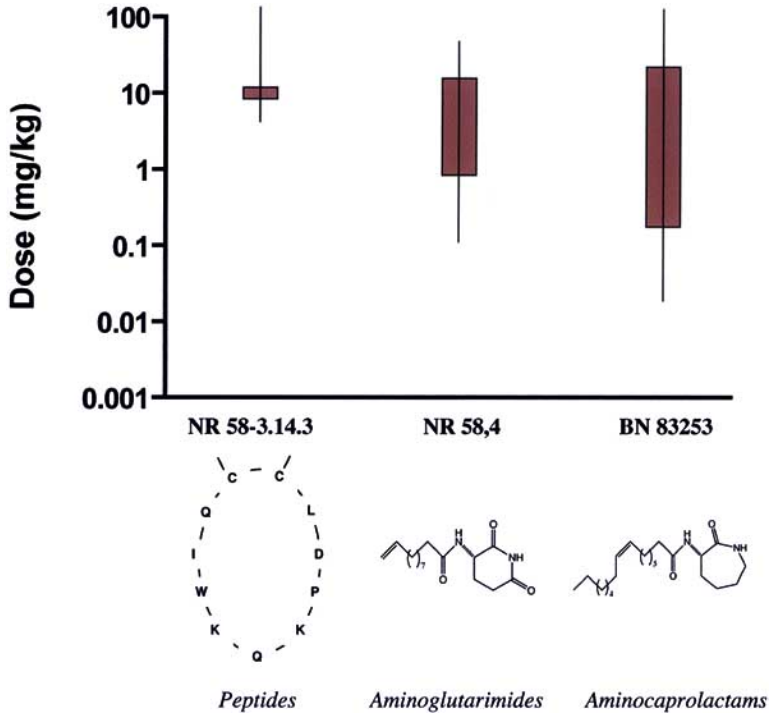

Fig. (1). Therapeutic index of various classes of broad-spectrum chemokine inhibitors (BSCIs). The therapeutic index for a representative member of three structural families of BSCI compounds (NR58-3.14.3 representing peptide BSCIs, NR58,4 representing N-substituted aminoglutarimides and BN 83253 representing N-substituted aminocaprolactams) are estimated, following sub-cutaneous administration of the compounds. The solid block represents a conservative estimate of the therapeutic index, where the top of the bar indicates the no-effect level estimated using a sensitive assay (such as quantitative actimetry) and the bottom of the bar represents the lowest dose which yields maximum anti-inflammatory effect in the LPS-induced endotoxemia model (47). The vertical line represents an alternative estimate, where the top of the line represents the no-effect level by direct observation, and the bottom of the line represents the ED50 for the anti-inflammatory effects in the LPS-induced endotoxemia model.

molecular mechanism of action of these compounds. Neither NR58-3.14.3, nor the newer non-peptide analogs such as NR58,4 or BN 83253 bind directly to chemokine receptors, nor do they block chemokine ligand binding [6]. As a result, a number of second messenger signals (such as increased calcium flux) remain intact, even though the ability of leukocytes to undergo directional migration in the presence of the BSCI is profoundly inhibited. It is likely, therefore, that BSCIs are acting at a distinct cell surface receptor and eliciting signals which specifically block the migratory response to chemokines (but not to classical chemoattractants such as C5a or fMLP). As a result, chronic treatment with BSCIs is not functionally equivalent to multiple gene deletions in a range of chemokine receptors, since at least some receptor-mediated events remain intact. As a result, any attempt to infer the likely toxicological profile of BSCIs from analysis of genetically modified mice has been largely misleading.

The LD50 for NR58-3.14.3 has been determined as approximately 510 mg/kg in mice by single intravenous injection in saline. This represents the limiting solubility of the compound, and the toxicity observed was, at least in part, due to the precipitation of the drug in lungs. The no-effect level for a single intravenous injection of NR58-3.14.3 is approximately 10mg/kg, if a sensitive method of detecting transient hypoactivity (such as actimetry) is used, although the hypoactivity only becomes visually detectable at doses around 100mg/kg and above. The hypoactivity shows rapid onset, but quickly resolves over the first three hours after dosing, with more rapid recovery at lower doses. Interestingly, this transient hypoactivity effect is shared by all of the structural BSCI classes described to date, although the window between the efficacious doses and the no-effect level varies considerably between the families (Fig. **1**), with the N-acylaminocaprolactams showing the greatest safety margin (as high as 10,000 fold).

Neither the molecular nor physiological origins of the transient hypoactivity effect are understood at present, although we have ruled out significant cardiovascular effects as a possible cause. However, it seems unlikely that this represents a limiting toxicity for the use of BSCIs in vivo, and quantitatively assessing the impact on susceptibility to infection seems to be a higher priority.

CONCLUSION

Although the development of chemokine inhibitors as clinically useful anti-inflammatory agents is in its infancy, all the available data suggest that this approach may indeed realize the goal of anti-inflammatory effects comparable with glucocorticoids, but with a reduced side-effect profile. Studies of mouse models with deletions of chemokine receptors or ligands, as well as in vivo neutralization studies and extensive experimentation with broad-spectrum chemokine inhibitors all point in the same direction: chemokine blockade results in impaired inflammatory responses, but without substantial changes in the basal function of the immune system or other tissues. However, in common with steroids, increased susceptibility to infection seems to be a common feature of reduced chemokine function. It will be important to define the extent of this side-effect once human trials of these agents are underway, although it seems unlikely that they will be sufficiently severe to preclude the use of the compounds at least in indications where steroids are currently used.

It is important, however, to note that certain of the possible side effects associated with reduced chemokine function (such as osteoporosis as a result of reduced osteoclast recruitment [1]) would not readily be detected by the analyses that have been carried out to date. Particularly, where chronic use of chemokine inhibitors is envisioned, it will be important to systematically evaluate their impact on tissues other than the immune system where interference with leukocyte trafficking could plausibly affect the tissue architecture in the longer term. As with any class of drugs acting on a family of molecular targets not presently known to be the site of action of clinically used therapeutics, toxicological studies will be required to confirm whether these agents are as safe as they appear to be at first glance.

REFERENCES

[1] Goldring, S.R. *Calcif Tissue Int.* **2003**, *73*, 97.
[2] Schimmer, B.P.; Parker, K.L. In *The Pharmacological Basis of Therapeutics*, Eds. Hardman, J.G. & Limbird L.E. 9th Edition. McGraw-Hill. New York. **1996**, 1459.
[3] Goyal, R.; Watts, P.; Lane, C.M.; Beck, L.; Gregory, J.W. *Ophthalmology* **2004**, *111*, 389.
[4] Rot, A.; Von Andrian, U.H. *Annu. Rev. Immunol*. **2004**, *22*, 891.
[5] Horuk, R.; Ng, H.P. *Med. Res. Rev*. **2000**, *20*, 155.
[6] Grainger, D.J.; Reckless, J. *Biochem. Pharmacol*. **2003**, *65*, 1027.
[7] Horuk, R. *Mini-Rev. Med. Chem.* **2005**, *5*, 791-804.
[8] Clercq, E.D. *Mini-Rev. Med. Chem.* **2005**, *5*, 805-824.
[9] Shang, X.; Qiu, B.; Frait, K.A.; Hu, J.S.; Sonstein, J.; Curtis, J.L.; Lu, B.; Gerard, C.; Chensue, S.W. *Am. J. Pathol.* **2000**, *157*, 2055.
[10] Gao, J.L.; Wynn, T.A.; Chang, Y.; Lee, E.J.; Broxmeyer, H.E.; Cooper, S.; Tiffany, H.L.; Westphal, H.; Kwon-Chung, J.; Murphy, P.M. *J. Exp. Med*. **1997**, *185*, 1959.
[11] Gao, W.; Topham, P.S.; King, J.A.; Smiley, S.T.; Csizmadia, V.; Lu, B.; Gerard, C.J; Hancock, W.W. *J. Clin. Invest*. **2000**, *105*, 35.
[12] Rottman, J.B.; Slavin, A.J.; Silva, R.; Weiner, H.L.; Gerard, C.G.; Hancock, W.W. *Eur. J. Immunol*. **2000**, *30*, 2372.
[13] Gerard, C.; Frossard, J.L.; Bhatia, M.; Saluja, A.; Gerard, N.P.; Lu, B.; Steer, M. *J. Clin. Invest*. **1997**, *100*, 2022.
[14] Blease, K.; Mehrad, B.; Standiford, T.J.; Lukacs, N.W.; Kunkel, S.L.; Chensue, S.W.; Lu, B.; Gerard, C.J.; Hogaboam, C.M. *J. Immunol*. **2000**, *165*, 1564.
[15] Khan, I.A.; Murphy, P.M.; Casciotti, L.; Schwartzman, J.D.; Collins, J.; Gao, J.L.; Yeaman, G.R. *J. Immunol*. **2001**, *166*, 1930.
[16] Boring, L.; Gosling, J.; Cleary, M.; Charo, I.F. *Nature* **1998**, *394*, 894.
[17] Dawson, T.C.; Kuziel, W.A.; Osahar, T.A.; Maeda, N. *Atherosclerosis* **1999**, *143*, 205.
[18] Izikson, L.; Klein, R.S.; Charo, I.F.; Weiner, H.L.; Luster, A.D. *J. Exp. Med*. **2000**, *192*, 1075.
[19] Traynor, T.R.; Kuziel, W.A.; Toews, G.B.; Huffnagle, G.B. *J. Immunol*. **2000**, *164*, 2021.
[20] Kurihara, T.; Warr, G.; Loy, J.; Bravo, R. *J. Exp. Med*. **1997**, *186*, 1757.
[21] Zhou, Y.; Kurihara, T.; Ryseck, R.P.; Yang, Y.; Ryan, C.; Loy, J.; Warr, G.; Bravo, R. *J. Immunol*. **1998**, *160*, 4018.
[22] Huffnagle, G.B.; McNeil, L.K.; McDonald, R.A.; Murphy, J.W.; Toews, G.B.; Maeda, N.; Kuziel, W.A. *J. Immunol.* **1999**, *163*, 4642.
[23] Hancock, W.W.; Lu, B.; Gao, W.; Csizmadia,V.; Faia, K.; King, J.A.; Smiley, S.T.; Ling, M.; Gerard, N.P.; Gerard, C. *J. Exp. Med*. **2000**, *192*, 1515.
[24] Cook, D.N.; Prosser, D.M.; Forster, R.; Zhang, J.; Kuklin, N.A.; Abbondanzo, S.J.; Niu, X.D.; Chen, S.C.; Manfra, D.J.; Wiekowski, M.T.; Sullivan, L.M.; Smith, S.R.; Greenberg, H.B.; Narula, S.K.; Lipp, M.; Lira, S.A. *Immunity* **2000**, *12*, 495.
[25] Forster, R.; Schubel, A.; Breitfeld, D.; Kremmer, E.; Renner-Muller, I.; Wolf, E.; Lipp, M. *Cell* **1999**, *99*, 23.

[26] Saeki, H.; Moore, A.M.; Brown, M.J.; Hwang, S.T. *J. Immunol.* **1999**, *16*, 2472.
[27] Lukacs, N.W.; Prosser, D.M.; Wiekowski, M.; Lira, S.A.; Cook D.N. *J. Exp. Med.* **2001**, *194*, 551.
[28] Chensue, S.W.; Lukacs, 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**, *19*, 573.
[29] Gerard, C.; Rollins, B.J. *Nat. Immunol.* **2001**, *2*, 108.
[30] Hall, L.R.; Diaconu, E.; Patel, R.; Pearlman, E. *J. Immunol.* **2001**, *166*, 4035.
[31] Boisvert, W.A., Santiago R.; Curtiss, L.K.; Terkeltaub, R.A. *J. Clin. Invest.* **1998**, *101*, 353.
[32] Del Rio, L.; Bennouna, S.; Salinas, J.; Denkers, E.Y. *J. Immunol.* **2001**, *167*, 6503.
[33] Frendeus, B.; Godaly, G.; Hang, L.; Karpman, D.; Svanborg C. *J. Infect. Dis.* **2001**, *183* (Suppl. 1), S56.
[34] Nagasawa, T.; Hirota, S.; Tachibana, K.; Takakura, N.; Nishikawa, S.; Kitamura, Y.; Yoshida, N.; Kikutani, H.; Kishimoto, T. *Nature* **1996** *382*, 635.
[35] Tachibana, K.; Hirota, S.; Iizasa, H.; Yoshida, H.; Kawabata, K.; Kataoka, Y.; Kitamura, Y.; Matsushima, K.; Yoshida, N.; Nishikawa, S.; Kishimoto, T; Nagasawa, T. *Nature* **1998**, *393*, 591.
[36] Forster, R.; Mattis, A.E.; Kremmer, E.; Wolf, E.; Brem, G.; Lipp, M. *Cell* **1996**, *87*, 1037.
[37] Topham, P.S.; Csizmadia, V.; Soler, D.; Hines, D.; Gerard, C.J.; Salant, D.J.; Hancock, W.W. *J. Clin. Invest.* **1999**, *104*, 1549.
[38] Bird, J.E.; Giancarli, M.R.; Kurihara, T.; Kowala, M.C.; Valentine, M.T.; Gitlitz, P.H.; Pandya, D.G.; French, M.H.; Durham, S.K. *Kidney Int.* **2000**, *57*, 129.
[39] Cook, D.N.; Beck, M.A.; Coffman, T.M.; Kirby, S.L.; Sheridan, J.F.; Pragnell, I.B.; Smithies, O. *Science* **1995**, *269*, 1583.
[40] Domachowske, J. B.; Bonville, C.A.; Gao, J.L.; Murphy, P.M.; Easton, A.J.; Rosenberg, H.F. *J. Immunol.* **2000**, *165*, 2677.
[41] Mehrad, B.; Moore, T.A; Standiford, T.J. *J. Immunol.* **2000**, *165*, 962.
[42] Lindell, D. M.; Standiford, T.J.; Mancuso, P.; Leshen, Z.J.; Huffnagle, G.B. *Infect. Immun.* **2001**, *69*, 6364.
[43] Dufour, J.H.; Dziejman, M.; Liu, M.T.; Leung, J.H.; Lane, T.E.; Luster, A.D. *J. Immunol.* **2002**, *168*, 3195.
[44] Ma, Q.; Jones, D.; Borghesani, P.R.; Segal, R.A.; Nagasawa, T.; Kishimoto, T.; Bronson, R.T.; Springer, T.A. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9448.
[45] Nagasawa, T.; Hirota, S.; Tachibana, K.; Takakura, N.; Nishikawa, S.; Kitamura, Y.; Yoshida, N.; Kikutani, H.; Kishimoto, T. *Nature* **1996**, *382*, 635.
[46] Reckless, J.; Tatalick, L.M.; Grainger, D.J. *Immunology* **1999**, *103*, 244.
[47] Fox, D.J.; Reckless, J.; Warren, S.G.; Grainger, D.J. *J. Med. Chem.* **2002**, *45*, 360.
[48] Beech, J.S.; Reckless, J.; Mosedale, D.E.; Grainger, D.J.; Williams, S.C.; Menon, D.K. *J. Cereb. Blood Flow Metab.* **2001**, *21*, 683.
[49] Naidu, B.V.; Farivar, A.S.; Krishnadasan, B.; Woolley, S.M.; Grainger, D.J.; Verrier, E.D.; Mulligan, M.S. *Ann. Thorac. Surg.* **2003**, *75*, 1118.
[50] Sato, N.; Ahuja, S.K.; Quinones, M.; Kostecki ,V.; Reddick, R.L.; Melby, P.C.; Kuziel, W.A.; Ahuja, S.S. *J. Exp. Med.* **2000**, *192*, 205.
[51] Campbell, E.M.; Charo, I.F.; Kunkel, S.L.; Strieter, R.M.; Boring, L.; Gosling, J.; Lukacs, N.W. *J. Immunol.* **1999**, *163*, 2160.
[52] Blease, K.; Mehrad, B.; Standiford, T.J.; Lukacs, N.W.; Gosling, J.; Boring, L.; Charo, I.F.; Kunkel, S.L.; Hogaboam, C.M. *J. Immunol.* **2000**, *165*, 2603.
[53] Humbles, A.A.; Lu B.; Friend, D.S.; Okinaga, S.; Lora, J.; Al-Garawi, A.; Martin, T.R.; Gerard, N.P.; Gerard, C. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 1479.
[54] Chvatchko, Y.; Hoogewerf, A.J.; Meyer, A.; Alouani, S.; Juillard, P.; Buser, R.; Conquet, F.; Proudfoot, A.E.; Wells, T.N.; Power, C.A. *J. Exp. Med.* **2000**, *191*, 1755.
[55] Wurbel, M.A.; Malissen, M.; Guy-Grand, D.; Meffre, E.; Nussenzweig, M.C.; Richelme, M.; Carrier, A.; Malissen, B. *Blood* **2001**, *98*, 2626.
[56] Cacalano, G.; Lee, J.; Kikly, K.; Ryan, A.M.; Pitts-Meek, S.; Hultgren, B.; Wood, W.I.; Moore, M.W. *Science* **1994**, *265*, 682.
[57] Devalaraja, R.M.; Nanney, L.B.; Du, J.; Qian, Q.; Yu Y.; Devalaraja, M.N.; Richmond, A. *J. Invest. Dermatol.* **2000**, *115*, 234.
[58] Haskell, C.A.; Hancock, W.W.; Salant, D.J.; Gao, W.; Csizmadia, V.; Peters, W.; Faia, K.; Fituri, O.; Rottman, J.B.; Charo, I.F. *J. Clin. Invest.* **2001**, *108*, 679.
[59] Gu, L.; Okada, Y.; Clinton, S.K.; Gerard, C.; Sukhova, G.K.; Libby, P.; Rollins, B.J. *Mol. Cell* **1998**, *2*, 275.
[60] Gosling, J.; Slaymaker, S.; Gu, L.; Tseng, S.; Zlot, C.H.; Young, S.G.; Rollins, B.J.; Charo, I.F. *J. Clin. Invest.* **1999**, *103*, 773.
[61] Boring, L.; Gosling, J.; Cleary, M.; Charo, I.F. *Nature* **1998**, *394*, 894.
[62] Low, Q.E.; Drugea, I.A.; Duffner, L.A.; Quinn, D.G.; Cook, D.N.; Rollins, B.J.; Kovacs, E.J.; DiPietro, L.A. *Am. J. Pathol.* **2001**, *159*, 457.
[63] Lu, B.; Rutledge, B.J.; Gu, L.; Fiorillo, J.; Lukacs, N.W.; Kunkel, S.L.; North, R.; Gerard, C.; Rollins, B.J. *J. Exp. Med.* **1998**, *187*, 601.
[64] DiPietro, L.A.; Burdick, M.; Low, Q.E.; Kunkel, S.L.; Strieter, R.M. *J. Clin. Invest.* **1998**, *101*, 1693.
[65] Makino, Y.; Cook, D.N.; Smithies, O.; Hwang, O.Y.; Neilson, E.G.; Turka, L.A.; Sato, H.; Wells, A.D.; Danoff, T.M. *Clin. Immunol.* **2002**, *102*, 302.
[66] Yang, Y.; Loy, J.; Ryseck, R.P.; Carrasco, D.; Bravo, R. *Blood* **1998**, *92*, 3912.
[67] Tomkinson, A.; Duez, C.; Cieslewicz, G.; Gelfand, E.W. *Int. Arch. Allergy Immunol.* **2001**, *126*, 119.
[68] Cook, D.N.; Chen, S.C.; Sullivan, L.M.; Manfra, D.J.; Wiekowski, M.T.; Prosser, D.; Vassileva, G.; Lira, S.A. *Mol. Cell Biol.* **2001**, *21*, 3159.
[69] Soriano, S.G.; Amaravadi, L.S.; Wang, Y.F.; Zhou, H.; Yu, G.X.; Tonra, J.R.; Fairchild-Huntress, V.; Fang, Q.; Dunmore, J.H.; Huszar, D.; Pan, Y. *J. Neuroimmunol.* **2002**, *125*, 59.
[70] Combadiere, C.; Potteaux, S.; Gao, J.L.; Esposito, B.; Casanova, S.; Lee, E.J.; Debre, P.; Tedgui, A.; Murphy, P.M.; Mallat, Z. *Circulation* **2003**, *107*, 1009.