Commentary

Broad-spectrum chemokine inhibitors (BSCIs) and their anti-inflammatory effects *in vivo*

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Abstract

Inappropriate inflammation is a component of a wide range of human diseases, including autoimmune disease, atherosclerosis, osteoporosis and Alzheimer's disease. Chemokines play an important role in orchestrating leukocyte recruitment during inflammation, and therefore represent an important target for anti-inflammatory therapies. Unfortunately, the chemokine system is complex, with about 50 ligands and 20 receptors, often acting with redundancy, making selection of appropriate specific antagonists difficult. One approach to overcoming this difficulty may be the development of broad-spectrum chemokine inhibitors (BSCIs). Here we review the present state of knowledge on BSCIs, including their activity *in vitro* and their anti-inflammatory effects *in vivo*, and discuss the future development of BSCIs as anti-inflammatory therapies for use in the clinic.
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Keywords: Chemokine; Antagonist; Migration; Inflammation; Therapy

1. Introduction

Inflammation that is quantitatively, spatially, or temporally inappropriate is a component of many prevalent human diseases, including atherosclerosis, autoimmune disorders, osteoporosis and Alzheimer's disease [1–7]. As a result, the molecules responsible for orchestrating the immune response have become not only important subjects for biomedical research, but also therapeutic targets for the pharmaceutical industry.

One of the most exciting developments of the 1990s was the discovery and subsequent enlargement of the family of immune regulatory molecules related to IL-8 [8–12]. This family of cytokines, collectively called chemokines, provide important regulatory cues to a wide range of leukocytes [8,9]. Chemokines can instruct leukocytes to move from one tissue to another, to stay where they are, to become activated, or perhaps, in a few cases, to become quiescent [11,13].

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Abbreviations: BSCI, broad-spectrum chemokine inhibitor; C5a, complement factor 5a; fMLP, formyl-methionyl-leucyl-phenylalanine; IL, interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; RANTES, regulated and normal T cell expressed and secreted protein; SDF-1α, stromal-derived factor 1α; and TGF-β, transforming growth factor-β.

One measure of the likely importance of the chemokine family in regulating the immune system is the large number of ligands and receptors that have been discovered. To date more than 50 chemokines have been identified, signalling through some 20 distinct receptors [8,10,12]. Such a signalling system has the informational density necessary to provide the tight temporal and spatial regulation of the immune system, which is essential for its normal function.

Genetic knockout strategies have, to some extent, confirmed this view that chemokines are important regulators of immune function [9], although the deletion of specific chemokines has led to relatively mild and specific defects in the inflammatory response, emphasising the complex redundancy of the system [9]. Nevertheless, there has been a considerable effort to develop specific inhibitors of chemokine function that can be used both as tools to further understand the contribution of individual receptors to the regulation of immune responses and also, ultimately, as lead compounds for the development of new anti-inflammatory drugs [14–21].

The chemokine inhibitors that have been developed to date fall into two broad classes: (a) inhibitors specific for one or a small number of chemokine receptors, and (b) inhibitors that broadly inhibit the function of a wide range of chemokines (BSCIs). The receptor-specific inhibitors, which have mostly been identified by conventional high

throughput screening for receptor antagonists, have been reviewed extensively elsewhere [17,19,20,22–24]. Here, we review the present knowledge of the BSCIs.

Both the receptor-specific inhibitors and the BSCIs have a number of advantages over the other. For example, receptor-specific inhibitors may allow more subtle control over immune function, but the role of individual receptors may be so subtle that the resultant molecules have little therapeutic benefit. As an example, CCR5Δ32 polymorphism results in clinical protection from infection by the HIV virus, which uses CCR5 as a co-receptor for cellular entry [25]. Individuals homozygous for this polymorphism have no detectable CCR5 present on the surface of their cells. Since these individuals appear phenotypically normal, it is possible that the CCR5-specific antagonists that are presently being developed will have relatively little impact as anti-inflammatory agents, although they may have some utility in fighting HIV infection.

In marked contrast, the first generation of BSCIs have been shown to have anti-inflammatory activity in a range of different animal models [26,27]. These molecules suppress the recruitment of leukocytes to sites of acute or chronic inflammation in rodent models of asthma, cerebral ischemia, atherosclerosis, sepsis, and transplant-induced fibrosis [26,27]. Furthermore, preliminary toxicological evaluation in animals has suggested that broad-spectrum chemokine inhibition is remarkably free of acute or chronic side-effects. Hence, BSCIs hold the promise of providing wide-range anti-inflammatory function similar to steroids but with reduced side-effects [28]. Studies of these molecules are in their infancy, but the results published to date are sufficiently promising to merit further investigation.

2. The first BSCIs

In contrast to receptor-specific antagonists, which have been identified by traditional screening approaches, broad-spectrum chemokine inhibitory activity was identified as the ability to inhibit chemokine-induced leukocyte migration *in vitro*, while leaving migration in response to non-chemokine signals unaffected. The first molecules with such properties to be identified were peptides derived from a conserved region of the chemokine MCP-1 [29]. One such peptide, termed peptide 3, with the sequence EICADPKQKWVQ, inhibited leukocyte migration induced by any of five chemokines tested (MCP-1, MIP-1α, RANTES, IL-8, and SDF-1α), albeit with low potency ($\sim$10 μM) [29]. This effect, however, was highly specific for the chemokines: peptide 3 had no effect at all on migration induced by a range of non-chemokine chemoattractants, including fMLP, C5a, and TGF-β, even at 100 μM [29]. This effect was apparently not cell-type specific, since chemokine-induced migration was blocked both for cell lines (human myelomonocytic THP-1 cells, and Jurkat T cells) as well as for freshly prepared human peripheral blood leukocytes (a monocyte-enriched mononuclear cell fraction and a polymorphonuclear cell fraction) [29].

Peptide 3 and its sequence analogues lacked sufficient potency for use *in vivo*. Unexpectedly, however, the cyclic retroinverso analogue, constructed of D-amino acids in the reverse sequence, termed NR58-3.14.3, was found not only to retain the chemokine-specific inhibitory properties of the parent peptide 3, but also to be at least three orders of magnitude more potent [27]. NR58-3.14.3 inhibited chemokine-induced leukocyte migration with a potency of 1–10 nM, but did not affect migration in response to other chemoattractants even at 100 μM, indicating a chemokine specificity of well over 10,000-fold [27].

Although peptides have a number of disadvantages for use *in vivo*, the potency of NR58-3.14.3 allowed it to be used for proof-of-concept studies in animal models [26,27]. Systemic administration of NR58-3.14.3 blocked the recruitment of leukocytes in response to chemokines injected into rat skin [27]. These studies suggest that NR58-3.14.3 and other BSCIs may be anti-inflammatory agents with a novel mechanism of action.

A short time after peptide 3 was first described, Mantovani and colleagues [30] demonstrated that the cytokine IL-10 had similar properties. Specifically, incubation with IL-10 blocked chemokine-induced migration but not leukocyte migration induced by other chemoattractants [30]. Tantalising, both IL-10 and peptide 3 have been shown to block chemokine-induced migration without affecting chemokine binding to their specific G-protein-coupled receptors. Both inhibitors, therefore, share the property of converting the high-affinity chemokine receptors into inactive sinks that bind chemokines but do not elicit a signal [30]. Such a property could lead to an inhibitory effect *in trans*, where removal of chemokine by inactive surface chemokine receptors on one population of cells could prevent signalling to a second population. This effect would amplify the inhibitory properties of the BSCI molecule.

A third class of molecules that have been described as having broad-spectrum chemokine inhibitory properties are the virally encoded chemokine antagonist proteins, such as the herpes virus M3 protein and the KSHV-encoded vMIP proteins [31,32]. Some of these proteins, such as M3, bind directly to the chemokine ligand, preventing interaction with the signalling receptor [31], while others bind to several chemokine receptors. Therefore, in some sense, these proteins lie midway between the specific receptor antagonists and the true BSCIs (such as peptide 3 and IL-10), because the viral chemokine inhibitors described to date tend to inhibit the function of just a subset, albeit a large one, of the chemokine superfamily. Despite the somewhat narrower chemokine specificity and the difficulties of using proteins as therapeutics, some progress has been made towards using virally encoded chemokine inhibitors as anti-inflammatory agents *in vivo* [33–35].

3. Molecular mechanism of broad-spectrum chemokine inhibition

Despite the observation that both peptide 3 derivatives and IL-10 block chemokine-induced migration without blocking chemokine binding to its receptors, it remains entirely unclear whether these two classes of BSCIs share a common molecular mechanism. Comparison of the two is hampered by the fact that the molecular pathways leading to blockade of the migratory response specifically to chemokines remains unknown for either IL-10 or the peptide 3 derivatives.

Nevertheless, a number of plausible mechanisms of action for peptide 3 derivatives have been ruled out (Fig. 1). First, they do not directly bind to or modulate the properties of the chemokine receptors. Binding of biotinylated peptide 3 to different cell lines, each over-expressing a single recombinant chemokine receptor, was characterised, and although peptide 3 bound to the parental cells (which do not express any chemokine receptors) with an apparent affinity constant of 9 μM, there was no additional binding detected when chemokine receptors were expressed at over 1 million copies per cell. Furthermore, as for IL-10, the binding of chemokines to the receptors was unaffected by the presence of peptide 3 derivatives, and the level of chemokine receptors on peripheral blood leukocytes was unaffected by incubation with peptide 3 derivatives over a wide concentration range. Hence, although the initial

strategy that led to the identification of peptide 3 was intended to identify receptor-binding antagonists, nevertheless we conclude that BSCIs do not function by directly interacting with chemokine receptors (Fig. 1). This conclusion is supported by the structure–activity relationships among BSCIs (see below) where any modification that affects the potency against one chemokine has similar effects on the potency against all of the others. This strongly suggests that BSCIs interact with a single target common to chemokine signalling pathways, but which does not participate in the instigation of migration in response to non-chemokine chemoattractants.

Interestingly, the inhibitory effects of BSCIs appear to be limited to the migratory response to chemokine signalling (Fig. 1). The presence of peptide 3 derivatives does not block the transient increase in intracellular calcium ion concentration induced by chemokines, nor do they block the chemokine-induced down-regulation of cell surface chemokine receptors, suggesting that at least some of the early intracellular second messenger pathways remain intact. Similarly, the acute stimulation of DNA synthesis in response to MCP-1 in cultured smooth muscle cells is unaffected by NR58-3.14.3, as is the stimulation of macrophage phagocytosis by a range of chemokines *in vitro*. These observations have led to the suggestion that BSCIs target a component of the chemokine signalling pathway that couples to the migratory response. They are unlikely, however, to target any component of the effector apparatus
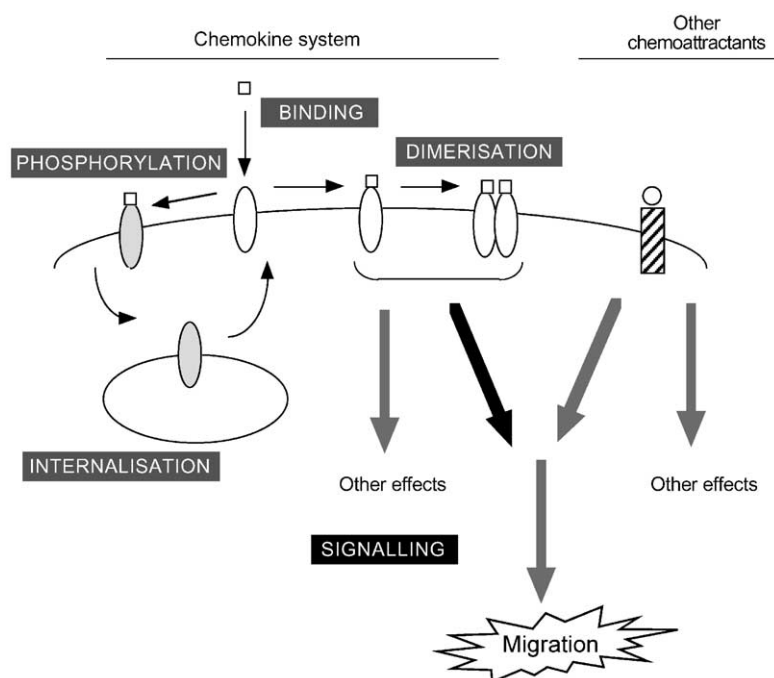

Fig. 1. Cellular pathways for BSCIs. A simplified overview of the receptor signalling pathway for chemokine receptors (ovals) and receptors for other chemoattractants (rectangles) is shown. A range of potential mechanisms by which BSCIs might specifically inhibit chemokine-induced migration have been excluded (grey boxes), suggesting that they may act as signalling inhibitors (black box). Since BSCIs do not affect migration induced by non-chemokine chemoattractants, they must target a component of the intracellular signalling pathway that is unique to chemokines and that is specifically linked to the migration response (black arrow).

of migration (such as the formation of pseudopods, cytoskeletal rearrangements, or membrane trafficking), since these are likely to be required for migration irrespective of the inducing signal.

Although these studies substantially narrow down the search for the molecular target of BSCIs, the precise mechanism by which they specifically block chemokine-induced migration remains elusive. Identifying this mechanism remains a key hurdle both to understanding this class of molecules and to their application as anti-inflammatory agents *in vivo*.

4. Structure–function relationship of BSCIs

A comprehensive analysis of the relationship between structure and function of the peptide 3 sequence has been published recently [36]. Two parallel strategies were adopted to identify the key structural motifs required for inhibitory activity. First, various deletions were performed which demonstrated that the bulk of the activity was retained in a tripeptide sequence at the C-terminus of the original dodecapeptide [36]. This tripeptide, WVQ, retained the broad-spectrum chemokine inhibitory properties of the parent sequence, with similar potency, while having no effect on migration induced by other chemoattractants [36]. Second, a mutagenesis strategy was pursued in which every amino acid in the parent sequence was substituted with the other 19 proteogenic amino acids in turn [36]. A total of 261 peptides were then tested for BSCI activity. Encouragingly, this strategy identified the same important structural motifs as the deletion approach, with particular focus on the tryptophan at position 10 and the glutamine at position 12 [36].

Based on the structure–function relationship of the peptides, Fox and colleagues have developed a series of low molecular weight compounds with potent BSCI activity. All of these molecules described to date are *N*-acyl derivatives of aminoglutarimide. The best characterised is *N*-undec-10-enoyl-L-3-aminoglutarimide (termed NR58,4) [36]. This compound inhibits chemokine-induced leukocyte migration *in vitro* with a potency of 1–10 nM (similar to the retroinverso peptide analogue NR58-3.14.3) but does not affect migration induced by fMLP, C5a, or TGF-β. NR58,4 does not bind to chemokine receptors, but competes for binding to the NR58-3.14.3 binding site on the cell surface. This suggests that the non-peptide *N*-acyl-aminoglutarimides are likely to act by a similar mechanism to the peptide 3 derivatives, and possibly IL-10, although more subtle variations in mechanism between the classes of BSCIs remain entirely possible.

Analysis of the relationship between structure and function of the aminoglutarimide derivatives has already begun (Fig. 2). *N*-Substitution with a wide range of simple acyl groups as well as more complicated peptidyl structures retains BSCI activity with a potency below 100 nM

(provided that the α-carbonyl group is retained), but modifications to the aminoglutarimide ring (including substitution of the ring nitrogen) readily reduce potency as chemokine inhibitors.

NR58,4 has been shown to reduce the systemic increase in tumour necrosis factor-α (TNF-α) in response to the non-specific inflammatory stimulus LPS in mice, active at doses below 0.3 mg/kg, suggesting that it may have useful anti-inflammatory properties similar to NR58-3.14.3 [36]. Certainly, NR58,4 is considerably cheaper to synthesise in large quantities than the D-amino acid-containing peptides, which may facilitate further studies of BSCIs *in vivo*. However, it has become clear recently that substituted aminoglutarimides are subject to enzymatic ring-opening in serum (unpublished observations), which may limit their use *in vivo*, although it may be possible to design NR58,4 analogues that retain BSCI activity but have increased stability.

5. Therapeutic properties of BSCIs in animal models of inflammation

Chemokines have been implicated in such a wide range of pathological inflammatory processes that it is difficult to decide in which model of inflammation BSCIs might be most effective. However, the availability of NR58-3.14.3 has allowed BSCIs to be tested in a wide range of animal models of inflammation, including atherosclerosis, asthma, stroke, sepsis, and osteoporosis [26,27].

The first studies of BSCIs *in vivo* utilised dermal injection of various inflammatory stimuli in rats as a proof of concept [27]. These studies demonstrated that a single systemic dose of NR58-3.14.3 (either intravenous or intraperitoneal) could completely abolish leukocyte recruitment into the dermis, whether the inflammatory challenge was a chemokine (MCP-1) or relatively non-specific (such as the bacterial endotoxin LPS) [27]. MCP-1 induced a monocyte/macrophage-rich infiltrate with some contribution from B- and T-lymphocytes; however, the dermal levels of all these leukocyte subpopulations remained at or below baseline following NR58-3.14.3 pretreatment [27]. LPS induced an even stronger inflammatory response, with the addition of a large neutrophil component. However, NR58-3.14.3 completely suppressed leukocyte recruitment to this challenge as well, inhibiting neutrophil accumulation to the same extent as monocytes and lymphocytes [27]. These observations strongly suggest that multiple chemokines acting in parallel lie upstream of TNF-α. If such a model is correct, then BSCIs would be expected to possess more powerful anti-inflammatory activity than many of the receptor-specific chemokine antagonists.

BSCIs have also been demonstrated to have anti-inflammatory activity in more disease-relevant animal models. Cerebral ischemia, induced by middle cerebral artery occlusion, has a neutrophil-rich inflammatory component
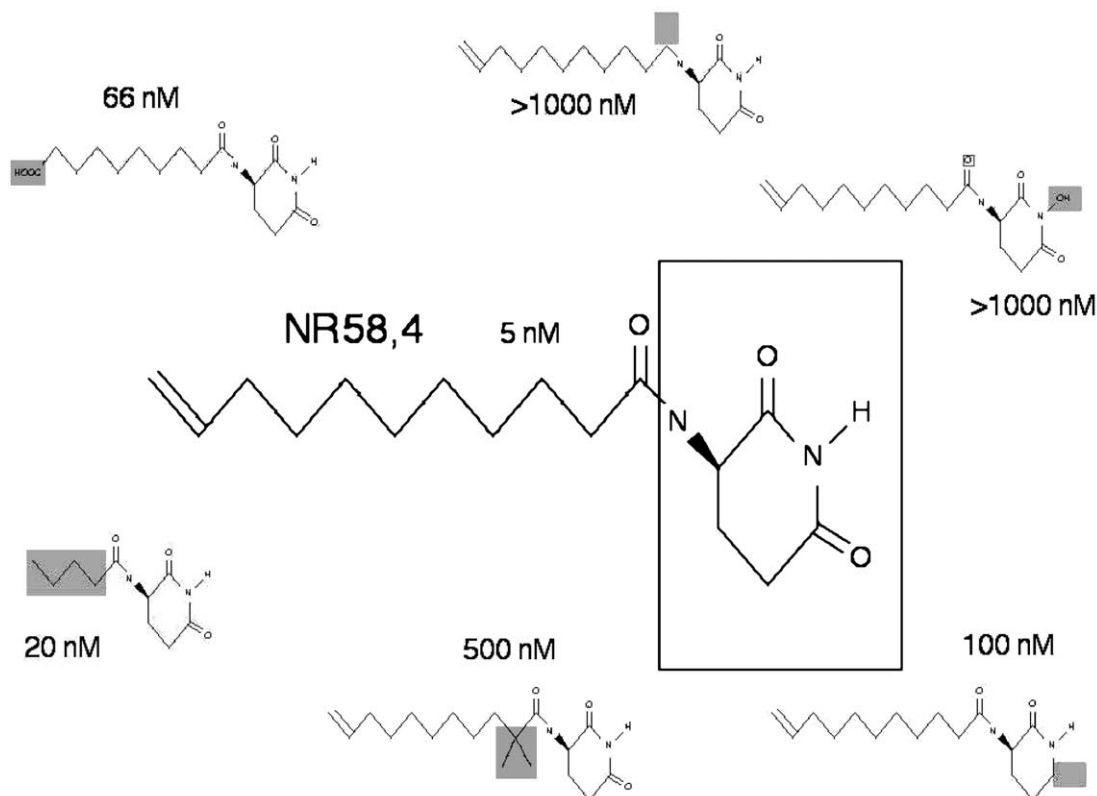
Fig. 2. Structure–activity relationship of *N*-acyl-L-aminoglutarimides. The most potent *N*-acyl-aminoglutarimide derivative described to date is NR58,4, which inhibited MCP-induced THP-1 migration with an EC_{50} of 5 nM. Various analogues of this compound have been described [36], which suggest that the key structural elements for BSCI activity reside in the aminoglutarimide ''head group'' (boxed) and the amide linker. Analogues with a wide variety of ''tail groups'' retain potency as BSCIs, although the presence of substituents at the α-carbon has a significant impact on BSCI activity. For each analogue shown, key structural differences from NR58,4 are highlighted with grey shading.

which results in the size of the infarct doubling over 48 hr post-reperfusion (presumably due to damaging effects of the activated neutrophils) [37]. Treatment with NR58-3.14.3 from the point of reperfusion onwards completely abolished leukocyte recruitment in the penumbral regions of the brain lesion, resulting in a 50% reduction in infarct size at 72 hr and a marked improvement in the behavioural markers of neural function [26]. Whether cerebral infarction in humans is accompanied by such a pronounced inflammatory response remains controversial, but if leukocyte recruitment has a negative impact during reperfusion in humans, then BSCIs may find application in the immediate aftermath of stroke or heart attack.

Chemokine inhibition may be beneficial during chronic (as well as acute) cardiovascular disease. Macrophage-derived proteases may play a key role in destabilising atherosclerotic plaques [38], triggering plaque rupture and hence stroke or heart attack. Recruitment of macrophages to the atherosclerotic plaque to some extent depends on chemokine function, at least in mice, since mice lacking MCP-1 or its receptor (CCR2) develop less severe lesions with reduced macrophage numbers when challenged to develop vascular lipid lesions [39–42]. Treatment with NR58-3.14.3 rapidly reduces the number of macrophages in vascular lipid lesions in mice (a 30–40%

reduction in less than 1 week), with accompanying beneficial changes in extracellular matrix turnover (unpublished observations). Although this reduction is not accompanied by reduction in lipid lesion size, even when the treatment is continued for 6 months, any improvement in plaque stability would likely translate into clinical benefit in humans with atherosclerosis.

The other major area where BSCIs have been studied is lung inflammation. In ovalbumin-induced asthma, NR58-3.14.3 substantially reduces the number of leukocytes in the lungs (including a 90% reduction in eosinophils). Surprisingly, this did not appear to result in any improvement in lung function (assessed as methacholine-induced airway hyper-reactivity), suggesting that the presence of leukocytes in general, and eosinophils in particular, is unlikely to be responsible for acute airway hyper-responsiveness. Instead, changes in lung architecture (such as hypertrophy of the lung epithelium and goblet cells, increased mucous secretion, and deposition of fibrous extracellular matrix) normally associated with chronic asthma were avoided (Fig. 3). Combination therapy using agents that control acute airway hyper-reactivity (such as salbutamol) together with BSCIs to prevent chronic changes in lung architecture appears to be an appealing avenue for further research.
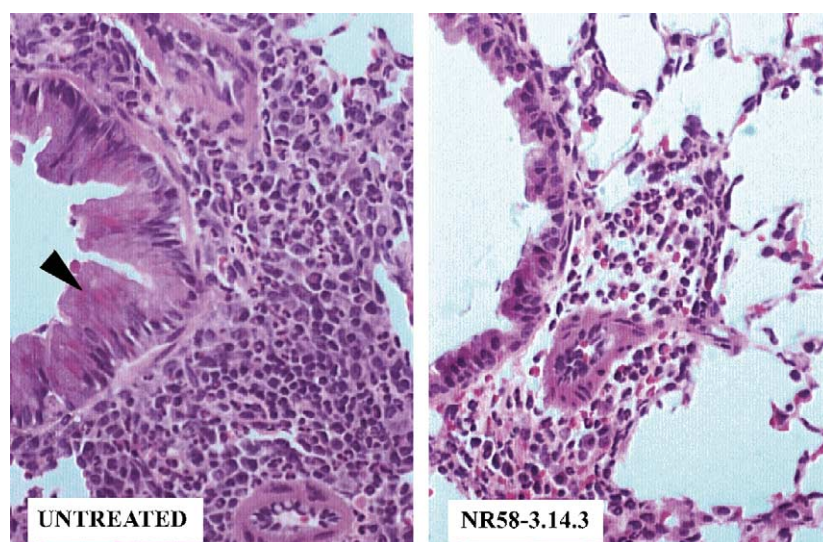
Fig. 3. Effect of NR58-3.14.3 on lung histology following ovalbumin sensitisation. Haematoxylin and eosin-stained lung sections from mice that had been sensitised to ovalbumin by i.p. injection and then exposed to an intratracheal challenge with ovalbumin. Mice that received no anti-inflammatory therapy (left panel) had massive eosinophil-rich leukocyte infiltrate and hypertrophy of the bronchiolar epithelium with enlarged mucous-producing cells (arrowhead). Treatment with the BSCI NR58-3.14.3 prior to the intratracheal challenge reduced the inflammatory infiltrate and maintained a normal epithelial morphology (right panel). Data courtesy of the NeoRx Corp., Seattle, WA, USA. Cited with permission of the NeoRx Corp.

Consistent with the effects of BSCIs in animal models of asthma, recent studies of the effect of NR58-3.14.3 in ectopic lung transplantation models suggest that these agents possess a significant anti-fibrotic effect. Ectopic transplant of an HLA mismatched lung results in rapid stenosis of the airways due to exuberant extracellular matrix deposition. However, treatment with NR58-3.14.3 reduces airway stenosis by 50–60%. The extent to which ectopic transplantation is a model for human brochiolitis obliterans syndrome (BOS), a complication of lung transplantation, is unclear. Nevertheless, leukocyte-induced fibrosis is a component in both rodents and humans, and it is plausible that broad-spectrum chemokine inhibition may be beneficial in BOS.

BSCIs have been studied already in 10 different animal models of acute and chronic inflammation, with a common pattern emerging [26,27]. Irrespective of the dominant leukocyte populations involved, BSCI administration reduces inflammatory cell recruitment and with it many of the side-effects of inappropriate inflammation, such as fibrosis and neutrophil-mediated cytotoxicity. Any malevolent consequences of reducing leukocyte accumulation in these disease models are currently less clear. Steroids have benefits similar to BSCIs, but the side-effects of steroids are well documented [28]. The side-effects of BSCIs, for the most part, remain to be defined.

6. Toxicological profile of broad-spectrum chemokine inhibition

Perhaps surprisingly, broad-spectrum chemokine inhibition seems to be remarkably free of acute side-effects. The best-studied BSCI, NR58-3.14.3, has an LD_{50} of greater than 50 mg by i.v. injection in mice (equivalent to more than 50 g in humans). At such doses, what toxicity is observed likely results from limiting solubility of the drug, leading to precipitation in the lungs and kidneys.

Chronic administration of BSCI (for up to 6 months in rodents) also has surprisingly few obvious side-effects (unpublished observations). Blood biochemistry and blood cell counts remain unaffected even though leukocyte recruitment to a pro-inflammatory challenge, such as LPS is blunted. No histological changes were noted in a range of tissues, confirming that broad-spectrum chemokine inhibition has fewer physiological consequences than might have been expected (unpublished observations). Most importantly, the levels of patrolling leukocytes in a range of normal tissues (including Kupffer cells in liver, microglia in brain, osteoclasts in bone, and alveolar macrophages in lungs) are unaffected by long-term administration of NR58-3.14.3. This suggests that chemokines (or at least those known to be inhibited by BSCIs) are not responsible for setting the levels of physiological leukocyte populations in a range of tissues. Intriguingly, the chemokine superfamily can be split on functional, rather than structural, criteria into "constitutive" chemokines involved in basal trafficking and homing, and "inducible" chemokines involved in the inflammatory response. Most of the chemokines whose pro-migratory activity has been shown to be inhibited by BSCIs (with the probable exception of SDF-1α) are of the "inducible" class, raising the possibility that "basal" chemokine function is not inhibited by the BSCIs reported to date.

Despite the lack of crude toxicological side-effects of chronic broad-spectrum chemokine inhibition, it remains

entirely possible that subtle, but nevertheless important, side-effects do exist. For example, reduced mucosal immunity may result from reduced ability to mount a leukocyte-rich inflammatory response in epithelial tissues. Similarly, dendritic cell maturation would be expected to be inhibited, as has been shown with IL-10 [30]. The structure of secondary lymphoid organs may also be disrupted, as seen in mice with genetic deletions of certain chemokines [43,44], and these changes may reduce or prevent the ability to produce an effective antibody response to antigen challenge.

When considering side-effects, it may be useful to compare BSCIs with steroids, since these are the best-studied molecules with comparable therapeutic benefits. Some of the side-effects of steroids are likely to result from their direct effects on transcription (e.g. alterations in dermal architecture), and such effects are unlikely to be shared by BSCIs [28]. Other side-effects, though, may result from their systemic anti-inflammatory activity: for example, increased susceptibility to opportunistic infections, such as candidiasis [45]. It remains entirely possible that BSCIs will share these side-effects with anti-inflammatory agents, such as steroids.

7. Future prospects for the clinical development of BSCIs

The limited experience of BSCIs in animal models of inflammation suggests that this class of molecules has significant potential for development as anti-inflammatory agents for use in human therapy [26,27]. Their broad efficacy in a wide range of different models of inflammation, coupled with their apparent paucity of side-effects, together suggest that BSCIs are leading candidates for further pre-clinical and, ultimately, clinical development.

Successfully transferring BSCIs, such as NR58-3.14.3, into the clinic, however, will depend on successfully dealing with a number of outstanding issues. Which molecules shall we use? Which disease shall we treat? What is their mechanism of action? The first of these questions is likely to be answered by a classical medicinal chemistry programme, already underway, in which a comparison of the pharmacology of BSCI analogues is performed, and both peptides derived from the NR58-3.14.3 structure and non-peptides related to NR58,4 are likely to be found that are suitable for administration in humans.

Ironically, the broad anti-inflammatory activity of BSCIs makes selection of a disease target for clinical trials more difficult. Drugs with highly specific targets are likely to be useful in only one or a small range of related pathologies, making clinical development comparatively straightforward. The range of diseases which could, in principle, be amenable to therapy with BSCIs is so vast (highlighted by the range of animal models in which BSCIs have been effective) that selection is difficult. Proof of the anti-inflammatory activity of these molecules will likely come in a disease accessible using small, quantitative clinical trials (such as lung fibrosis following transplantation), and only after successful intervention in such a "gateway" indication would trials of BSCIs in larger indications, such as chronic obstructive pulmonary disease (COPD), asthma, or atherosclerosis, be considered.

Finally, an understanding of the mechanism of action of BSCIs would be helpful. Such knowledge would help predict potential side-effects, as well as guiding in the development of treatment strategies. The precise molecular mechanism of many successful drugs was not known during, or in some cases, long after, clinical development. Indeed, in many instances, the molecular mechanisms of today's drugs are still the subject of debate and controversy. A better understanding of the mechanism would be advantageous, but it is unlikely, by itself, to preclude successful clinical development.

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