Short Communication

# Interleukin-8-like activity in a filarial asparaginyl-tRNA synthetase

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**Abstract**

A wide range of secondary biological functions have been documented for eukaryotic aminoacyl-tRNA synthetases including roles in transcriptional regulation, mitochondrial RNA splicing, cell growth, and chemokine-like activities. The asparaginyl-tRNA synthetase (AsnRS) of the filarial nematode, *Brugia malayi*, is a highly expressed excretory-secretory molecule which activates interleukin 8 (IL-8) receptors via extracellular domains that are different from those used by IL-8. Recent success in determining the complete atomic structure of the *B. malayi* AsnRS provided the opportunity to map its chemokine-like activity. Chemotaxis assays demonstrated that IL-8-like activity is localized in a novel 80 amino acid amino terminal substructure. Structural homology searches revealed similarities between that domain in *B. malayi* AsnRS and substructures involved in receptor binding by human IL-8. These observations provide important new insights into how parasite-derived molecules may play a role in the modulation of immune cell function.

**Keywords**: aminoacyl-tRNA synthetase; chemokine; interleukin-8 (IL-8); filaria; immunoregulation; receptors

Chronic infectious diseases, such as those caused by human parasites, induce an array of immunopathologic phenomena including immune tolerance and antigen-specific immunosuppression [1]. Such phenomena are believed to represent ways by which infecting agents down-regulate the host immune response in order to help establish a non-lethal but persistent infection [2]. The exact mechanisms by which specific microbes cause immunomodulation are not completely known.

Aminoacyl-tRNA synthetases (AARS) are a family of enzymes that exemplify a classic paradox of structure and function, exhibiting both great structural diversity and extraordinary substrate specificity. Evolutionary biologists suggest that as primordial class I and class II enzymes evolved over time, domains have “broken loose” to perform unexpected catalytic or regulatory functions [3]. A wide range of secondary functions for AARS have been documented in various species, including roles in transcriptional regulation, mitochondrial RNA splicing, processivity in mitochondrial DNA polymerization, control of cell growth, and cytokine- or chemokine-like activity [4]. Chemokines are chemotactic cytokines that play a role in diverse physiological processes because their corresponding G protein coupled receptors are ubiquitous, occurring on both hematopoeitic and endothelial cells. The monomeric forms of chemokines are known to have similar 3-dimensional structures, while their oligomeric forms often differ [5].

Cytoplasmic asparaginyl-tRNA synthetase (AsnRS) from the human filarial parasite, *Brugia malayi*, is a highly expressed excretory-secretory protein that (1) *in vitro* specifically interacts with IL-8 chemokine receptors, CXCR1 and CXCR2, using extracellular loops of the receptor that differ from those utilized by IL-8, (2) activates

MAP kinases upon receptor binding, and (3) blocks the normal calcium transient generated by the binding of a native ligand [6]. Recently we have solved the complete atomic structure of the *B. malayi* AsnRS (GenBank accession number P10723) which reveals an N-terminal domain structure unlike that in other AARS, and part of that domain is involved in tRNA binding [7]. The two known functions of the N-terminal domain of AsnRS, chemokine receptor activation [6] and tRNA binding [7], likely are exerted under different conditions. Transfer RNA binding activity is relevant inside the cell, whereas chemokine-like activity becomes relevant once the protein is secreted/excreted from the parasite where it can interact with the host's immune defenses. The availability of structures for all domains of the *B. malayi* AsnRS provides the opportunity to dissect the structural basis for the IL-8-like immunological activity observed in the full-length AsnRS.

Three different cDNA constructs derived from the *B. malayi* AsnRS amino acid sequence were designed to express the N terminal region (residues 1-111), C terminal region (residues 112-548), or the entire wild type cytoplasmic AsnRS (548 aa) in pET28A expression systems. Each of these constructs yielded soluble recombinant protein of the expected molecular mass, which was then purified as an endotoxin-free reagent (<0.001 IU/μg) for use in immunological assays. Endotoxn free proteins were purified by using magnesium sulfate precipitation, followed by sequential rounds of size selection-, nickel affinity- and anion- exchange chromatography, and final adsorption using Endotrap® (Hygiene Biotech Company, Ltd.) Chemotaxis assays were designed to measure the migration of purified human neutrophils in response to varying concentrations of IL-8 or *B. malayi* AsnRS.

Chemotaxis assays demonstrated that both the full length *B. malayi* AsnRS (Fig. 1 A) and the amino-terminal 111 amino acid eukaryote-specific domain (Fig. 1B), but not the carboxy-terminal domain (Fig. 1C, residues 112-548), induced migration of human neutrophils in a concentration-dependent pattern typical of known IL-8 chemokines. From prior structural studies of the *B. malayi* AsnRS, it was known that N terminal eukaryote specific extension region of this enzyme, residues 1-111, exists as a structured 80 amino acid domain involved in tRNA binding, connected by a 33 residue unstructured tether to the catalytic domain [7]. The amino- terminal AsnRS fragment (111 residues) induced maximum chemotaxis at a ten fold lower concentration than the wild type BmAsnRS (548 residues), which could be explained by their difference in molar concentration.

Computational analysis of the *B. malayi* N-terminal structure using DaliLite [8] (http://ekhidna.biocenter.helsinki.fi/dali_lite/start) identified a region (Fig. 2) that overlaid with the chemokine SDF-1 (Stromal Cell Derived Factor-1), consisting of the β hairpin-α helix motif common to chemokines. The same structural fold found in IL-8 (Fig. 2A) is shown overlaid on the N-terminal region in *B. malayi* AsnRS and human SDF-1 [9]. DaliLite superimposed the backbone of residues Lys A36-Lys A45, Lys A48-Ser A55, and Lys A56-Gln A68 of *B. malayi* AsnRS onto residues Ala A35-Asn A44, Asn A45-Asp A52, and Leu A55-Asn A67 of SDF-1 (PDB entry 2k03; [10]), with a $C_\alpha$ RMSD of 2.3Å (Fig. 2A).

The LIRTKKDGKQ(V/I)W amino acid sequence of the β hairpin in the *B. malayi* AsnRS chemokine motif is similar to that of other CXC chemokines, matching all but the

final position in the pattern:

(**V**,I,L)(**I,**V,A)(**A**,V,R)(**T**,S,K,W,L)(**L**,K,M)(**K**,N,S)(**N**,D)(**0**,K,N)(**G**,0)

(**R**,K,E,Q,S,V)(**K**,Q,E,I) (**V**,I,L) (**C**).

In this pattern deduced from an alignment of 13 CXC chemokines including IL-8 [11], boldface indicates the dominant residue in the alignment, an underscore indicates the residue in AsnRS, and 0 indicates a deletion at this position in some chemokines. A tryptophan residue near the C-terminal end of the β hairpin in *B. malayi* AsnRS replaces the conserved, disulfide-forming Cys residue in other CXC chemokines, which links the N-terminal end of the β hairpin in one monomer to the C-terminal end of the helix in another monomer, stabilizing the dimer. Thus the tethered N-terminal region in filarial AsnRS likely does not adopt the same type of dimer as IL-8, though it may form an alternative dimer, given that the enzymatically active form of AsnRS is known to involve a homodimer of the catalytic domain (PDB entry 2xgt; [7]). Filarial AsnRS lacks the ELR sequence in the N-terminal region (N-loop) of human tyrosyl-tRNA synthetase (TyrRS, GeneBank accession number U89436) which also interacts with IL-8 receptors [4,7]) and in other chemokines that interact with Site 2, which is formed by two disulfide-linked extracellular loops on CXCR1/2 [12,13]. Thus it is expected that AsnRS either does not interact with Site 2 loops on the receptor, or interacts with them differently from IL-8. The AsnRS helical sequence is also not clearly similar in sequence to the helix in other CXC chemokines, though it does contain a sequence, SWKR, resembling the (polar)(**W**)(**V**,Q)(**Q**,R,K,P) motif found in this region of CC chemokines such as RANTES [11]. The NMR structure of the IL-8 interaction with a CXCR1/2 peptide (PDB entry 1ilp; [14]) supports that the N-terminal region of the chemokine (N-loop) interacts with Site 1,

which is the extracellular binding surface of the receptor formed by the receptor's N-terminal peptide [12,13].

The solution structure of IL-8 in complex with an N-terminal peptide from CXCR1 [14, PDB entry 1ilp] provides more detail on their interaction: residues Phe C4 to Pro C17 from CXCR1 bind in an extended fashion parallel to the N-loop against the β hairpin, interacting with its solvent-exposed edge, on the opposite side relative to where the α helix packs (Fig. 2B). Based on the superposition between the *B. malayi* AsnRS N-terminal chemokine motif and the IL-8 complex with the CXCR1 peptide (Fig. 2B), CXCR1 could interact similarly with AsnRS, forming the following clusters of favorable contacts: CXCR1 Glu C13 with AsnRS Lys A45 and Thr A43; CXCR1 Tyr C15 with AsnRS Glu A52 and Ala A53; CXCR1 Ser C16 with AsnRS Trp A51; and CXCR1 Pro C17 with AsnRS Leu A40. Thus, the structural correspondence between the hairpin-helix motif in AsnRS with IL-8 predicts the epitope on AsnRS for CXCR1 binding. Together, these results support the presence of a minimalist hairpin-helix motif in AsnRS that can mimic IL-8 structurally by interacting with the Site 1 region of CXCR1/2 primarily via the edge of the AsnRS β-hairpin.

The concept that pathogenic microbes can subvert the chemokine system to their own advantage has been best illustrated in the context of viral infections [15]. Similar chemokine-related immune phenomena have been reported less commonly for both unicellular and multi-cellular parasites including *Plasmodium vivax*, *Toxoplasma gondii* and *Schistosoma mansoni* [2,16]. The chemokine-chemotaxis response is an obvious point of attack by microbial pathogens since it is a saturable phenomenon in which binding of a specific chemokine or chemokine mimic results in receptor

158 desensitization/internalization. Receptor desensitization effectively would inhibit further
159 cellular migration in the direction of an invading microbe. Thus the interaction between
160 *B. malayi* AsnRS and IL-8 receptors may explain in part the complex
161 immunomodulatory effects of filarial nematode infections.

162 The β hairpin + α helix structure in *B. malayi* AsnRS amino terminus may
163 represent a minimal motif for CXCR1 binding and activation of corresponding signal
164 transduction cascades. That an *in vivo* immunological role exists for excreted/secreted
165 *B. malayi* AsnRS is consistent with several other previous observations: (1) AsnRS is
166 ten times more highly expressed than any other filarial AARS and (2) virtually 100% of
167 persons with lymphatic filariasis develop IgG antibodies against AsnRS [17,18]. This
168 hypothesis is supported also by our more recent unpublished data that suggests (1)
169 filarial AsnRS is not pro-inflammatory when injected into mice, (2) *in vitro* filarial AsnRS
170 induces the maturation of IL-10$^{+}$ T regulatory cells via immature dendritic cells that
171 express IL-8 receptors, and (3) *in vivo* filarial AsnRS enhances IL-4 and IL-10
172 expression and induces a shift from a T helper (Th)1/Th17 cytokine pattern towards the
173 Th2 pattern in the B6 Rag1$^{-/-}$ mouse T cell transfer model [Kron, Elliott, Metwali, et al.
174 unpublished.] In comparison, two other human autoantigenic AARS, histidyl-tRNA
175 synthetase (HisRS) and AsnRS, have overall protein domain organizations similar to *B.*
176 *malayi* AsnRS [ 7 ] including similar predicted secondary structures in their N termini
177 [Supplementary electronic Fig. 2]. However, only a select few eukaryotic AARS have
178 been shown to elicit chemotaxis. Neither the human lysyl-tRNA synthetase (GeneBank
179 NP005539, LysRS) nor the human aspartyl-tRNA synthetase (GeneBank P14868,
180 AspRS) are chemoattractants [19] in spite of some amino acid level homology to AsnRS

in the ATP binding domain. On the other hand, both human HisRS (GenBank P12081) and human AsnRS (UniProt O43776) have been shown to interact with specific chemokine receptors, CCR5 and CCR3 respectively [19]. Human HisRS does not attract neutrophils because they lack CCR5 receptors, in contrast to filarial AsnRS which attracts neutrophils *in vitro* via IL-8 receptors. The autoantigenic epitope of HisRS also has been localized in an amino-terminal substructure. This correspondence suggests that 3-dimensional chemokine motifs also may be present in human HisRS and AsnRS.

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## Figure legends

**Figure 1**. Results of human neutrophil chemotaxis experiments using (A) whole recombinant AsnRS (548 amino acids), (B) N terminal (1-111) eukaryote extension domain, and (C) C terminal (112-548) catalytic domain. The concentration in ng/ml is given following the construct name in the x-axis labels. Whereas both the full length (Panel A) and N-terminal extension domain (Panel B and left half of Panel C) exhibit a typical concentration-dependent chemotaxis curve with saturation, the C terminal domain (right half of Panel C) does not induce chemotaxis. To express the *B malayi* N-terminus (amino acids 1-111) and the C terminal region (amino acids 112-548) the published DNA sequence of the *B. malayi* AsnRS (accession number P10723) was used to design oligonucleotide primers specific for the two regions [7]. Asterisk (*) refers to statistically significant differences obtained using a two tailed T-test when comparing the chemotaxis induced by an AsnRS protein versus the media control.

**Figure 2.** Regions of superimposition of *Brugia malayi* AsnRS with IL-8 and SDF-1. Panel A shows Dali superimposable motifs of AsnRS (blue), IL-8 (green) and SDF-1 (pink; from PDB entry 2k03; [10].) Panel B shows the detailed correspondence between the IL-8 (green) and Asn-RS (blue) structures (rotated by 90 degrees relative to panel A), focusing on contacts with the CXCR1 peptide (light green) bound in the IL-8 NMR structure (PDB entry 1ilp; [14]). Side chains are shown for all side chain contacts predicted between AsnRS and the CXCR1 peptide, based on the IL-8 alignment. In the case of IL-8, its N-loop is also involved in binding to CXCR1.

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Figure(s)

**Figure 1.**

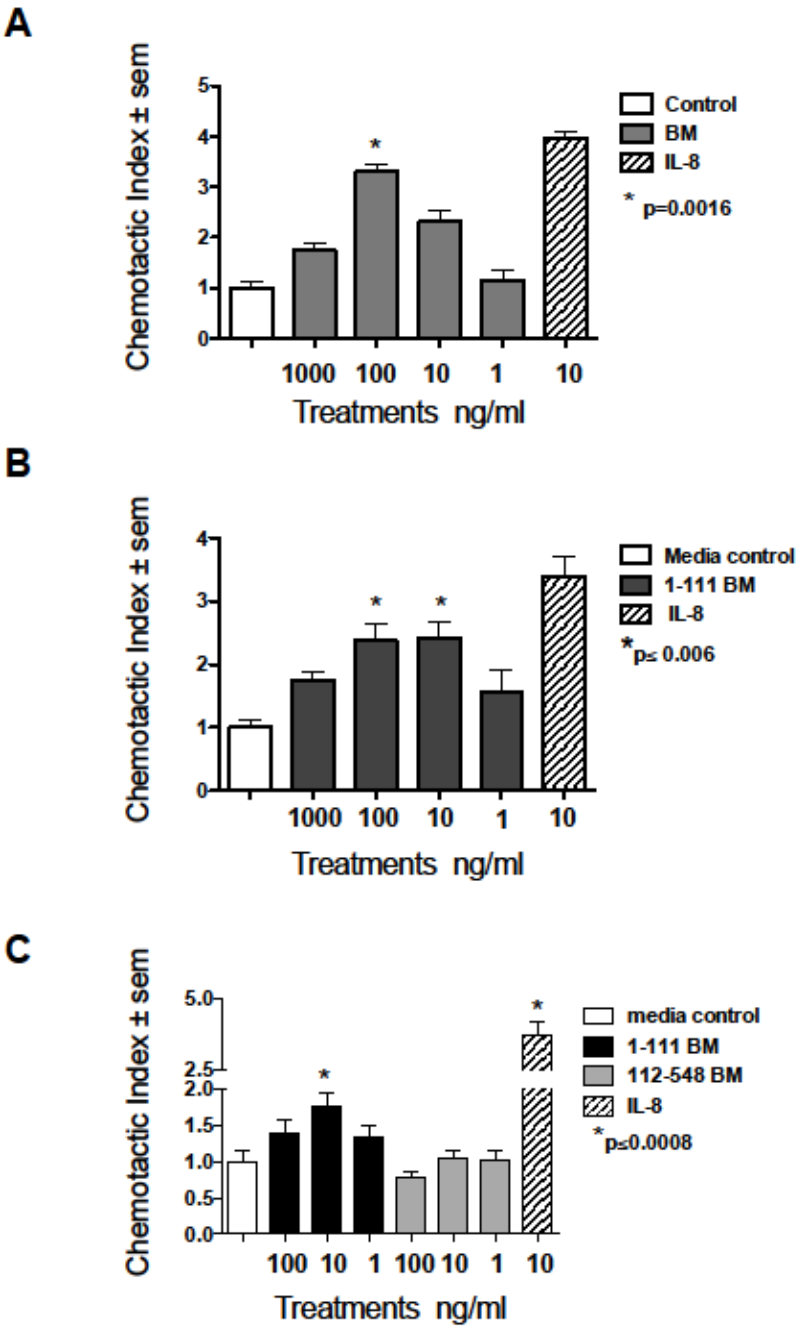

**Figure 2.** COLOR FIGURE