Eur Respir J 2005; 26: 933–947
DOI: 10.1183/09031936.05.00120204

SERIES "SIGNALLING AND TRANSCRIPTIONAL REGULATION IN INFLAMMATORY AND IMMUNE CELLS: IMPORTANCE IN LUNG BIOLOGY AND DISEASE"
Edited by K.F. Chung and I.M. Adcock
Number 1 in this Series

The role of post-transcriptional regulation in chemokine gene expression in inflammation and allergy

J. Fan*, N.M. Heller*, M. Gorospe#, U. Atasoy¶ and C. Stellato*

ABSTRACT: The aim of this review is to discuss recent advances in the understanding of the regulation of chemokine expression occurring during chronic inflammatory conditions, such as allergic diseases. The focus will be on current data, which suggest that post-transcriptional regulation plays a larger role in chemokine gene regulation than previously recognised. In particular, a growing body of data indicates that mechanisms controlling mRNA stability may be relevant in determining, or maintaining, the increased levels of chemokine gene expression in this context. Such regulatory pathways may be important targets of novel anti-inflammatory strategies.

KEYWORDS: Allergy, chemokines, inflammation, post-transcriptional regulation, RNA stability

BACKGROUND

Since the 1980s, research on the superfamily of small, secreted proteins known as the chemokines has steadily grown into a research world of its own. It is now clearly established that these molecules play a central role in many homeostatic and pathological processes in human biology. Chiefly, chemokines shape the way in which the immune system responds to an inflammatory insult, by coordinating the recruitment, activation and homing of leukocytes during the different phases of both innate and adaptive inflammatory responses [1]. It is easy to envision how an alteration of chemokine expression or function might lead to the persistence of an inflammatory reaction well beyond its original purpose, therefore creating a key pathogenetic event for the establishment of chronic inflammation [2].

Given the central role of inflammation in the pathogenesis of allergic airway diseases, and of bronchial asthma in particular, the molecular pathways driving the expression and function of chemokines are now viewed as primary front-line candidates in the development of anti-inflammatory strategies. Every research breakthrough, however, reveals a new level of complexity in the chemokine network and therefore, adds a new challenge in the identification and design of targeted therapies.

In the past few years, a growing body of data suggests that post-transcriptional regulation plays an important role in determining the levels of chemokine expression. In particular, the mechanisms controlling mRNA stability appear to be crucially involved in determining the timing and the levels of chemokine gene expression during an inflammatory response. Although data on regulation of chemokine translation are still lacking, increased knowledge on how RNA turnover and translation are integrated [3, 4] will probably lead to new insights in this area in the very near future, and further expand the identification of pro-inflammatory mechanisms potentially targetable by therapeutic intervention.

CHEMOKINES: MULTI-TASKING MOLECULES IN ALLERGIC AIRWAY INFLAMMATION

Chemokines were initially identified as regulators of leukocyte trafficking [5]. Subsequently, it has been recognised that these molecules also contribute to other aspects of the inflammatory

For editorial comments see page 762.

AFFILIATIONS
*Division of Allergy and Clinical Immunology, The Johns Hopkins University, and
#National Institute of Aging, National Institutes of Health (NIH), Baltimore, MD, and
¶Dept of Surgery, Molecular Microbiology and Immunology, University of Missouri-Columbia, Columbia, MO, USA.

CORRESPONDENCE
C. Stellato
Johns Hopkins Asthma and Allergy Center
5501 Hopkins Bayview Circle
Baltimore
MD 21224
USA
Fax: 1 4105502130
E-mail: stellato@jhmi.edu

Received:
October 21 2004
Accepted after revision:
June 28 2005

SUPPORT STATEMENT
C. Stellato's work is supported by National Institutes of Health Grants AI 44242-01A2 and AI 060990-01A1 (Bethesda, MD, USA).

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

process, such as fibrosis, tissue remodelling and angiogenesis [6]. The multiple actions and functions of chemokines are now recognised as playing a major role in diverse conditions such as vascular, neoplastic, infectious and allergic disorders, and to be involved in the immunopathology of transplant rejection and auto-immune diseases [2].

So far, >40 chemokines and >20 chemokine receptors have been identified and cloned [7]. The chemokine superfamily is divided into four branches, based upon the number and spacing of conserved cysteine residues present in their amino acid sequence (table 1). These subfamilies are referred to as CC (or β), CXC (or α), C (or γ), and CX_3C. The absence or presence of an intervening amino acid between the first two out of four conserved cysteines characterises the CC and the CXC family, respectively. The C (or γ) subfamily includes lymphotactin, in which only two cysteines are conserved. Fractalkine is the only member of the CX_3C subfamily and it is the only membrane-bound chemokine, since it possesses a transmembrane domain linked to a CC-like domain *via* a long mucin-rich region.

The upregulated expression of a relatively specific subset of chemokines within inflammatory sites has been identified in a variety of human chronic inflammatory diseases, as well as in animal models of inflammation. This pattern of expression correlated with the selective recruitment of distinct inflammatory cells types [9]. In the case of allergic disease, such as asthma, which is characterised by a predominant influx of eosinophils, T-helper (Th)2 lymphocytes and basophils, the increased expression of several CC chemokines, such as CCL2 (monocyte chemoattractant protein (MCP)-1), CCL13 (MCP-4), CCL5 (regulated on activation, normal T-cell expressed and secreted), CCL11 (eotaxin-1), CCL24 (eotaxin-2), CCL26 (eotaxin-3), CCL17 (thymus and activation-regulated chemokine) and CCL22 (monocyte-derived chemokine) is firmly established [6, 10]. In particular, expression of CCL11 in the airways is strongly correlated with the presence of an eosinophilic infiltrate [11–14]. It is currently believed that, in chronic asthma, chemokines function as the link between the activation of Th2 lymphocytes and the recruitment to the tissue of eosinophils, basophils and additional Th2 lymphocytes [6, 9]. In fact, antigen-activated CD4+ Th2 cells produce interleukin (IL)-4 and IL-13, which potently synergise with proinflammatory cytokines, such as IL-1 and tumour necrosis factor (TNF)-α, and stimulate the production of eosinophil, basophil and Th2-specific CC chemokines from epithelial cells, endothelial cells and tissue macrophages [8]. Through the concerted expression of the appropriate chemokine receptors, primed eosinophils and basophils are recruited into the tissue and activated by these chemokines, an event that results in the well-known pathological hallmarks of allergic diseases [15]. Furthermore, Th2 cell-specific chemokines, CCL17 and CCL22 further amplify the recruitment of CD4+ Th2 cells, therefore generating a self-sustaining proinflammatory loop [6, 9, 10]. Animal models of IL-13 overexpression, as well as of certain chemokine knockouts, have confirmed the interdependence of the Th2-derived cytokines and the eosinophilic chemokines, such as the eotaxins, in the establishment of a lung inflammatory reaction [8]. These attractive models could provide the basis for testing a variety of pharmacological inhibitors of allergic inflammation [16].

A broad array of chemokines can be induced *in vitro* by proinflammatory stimuli in different types of leukocytes or resident cells, such as mast cells, dendritic cells, fibroblasts, epithelial cells, endothelial cells and smooth muscle cells [10]. However, examination of airway specimens from animal models and human subjects reveals a more narrow spectrum of cellular sources for chemokines, suggesting the existence of complex mechanisms regulating chemokine production *in vivo*, which control proper leukocyte trafficking in homeostasis and disease states. Different pathways participating in these regulatory mechanisms can be altered following the onset of inflammatory responses, and, thus, play a pivotal role in determining the abnormal expression of chemokines seen in chronic inflammatory diseases, such as asthma.

THE RISING ROLE OF POST-TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION IN INFLAMMATION AND ALLERGY

Post-transcriptional regulation of gene expression is a powerful adaptive mechanism implemented by eukaryotic cells in response to a variety of cell perturbations, such as stress, proliferation or immune activation [17]. This mechanism is known to be crucial in regulating the timing and the amount of expression of early-response genes, by acting in tight coordination with gene transcription and under the common control of phosphorylation-mediated signalling events [18]. The critical role of post-transcriptional regulation for gene expression has been highlighted in a recent gene array study, in which ~50% of stress-induced genes were found to be regulated primarily at the level of mRNA stability [19]. Changes in the half-life of labile mRNAs are often in the range of two- to four-fold fluctuations [20]. Though seemingly modest, alterations of this amplitude have been shown to result in greater than 1,000-fold differences in steady-state mRNA levels, which, in turn, can lead to dramatic changes in protein production [20].

The multiple stages of the life cycle of an mRNA molecule, such as transport, subcellular localisation, stability and translation, are dependent on the presence of cis-regulatory elements scattered throughout the molecule [21, 22]. Among these sequences, the adenylate-uridylate-rich elements (AREs) present within the 3′-untranslated region (UTR) of an mRNA represent the most conserved and well-studied group of RNA motifs shown to regulate a distinct subset of transcripts [23–25]. This group of sequences is very heterogeneous, and includes AUUUA pentamers and AT-rich stretches that can be found clustered in different combinations. ARE sequences were originally loosely classified in three classes as follows: class I and class II contained AUUUA pentamers either scattered (class I) or clustered as nonamers (UUAUUUAU/AU/A; class II), in association with U-rich regions, whereas in the class III transcripts the AUUUA pentamer was not present at all [25]. Recently, a database of ARE-bearing mRNA molecules has been established [26] and AREs were reclassified into five groups, based on reiterations of the AUUUA motif [27]. In an effort to merge the two classifications, AREs have been subsequently regrouped according to the number and configuration of these elements within the 3′-UTR (table 2) [17]. Ultimately, AREs gain their function due to the combination of multiple and distinct domains. Recent studies

TABLE 1 Human chemokines and their receptors

Family	Current nomenclature	Previous nomenclature(s)	Receptor[#]
CXC	CXCL1	GRO-α/MGSA-α	CXCR2
	CXCL2	GRO-β/MGSA-β	CXCR2
	CXCL3	GRO-γ/MGSA-γ	CXCR2
	CXCL4	PF4	Unknown
	CXCL5	ENA-78	CXCR2
	CXCL6	GCP-2	CXCR1, CXCR2
	CXCL7	NAP-2	CXCR2
	CXCL8	IL-8	CXCR1, CXCR2
	CXCL9	MIG	CXCR3, (CCR3)
	CXCL10	IP-10	CXCR3, (CCR3)
	CXCL11	I-TAC	CXCR3, (CCR3)
	CXCL12	SDF-1α/β	CXCR4
	CXCL13	BLC/BCA-1	CXCR5
	CXCL14	BRAK/bolekine	Unknown
	CXCL15[¶]		
	CXCL16	SR-PSOX	CXCR6
CC	CCL1	I-309	CCR8
	CCL2	MCP-1/MCAF	CCR2
	CCL3	MIP-1α/LD78α	CCR1,CCR5
	CCL4	MIP-1β	CCR5
	CCL5	RANTES	CCR1, CCR3, CCR5
	CCL6		
	CCL7	MCP-3	CCR1, CCR2, CCR3, (CCR5)
	CCL8	MCP-2	CCR3
	CCL9/10[¶]		
	CCL11	Eotaxin	CCR3, CCR5, (CCR2)
	CCL12[¶]		CCR2
	CCL13	MCP-4	CCR2, CCR3
	CCL14	HCC-1	CCR1
	CCL15	HCC-2/Lkn-1/MIP-1δ	CCR1, CCR3
	CCL16	HCC-4/LEC	CCR1
	CCL17	TARC	CCR4
	CCL18	DC-CK1/PARC/AMAC-1	Unknown
	CCL19	MIP-3β/ELC/exodus-3	CCR7
	CCL20	MIP-3α/LARC/exodus-1	CCR6
	CCL21	6Ckine/SLC/exodus-2	CCR7
	CCL22	MDC/STCP-1	CCR4
	CCL23	MPIF-1	CCR1
	CCL24	Eotaxin 2/MPIF-2	CCR3
	CCL25	TECK	CCR9
	CCL26	Eotaxin 3	CCR3
	CL27	CTACK/ILC	CCR10
	CCL28	MEC	CCR3, CCR10
C	XCL1	Lymphotactin/SCM-1α/ATAC	XCR1
	SCL2	SCM-1β	XCR1
CX3C	CX3CL1	Fractalkine	CX3CR1

GRO: growth-regulated oncogene; MGSA: melanoma growth stimulatory activity; PF: platelet factor; ENA: epithelial neutrophil activating; GCP: granulocyte chemotactic protein; NAP: neutrophil-activating peptide; IL: interleukin; MIG: monokine-induced by interferon (IFN)-γ; IP: IFN-γ-inducible protein; I-TAC: IFN-γ-inducible T-cell chemoattractant; SDF: stromal cell-derived factor; BLC: B-lymphocyte chemoattractant; BCA: B-cell-attracting chemokine; BRAK: breast and kidney-expressed chemokine; SR-PSOX: scavenger receptor for phosphatidylserine and oxidised lipoprotein; MCP: monocyte chemoattractant protein; MCAF: monocyte chemotactic and activating factor; MIP: macrophage inflammatory protein; RANTES: regulated on activation, normal T-cell expressed and secreted; HCC: human CC chemokine; Lkn: leukotactin; LEC: liver-expressed chemokine; TARC: thymus and activation-regulated chemokine; DC-CK1: dendritic cell-derived CC chemokine; PARC: pulmonary and activation-regulated chemokine; AMAC: alternative macrophage activation-associated CC chemokine; ELC: Epstein-Barr virus-induced molecule-1 ligand chemokine; LARC: liver and activation-regulated chemokine; SLC: secondary lymphoid tissue chemokine; MDC: monocyte-derived chemokine; STCP: stimulated T-cell chemoattractant protein; MPIF: myeloid progenitor inhibitory factor; TECK: thymus-expressed chemokine; CTACK: cutaneous T-cell-activating chemokine; ILC: IL-11 recepter alpha-locus chemokine; MEC: mucosae-associated epithelial chemokine; SCM: single C motif; ATAC: activation-induced, chemokine-related molecule. [#]: antagonist effect in parentheses; [¶]: human ligand has not yet been identified. Modified from [8].

TABLE 2 Adenylate-uridylate-rich elements[#]

Group	Motif	Representative transcripts
I	WAUUUAW with U-rich region	c-fos, c-myc
IIA	AUUUAUUUAUUUAUUUAUUUA	GM-CSF, TNF-α
IIB	AUUUAUUUAUUUAUUUA	IFN-α
IIC	WAUUUAUUUAUUUAW	COX-2, IL-2, VEGF
IID	WWAUUUAUUUAWW	FGF2
IIE	WWWWAUUUAWWWW	u-PA receptor
III	U-rich region, non-AUUUA	c-jun

GM-CSF: granulocyte macrophage-colony stimulating factor; TNF: tumour necrosis factor; IFN: interferon; COX: cyclooxygenase; IL: interleukin; VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; u-Pa: urokinase plasminogen activator. #: proposed classification by WILUSZ *et al*. [17].

underscore the importance of noncanonical AREs, such as U stretches, rather than AUUUA pentamers, as key elements in the formation of the secondary mRNA structures necessary for the recognition of the binding site by specific regulatory RNA-binding proteins [28]. The functional role of the ARE was first established *in vitro* by subcloning the ARE-containing sequences from the 3′-UTR of granulocyte macrophage-colony stimulating factor (GM-CSF) in a reporter gene construct, and examining the decrease in the stability of the chimeric reporter mRNA [29]. This method has been widely applied in the study of the signalling and of the RNA-binding factors involved in ARE-mediated decay [18, 30]. Besides mRNA turnover, AREs also participate in the regulation of translation through different signalling pathways, as shown for TNF-α, IL-2 and IL-3 [3, 31]. However, the specific interrelationship between the ARE-mediated control of mRNA turnover and translation is yet to be fully uncovered [17]. The pivotal role of AREs in the control of mRNA stability and translation has been confirmed *in vivo* in genetically altered mouse models of targeted ARE deletion (ΔARE): ablation of the ARE region in the TNF-α gene led to an increase in production of TNF-α, which was found to be caused by a decrease in the rate of the TNF-α mRNA decay and by loss of translational inhibition [32].

Recently, a database of ARE-bearing mRNA molecules has been established [26, 27]. This gene pool encodes proteins involved in different biological processes, such as cell cycle, cell activation, tumourigenesis and stress response, and many of these proteins are implicated in several disease states. The expression of many key genes in immunity and inflammation, such as IL-2, IL-3, IL-4, IL-5, and GM-CSF, whose mRNAs display AREs, is indeed regulated post-transcriptionally and, among these, chemokine genes are being increasingly identified [33]. The biological relevance of genes found to contain and to be regulated by AREs [27] points at these RNA sequences as central cis-regulatory elements in gene expression in immunity, and further indicates the potential impact of therapeutic intervention targeting ARE-mediated regulatory pathways.

The mechanism of ARE-dependent control of mRNA turnover, which is yet to be fully elucidated, relies on the formation of messenger ribonucleoprotein (mRNP) complexes, in which the mRNA is bound to ARE-binding proteins. Upon binding, these factors can either accelerate or, in turn, slow down deadenylation-dependent mRNA decay by influencing multiple steps of the decay process, including the accessibility of the RNA transcripts to specific mRNA degrading complexes [17, 34–37].

Several ARE-binding proteins have been recently cloned and functionally characterised, and they are emerging as key regulatory molecules in the organisation of post-transcriptional gene expression [23, 38]. These factors are involved in several critical steps of the mRNA life cycle, like the nuclear export and subcellular localisation of mature mRNAs, as well as the rate of mRNA decay and translation, and are situated downstream of signalling pathways that have been increasingly identified, as discussed below. ARE-binding proteins often act in association with other regulatory proteins and RNA recognition motifs to achieve full regulatory functionality [30, 37, 39].

Growing evidence indicates that the alteration of the expression and/or function of ARE-binding proteins, as well as that of the signalling pathways governing them, could potentially play a role in human disease [32, 40–44]. However, alterations of mRNA turnover and translation are greatly underexplored in human inflammatory and allergic processes, as it is the role of these mechanisms, and the pathways they use, as targets for anti-inflammatory therapy.

Following the termination of acute inflammatory responses, expression of early-response genes, such as protooncogenes, growth factors, cytokines and chemokines, are physiologically returned to low levels or kept silent, in concert with transcriptional repression, also through ARE-mediated acceleration of mRNA decay and translational silencing [17, 18]. Failure of these post-transcriptional mechanisms of rapid shut-off has been strongly associated with cancer and other diseases [45–47]. The relevance ascribed to the control of mRNA turnover in inflammation and immunity [18, 48] allows the hypothesis that, in inflammatory and allergic diseases, alteration of proper and timely mRNA degradation of inflammatory transcripts (chemokines included) could result in their aberrant stabilisation, leading ultimately to a chronic increase of inflammatory protein production. This view is also supported by several lines of evidence obtained *in vivo*: mice carrying a deletion of the ARE in the TNF-α gene manifested an early inflammatory response within the joints and the bowel, which strongly resembled the infiltrate present in human rheumatoid arthritis and Crohn's disease, respectively. These mutant mice also displayed high circulating levels of TNF-α, resulting from a decrease in the rate of TNF-α mRNA decay and from a loss of translational inhibition [32]. Interestingly, very similar inflammatory responses and alterations in the rate of TNF-α mRNA decay were found in mice lacking tristetraprolin (TTP), an ARE-binding protein that binds to the TNF-α 3′-UTR and promotes its rapid decay [49].

Furthermore, recent data point at the regulation of mRNA stability as an important, novel mechanism that can determine

the differential expression of Th1/Th2 cytokines. BUTLER *et al*. [50] reported that, in the Th2-biased mouse strain DBA/2, increased stabilisation of IL-4 and IL-13 mRNAs largely accounted for increased expression of these cytokines from activated T-cells, in comparison with the cytokine levels found in the Th1-biased C56BL/6 strain.

POST-TRANSCRIPTIONAL REGULATION OF CHEMOKINE EXPRESSION: TODAY'S VIEW

In vitro studies on the post-transcriptional regulation of chemokines are increasingly revealing the impact of this mechanism in determining chemokine expression. A wide spectrum of stimuli has been found, in different cell types, to trigger changes in mRNA turnover of several chemokines (table 3), as follows: proinflammatory and immunomodulatory cytokines, such as TNF-α, IL-1, IL-4, interferon (IFN)-γ and IL-10 [31, 51–60]; stress-related signals, like hypoxia [61, 62]; infectious agents, such as viruses [63, 64] or bacterial-derived products like lipopolysaccharide (LPS) or formyl-methionyl-leucyl-phenylalanine (FMLP) [65, 66]; and other stimuli, such as nitric oxide [67] and activated protein C [68]. Importantly, post-transcriptional regulatory mechanisms are also emerging as critical in the ability of glucocorticoids, the major therapeutic drug class for allergic diseases, to inhibit chemokine expression [33, 69]. It is also interesting to note that T-cell-derived products selectively expressed in polarised inflammatory responses, such IFN-γ and IL-4, may utilise post-transcriptional pathways to exert opposite effects on chemokine gene expression. For example, in human monocytes, IFN-γ upregulates the expression of CXCL8 by increasing in mRNA stability [56], whereas, in the same cell type, IL-4 downregulates CXCL8 expression by decreasing the half-life of its mRNA [54].

The available *in vitro* studies on chemokine mRNA turnover have been mostly performed using the transcriptional inhibitor, actinomycin D. According to this protocol, cells are treated with inducing stimuli to achieve steady-state mRNA levels for the gene of interest, and then the total mRNA is either harvested without further treatment at the end of the challenge period, or isolated at various time intervals after incubation with actinomycin D. This experimental approach delivers a fast and efficient blockade of transcription and has yielded a large and valuable body of data on the changes in the half-life of chemokine RNA transcripts following a variety of cell stimulation protocols. However, this approach has critical limitations due to its cellular toxicity and potential direct effects on the stability of some mRNAs [81]. Therefore, different approaches are needed to validate the results obtained with actinomycin D. A more recent set of studies has examined in greater detail the mechanisms regulating chemokine mRNA turnover at a molecular level. Most of these studies use a transcriptional pulsing strategy [81], a method that allows studying mRNA decay in isolation from transcriptional activation and identifies the regions that regulate this process. This experimental protocol employs transient transfections with chimeric constructs, in which the 3′-UTRs or other more specific regulatory sequences (*i.e.* ARE-rich sequences) of the gene of interest are inserted in a reporter gene that is under the control of transiently induced promoters, such as the c-fos serum-inducible or the tetracycline (Tet)-regulated promoter system. A transient burst of the reporter mRNA transcription is achieved by varying the amount of serum or Tet in the medium for a set time. The termination of the transcriptional activity of the reporter mimics the effect of the transcriptional inhibitors, without perturbing any transcriptional process other than that occurring in the transfected construct. The insertion of the regulatory sequence influences the rate of decay of the reporter mRNA, and allows the determination of the destabilising potential of that sequence. Modification of the inserted sequence, by deletion or mutation, can be used to uncover

TABLE 3 Studies on chemokine mRNA turnover

Chemokine	Alternative name	Reported stimuli		[Refs]
		Increase mRNA stabilisation	Induce acceleration of mRNA decay	
CXCL1	GRO-α	LPS, IL-1		[52, 65, 70, 71]
CXCL2	GRO-β	LPS, IL-1		[52, 65, 70, 71]
CXCL3	GRO-γ	LPS, IL-1		[52, 65, 70, 71]
CXCL8	IL-8	LPS, IL-1, TNF-α, IFN-γ, NO, adenovirus, hypoxia	IL-4, IL-10, GCs	[31, 54, 56, 60, 62, 64, 65, 72–75]
CCL2	MCP-1	APC, hyperoxia, IL-1, LPS	Hypoxia, GCs	[31, 61, 65, 68, 76, 77]
CCL3	MIP-1α	LPS	IL-10	[60, 65]
CCL4	MIP-1β	LPS	IL-10	[60, 65]
CCL5	RANTES	RSV		[63]
CCL11	Eotaxin	TNF-α, IL-4, IFN-γ	GCs	[55, 78, 79]
CCL13	MCP-4		GCs	[80]
CCL20	MIP-3α	FMLP		[66]

GRO: growth-related oncogene; IL: interleukin; MCP: monocyte chemotactic protein; MIP: macrophage inflammatory protein; RANTES: regulated upon activation, normal T-cell expressed and secreted; LPS: lipopolysaccharide; TNF: tumour necrosis factor; IFN: interferon; NO: nitric oxide; APC: activated protein C; RSV: respiratory syncitial virus; FMLP: formyl-methionyl-leucyl-phenylalanine; GC: glucocorticoid.

key structural regions involved in this process. Moreover, the involvement of signalling pathways governing these processes can be also studied using this system by co-transfection of either constitutively active or dominant negative forms of signalling molecules [51].

Post-transcriptional regulation of CXCL8: a model for the study of molecular mechanisms of chemokine mRNA turnover

Multiple, heterogeneous AREs are present in the 3′-UTRs of several chemokine transcripts, such as CXCL1, CXCL2 and CXCL3 (growth-related oncogene (GRO)-α, GRO-β and GRO-γ) [27], CXCL8 [82], CCL2 [83], CCL3 (macrophage inflammatory protein (MIP)-1α) [84], CCL11 [85] and CCL20 (MIP-3α). However, despite the absence of AREs in the 3′-UTR of CCL5 and CCL13 [86], CCL13 mRNA decay is accelerated in airway epithelial cells following incubation with glucocorticoids (GCs) [80], whereas CCL5 mRNA stability is increased after challenge with respiratory syncitial virus [63], pointing at the involvement of other RNA motifs in the regulation of the mRNA turnover of these transcripts.

As mentioned previously, very little is known about the functionality of the AREs and other RNA motifs present in these transcripts, the identity of the factors binding to them, and the signalling pathways regulating chemokine mRNA stability and/or translation. The largest body of data on this topic has so far been obtained using CXCL8 as a model, for which extensive studies have identified several of the mechanisms of post-transcriptional control [72, 87].

The mature CXCL8 transcript displays a long 3′-UTR that contains multiple and sometimes clustered AUUUA motifs, in a context of A- and U-rich sequence stretches. The expression of CXCL8 is chiefly characterised by rapid, wide variations in the mRNA and protein levels in response to cell stimulation by inflammatory cytokines, pathogens or stress. This chemokine is barely detectable in many cell types in homeostatic conditions, but according to the type of cell stimulation it can be upregulated up to 100-fold. CXCL8 mRNA displays a long half-life when induced by IL-1β or TNF-α [73, 88], indicating a potential role for mRNA stabilisation in the strong increase of steady-state mRNA levels in response to stimulation. HOFFMANN *et al*. [87] proposed a model, based on their studies as well as on data from several groups, in which the extent of CXCL8 production following a specific stimulus results from the combination of at least three regulatory mechanisms as follows: 1) the release of the gene promoter from transcriptional repression; 2) transcriptional activation by nuclear factor (NF)-κB- and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK)-mediated pathways; and 3) stabilisation of mRNA by the p38 mitogen-activated protein kinase (MAPK) pathways (also known as SAPK2). Collectively, the studies by HOFFMAN *et al*. [86] indicated that the relative contribution of these mechanisms changes with the activation state of the cell and the nature of the activating stimulus. Briefly, transcriptional repression, together with the rapid turnover of IL-8 mRNA accounts for the low levels of IL-8 in unstimulated cells. Transcriptional activation mediated by NF-κB and JNK pathways is indispensable for IL-8 induction, whereas the activating protein-1 and CCAAT-enhancer binding protein sites are not essential for induction, but appear to promote maximal transcriptional activation. The occurrence of a third signal mediated by the p38 MAPK, induced by strong inflammatory stimuli such as IL-1, TNF-α and LPS, mediates CXCL8 mRNA stabilisation. Importantly, integration of this latter mechanism with those occurring at transcriptional level provides the largest increase in CXCL8 protein output [87].

The transcriptional pulsing approach illustrated previously was used to demonstrate the involvement of the CXCL8 3′-UTR in mediating basal mRNA decay and stimulus-induced stabilisation, and to define the signalling pathways involved [51]. A cDNA fragment of the CXCL8 3′-UTR encompassing the ARE regions was inserted into the 3′-UTR of a rabbit β-globin DNA construct, under the transcriptional control of a Tet-responsive promoter. Half-life measurement of the β-globin mRNA in HeLa cells transfected with this chimeric construct revealed a sharp acceleration of the degradation of the insertless β-globin mRNA, which displays a long half-life, indicating that the subcloned region of the CXCL8 3′-UTR contains sequences that mediate mRNA decay. Interestingly, deletional mutants of the 3′-UTR region subcloned in the reporter construct revealed that the minimal destabilising sequence requires the presence of neighbouring regions surrounding the AU-rich element, indicating that AREs are an essential but not exclusive part of the RNA cis-elements used by regulatory RNA-binding proteins [51]. Furthermore, cell treatment with IL-1 significantly increased the reporter mRNA half-life, suggesting that the subcloned region of the CXCL8 3′-UTR was sufficient to infer stabilisation of the reporter transcript by activating stimuli.

IL-1 mediates many of its effects by activating signalling pathways under the control of the MAP kinase kinase kinase, MEKK1. In the study mentioned previously, transient co-expression of a constitutively active form of MEKK1 also induced a sharp increase in the half-life of the endogenous CXCL8 mRNA, as well as the β-globin mRNA bearing the CXCL8 3′-UTR sequence. MEKK1 regulates multiple regulatory pathways, such as NF-κB, SAPK/JNK kinase, extracellular signal-regulated kinase (ERK) and p38 MAPK pathways [51]. To further define which pathway was mediating the reporter mRNA stabilisation, WINZEN *et al*. [51] used overexpression of either dominant negative mutants or constitutively active kinases that selectively activate the different pathways. These experiments revealed that MKK6, a selective activator of the p38 MAPK pathway [89], was uniquely capable of inducing a marked stabilisation of the reporter mRNA, suggesting the specific involvement of the p38 MAPK pathway in CXCL8 mRNA stabilisation. In the same study, similar strategies were used to identify mitogen-activated protein kinase-activated protein kinase (MAPKAP)2, also known as MK2, as the downstream target of the p38 MAPK.

It is important to note that the MK2 kinase has been recognised to be crucial in the regulation of mRNA stability and translation of IL-6 and TNF-α, respectively [90], and it is increasingly viewed as an important gatekeeper in post-transcriptional regulation [91]. Furthermore, studies conducted in murine macrophages have shown that MK2 phosphorylates an ARE-binding protein, the heterogeneous nuclear ribonucleoprotein (hnRNP) A0. This protein binds *in vitro* to MIP-2, the mouse homologue of human CXCL1 (GRO-α), as well as to

TNF-α and cyclooxygenase (COX)-2 mRNAs [92]. In the study by ROUSSEAU *et al*. [91], the aforementioned mRNAs were stabilised following cell stimulation with LPS, and cell treatment with chemical inhibitors of the p38 MAPK cascade abolished the LPS-induced stabilisation of MIP-2 mRNA, as well as the production of MIP-2 protein. The same treatment also prevented MK2-dependent phosphorylation of hnRNP A0, as well as binding of this protein to the target mRNAs, suggesting that LPS-induced stabilisation of several inflammatory transcripts, including the MIP-2 chemokine, is a p38 MAPK-dependent process that ultimately targets regulatory proteins interacting with AREs [92].

The involvement of p38 MAPK in mRNA stabilisation and/or translation has been found to be shared by several inflammatory genes, such as COX-2, IL-6, TNF-α and GM-CSF [32, 51, 92, 93], indicating that the p38 MAPK pathway acts as a potent amplifier of the inflammatory response. The effect of p38 MAPK activation on the mRNA stability of ARE-bearing genes has been studied on a large scale by employing cDNA arrays highly enriched with such genes [65]. THP-1 monocytic cells were stimulated with LPS, and either harvested or further treated with actinomycin D, in the presence or absence of the p38 MAPK inhibitor SB203580. Interestingly, this study showed that the increase in mRNA stability of genes that were induced by LPS is regulated by p38 MAPK only in a subset of ARE-containing targets. Several chemokines, including CXCL1, CXCL2, CXCL3 and CXCL 8, together with CCL2, CCL3 and CCL4, were identified among those proinflammatory genes showing a clear-cut difference in the rate of mRNA decay following inhibition of p38 MAPK activity [65]. Further studies are necessary to validate the participation of AREs in the p38 MAPK-mediated stabilisation of these molecules, as demonstrated for CXCL8 mRNA [51], and to further evaluate the downstream signalling molecules and the RNA-binding proteins regulating the mRNA turnover of the newly identified chemokine targets.

Other stress-induced kinase pathways, such as ERK and JNK, have also been shown to regulate the cytoplasmic transport and turnover of mRNAs [18]. These signalling pathways could be an alternative or complementary to p38 MAPK in mediating changes in chemokine mRNA decay, possibly through factors interacting with different motifs present in other regions of the transcripts. For example, a JNK-responsive element present in the 5′-UTR of the IL-2 mRNA was found to convey stimulus-induced stability to this transcript in Jurkat cells through the interaction with the RNA-binding proteins nucleolin and YB-1 [37] (fig. 1), and in cooperation with elements present in the 3′-UTR of the IL-2 mRNA. Increased stability of CCL20 mRNA induced by FMLP appears to involve the ERK pathway [66]. Moreover, RNA-binding proteins that regulate RNA turnover are chiefly regulated by other post-translational modifications, such as methylation [94], and the role of these signalling pathways in regulating proteins involved in chemokine mRNA turnover is yet to be explored.

RNA-binding proteins as mediators of chemokine mRNA turnover: the role of HuR in cytokine-induced CCL11 mRNA stabilisation

The CC chemokine CCL11 displays strong and selective chemoattractant and activating properties towards eosinophils [95], and several studies have established a key role for this molecule in allergic inflammation [12] and other biological functions, such as angiogenesis [96]. CCL11 is strongly upregulated in airway epithelial cells by IL-4, especially in combination with TNF-α [80, 97], and its expression is profoundly inhibited by GCs [80]. Several studies using epithelial cells transfected with full-length CCL11 promoter constructs showed that cytokine-induced upregulation, as well as GC-mediated inhibition of the eotaxin gene, appeared to be mediated only partially through transcriptional regulation [78, 80]. The 3′-UTR of CCL11 contains a tandem AUUUA sequence in the context of a TA-rich region [85], indicating a potential post-transcriptional regulation. Indeed, it has been shown *in vitro* that CCL11 expression is critically regulated at the level of mRNA turnover in airway epithelial cells. The potent topical GC, budesonide, induced a significant decrease of cytokine-induced CCL11 production also by accelerating the decay of its mRNA [80]. On the contrary, the combination of TNF-α plus IL-4, which yields the strongest synergistic effect on eotaxin protein secretion [80], significantly increases the stability of eotaxin mRNA [55]. Such changes appear to be mediated by the 3′-UTR of the CCL11 mRNA, as both the acceleration of mRNA decay induced by budesonide and the cytokine-induced increase in mRNA stability were reproduced using the transcriptional pulsing approach, in which the expression of a chimeric β-globin reporter mRNA bearing the CCL11 3′-UTR was monitored in transfected National Institutes of Health 3T3 cells, following treatment with either budesonide or TNF-α plus IL-4 [55, 79].

It is well known that the synergism between TNF-α and Th1 or Th2 cell-derived products, such as IFN-γ and IL-4, provides a powerful amplifying signal for the upregulation of genes preferentially expressed during Th1/Th2 responses, as well as for the selective activation and recruitment of inflammatory cells during these responses [8, 10]. In particular, the combination of IL-4 and TNF-α upregulates the expression of vascular cell adhesion molecule-1 in endothelial cells and induces the expression of Th2 cell and eosinophil chemoattractants from epithelial cells, such as CCL17, CCL22 and the eotaxins [10]. In light of this relevant proinflammatory response, the mechanism by which this cytokine combination promotes CCL11 mRNA stabilisation might represent a significant proinflammatory pathway. ATASOY *et al*. [55] found that the ARE-binding protein HuR, which determines increased mRNA stability and/or increased translation of the target transcripts [98–100], plays a role in mediating the effect of cytokines on CCL11 expression. HuR (also known as HuA) is a member of the Hu protein family of RNA-binding factors, which is homologous to the Drosophila embryonic lethal abnormal vision (ELAV) protein family. HuR is the only ubiquitously expressed ELAV protein, while three additional members of this family (HuB, C and D) are expressed exclusively in neuronal tissue, with additional gonadal expression only for HuB [101]. HuR has been shown to bind *in vitro* to ARE elements in mRNAs encoding genes that critically regulate proliferation and stress responsiveness, such as p21, cyclin A, cyclin B1 [102, 103], as well as transcription factors, such as c-fos [104]. The unique mRNA-stabilising function of HuR has also been documented by using chimeric ARE-containing constructs and in cells overexpressing HuR [30, 55].

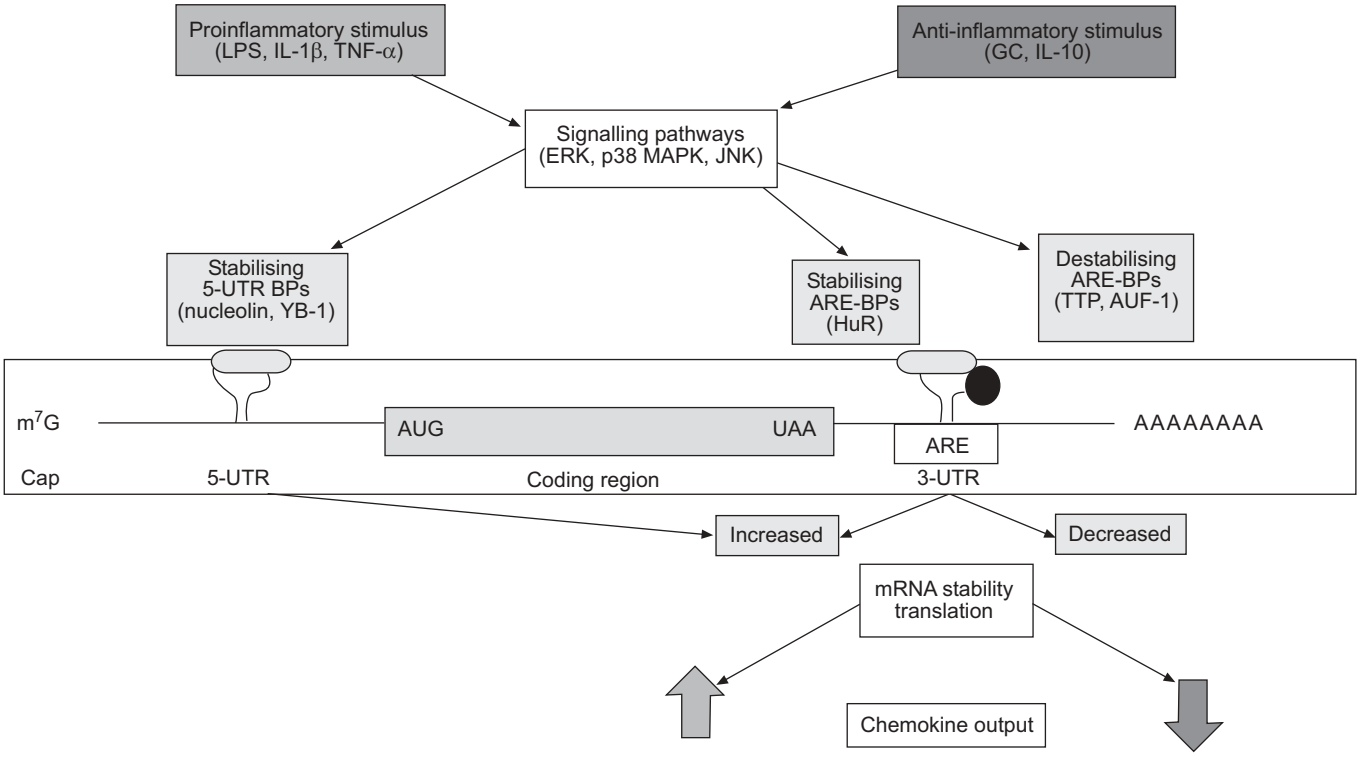

FIGURE 1. Regulatory signals and RNA determinants potentially mediating post-transcriptional regulation of chemokine mRNA. Post-transcriptional regulation, either mediated by adenylate-uridylate-rich elements (AREs) or by other regulatory sequences located in another part of the mRNA molecule such as the 5′-untranslated region (UTR), can mediate both pro- and anti-inflammatory signals aimed at rapidly adapting the level of chemokine expression in response to changes in tissue environment. Regulatory sequences in the 5′ or 3′ mRNA UTRs are designed as stem-loop structures to highlight the importance of their secondary structure, in addition to their sequence, for their functionality. Proinflammatory stimuli have been shown to activate different signalling pathways in order to carry on mRNA stabilisation and increased translation, in cooperation with increased gene transcription, to increase chemokine levels, as described for the involvement of p38 mitogen-activated protein kinase (MAPK) in CXCL8 mRNA stabilisation. Conversely, post-transcriptional regulation has also been shown to be utilised by anti-inflammatory signals, such as glucocorticoids (GCs) or interleukin (IL)-10, in order to decrease the output of chemokines. For the majority of the chemokines recognised to be post-transcriptionally regulated, the identity of the signalling pathways regulating this process is not yet fully uncovered, nor are the mechanism(s) by which these pathways affect the downstream RNA-binding factors that govern mRNA transport, stability and translation. An increase in the expression of RNA-stabilising factors, or the induction of post-translational modifications (phosphorylation, methylation) favourable for their competitive binding might be responsible for increased mRNA stabilisation and/or translation of chemokines, thus contributing to increased chemokine output (↑). The same outcome might be mediated by a decrease in the expression, or by displacement of mRNA-decaying factors from their RNA binding sites by stimulus-induced changes in binding affinity. Conversely, a decrease in chemokine output (↓) could be due to increased synthesis or increased binding activity of RNA decay-promoting factors, or an opposite effect on the expression or function of mRNA-stabilising proteins. Ultimately, it is the regulation of the interplay between factors that positively or negatively affect mRNA stability and/or translation that could determine how either pro- or anti-inflammatory mediators post-transcriptionally affect the level of chemokine production. LPS: lipopolysaccharide; TNF: tumour necrosis factor; ERK: extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; BP: binding protein; YB: Y-box binding protein; TTP: tristetraprolin; AUF: ARE/poly(U)-binding/degradation factor.

In the study by ATASOY *et al*. [55], treatment of human airway epithelial cells with TNF-α and IL-4 induced the cytoplasmic localisation of HuR, a process linked with activation of HuR function and previously described to occur under conditions of cellular stress [103]. Furthermore, such treatment enhanced binding of HuR to the endogenous CCL11 mRNA. In fact, CCL11 mRNA was amplified from the mRNA pool obtained by immunoprecipitation (IP) of endogenous mRNP complexes from lysates of cells stimulated with or without TNF-α plus IL-4, using a mouse monoclonal antibody specific for HuR. Cytokine stimulation resulted in >40-fold enrichment in CCL11 mRNA over unstimulated samples. Lastly, CCL11 expression was significantly increased upon treatment with TNF-α and IL-4 in epithelial cells overexpressing HuR when compared to the mock-transfected cells. This increase was due, at least in part, to increased mRNA stability, which was assessed using the actinomycin D protocol on cytokine-treated cells transfected with HuR [55].

The notion that cytokines can activate HuR provides direct evidence of the importance of post-transcriptional regulation in the mechanism by which T-cell-derived products, in conjunction with inflammatory mediators, cause strong upregulation of chemokine production from epithelial cells and, by doing so, sustain a proinflammatory loop. It is interesting to note that HuR has been increasingly recognised as being involved in the mRNA stabilisation of an impressive number of ARE-bearing genes that are critical mediators of inflammatory and immune reactions. The report on CCL11 [55] has added the first CC chemokine to a list of genes that, in the span of few years, has included TNF-α, IL-3, IL-6, CXCL8, GM-CSF, COX-2, vascular endothelium growth factor, transforming

growth factor-β, inducible nitric oxide synthase, CD154 (the CD40 ligand) and the β-adrenergic receptor as HuR targets [34, 41–43, 45, 105–112]. For these genes, binding of HuR to the 3′-UTR of their transcripts or to chimeric constructs containing the target 3′-UTRs was demonstrated, together with increased stability of their mRNAs. These data strongly suggest a regulatory role of HuR in inflammation. Currently, the role of HuR in the expression of epithelial-derived chemokines in experimental conditions that reproduce polarised inflammatory responses is under systematic study.

In an extensive study on the mechanism of HuR activation [113], stress-induced HuR translocation in the cytoplasm was found to be uniquely inhibited by the adenosine monophosphate-activated protein kinase (AMPK), an enzyme which functions as a cellular sensor of metabolic stress [114]. As a result, AMPK inhibits the downstream binding of HuR to its targets, as well as their expression and stability. Conversely, inhibition of AMPK markedly increases the cytoplasmic translocation of HuR, which correlated with increased HuR function [113]. In the study identifying AMPK as a regulator of HuR activation, other stress-regulated protein kinases that were critically involved in post-transcriptional regulation, such as protein kinase (PK)C and MAPK (p38, JNK, ERK), did not affect HuR cytoplasmic translocation [113]. Although HuR translocation appears to be exclusively AMPK dependent, several reports indicate that binding of HuR to its targets in the cytoplasm can be regulated by other signalling pathways. For example, HuR binding to several targets has been reported to be regulated by p38 MAPK [107, 115, 116] or by the ERK pathway [117]. This is probably due to interaction with yet unidentified targets of these pathways, as HuR is reported not to be a phosphoprotein, and to be regulated by methylation [94, 107, 116].

Studies currently underway are investigating whether cytokines promote cytoplasmic localisation of HuR in a fashion similar to stress-induced activation, that is, through inhibition of AMPK, or modulate its binding to inflammatory and chemokine targets by activation of the p38 MAPK cascade, given the established role of this kinase pathway in regulating the expression of CCL11 and other chemokines [118, 119].

Post-transcriptional regulation of chemokines as target of anti-inflammatory molecules: GCs and IL-10

Following activation of an immune response due to injury or infections, inflammatory and anti-inflammatory processes occur in a coordinate fashion, with the aim of fighting the offending cause while preventing or minimising tissue damage to the host, through a tight control of the duration and the intensity of the inflammatory response. Changes in gene expression occurring during these anti-inflammatory processes can also be achieved, just as for proinflammatory ones, through post-transcriptional regulatory mechanisms. The role of these mechanisms in anti-inflammatory action is a relatively new field of investigation, and it opens new and exciting research avenues aimed at the identification of novel regulatory pathways and molecules that are potentially targetable for therapeutic purposes. Given the importance of chemokines in the establishment and maintenance of chronic inflammation, it is valuable to understand to what extent the modulation of their post-transcriptional regulation may contribute to the inhibition of their expression by anti-inflammatory agents, such as GCs, or by endogenous immunomodulatory molecules, such as IL-10.

Chemokine genes are being increasingly identified among the inflammatory genes that are post-transcriptionally regulated by GCs (table 3) [69, 78]. However, the molecular mechanisms of the effect of GCs on chemokine mRNA stability and translation are yet to be fully characterised. Regarding the cis-elements involved, acceleration of chemokine mRNA decay by GCs could be ARE dependent, as previously demonstrated for IFN-β and COX-2 [93, 120]. Such elements may play a role in GC-induced eotaxin inhibition in airway epithelial cells, as GC treatment accelerated the decay of a reporter containing the ARE-bearing eotaxin 3′-UTR [79]. The presence of AREs in the 3′-UTR by itself is not always predictive of GC-mediated changes in chemokine mRNA turnover. For example, mRNA decay does not account for the inhibition of MIP-1α exerted by dexamethasone in human monocytes, despite the existence of four AUUUA sequences in the 3′-UTR of MIP-1α mRNA [121]. Similarly, rat MCP-1 mRNA stability was decreased by GCs in smooth muscle cells through a unique sequence in the 5′-UTR, not through the ARE-bearing 3′-UTR [76]. Furthermore, in airway epithelial cells, budesonide shortened the half-life of the ARE-less MCP-4 mRNA [80]. These data suggest that AREs may be, in some instances, either not involved or not sufficient in promoting the binding of regulatory proteins and conveying the regulatory effect on mRNA turnover. To take place, such a process may involve other yet uncharacterised cis-elements present in the 3′-UTR or in other parts of the mRNA of chemokines. These additional sequences could work in concert with AREs or independently, and would contribute to implement the post-transcriptional GC action. It is important to point out that the presence of sequences linked to changes in mRNA stability is not sufficient to infer post-transcriptional regulation of a certain mRNA. These determinants are crucial for the formation of secondary structures of the mRNA molecule (*i.e.* stem loops, or contact between the 5′- and 3′-UTRs), which ultimately allow the binding-site recognition by RNA-binding proteins that can either promote or delay mRNA stability and translation [17, 28, 122]. The secondary structure and the binding affinity of different proteins to these mRNA binding sites is, in turn, influenced by several intracellular parameters, such as ion strength and temperature [122] and, possibly, other unknown factors. Moreover, the expression of RNA-binding factors and their activation can also be stimulus and cell dependent, such that the contribution of post-transcriptional regulation to the expression of a single transcript may vary according to the cell source and environment. A good example of this level of selectivity is given by the different contribution of mRNA decay reported in literature on the inhibitory effect of GCs on CXCL8. In human fibroblasts, dexamethasone increased CXCL8 mRNA decay induced by TNF-α in a protein synthesis-dependent manner [74]. Similarly, for human bone marrow stromal cells, in nuclear run-on experiments, the transcription of CXCL8 induced by IL-1β was not inhibited by cell treatment with dexamethasone, whereas the chemokine mRNA decay was found to be accelerated by the GC treatment [123]. In contrast, in primary epithelial cells, inhibition of CXCL8 by GCs was not due to an effect on mRNA decay [124].

Most of the regulatory mRNA–protein interactions, the signalling pathways that regulate them and the effect of GCs on these parameters are still not determined in chemokine biology. There are multiple molecular mechanisms by which glucocorticoids act on post-transcriptional events, and they are still far from being fully understood. Studies aimed at the identification of these mechanisms have been recently reviewed [69].

Inflammatory and immune responses are also downregulated by endogenous immunomodulatory molecules, such as IL-10. This cytokine is produced by CD4+ T-regulatory and antigen-driven Th2 cells, as well as other numerous immune cells, and is a potent negative regulator of gene expression in macrophages [58, 125–127]. Post-transcriptional effects have been shown to play a relevant role in the mechanisms of IL-10-mediated gene regulation [128, 129]. IL-10 decreases the mRNA stability of mouse and human monocyte and macrophage-derived chemokines, such as CCL3, CCL4 and CXCL8, induced by inflammatory stimuli cells, such as LPS, IL-1β and the extracellular matrix component hyaluronan [58, 126, 127, 129]. The molecular mechanisms of the activity of IL-10 on chemokine mRNA decay have been further defined in a study that examined the opposite effects of LPS and IL-10 on the post-transcriptional regulation of KC (the murine CXCL1) in mouse macrophages [59]. In this study, LPS promoted the stability of the endogenous KC mRNA, as well as that of a transfected Tet-regulated construct expressing KC mRNA. Co-culture of LPS-treated cells with IL-10 indicated that this cytokine promoted KC mRNA decay primarily by antagonising LPS-induced mRNA stabilisation, rather than by a direct effect on the mRNA decay process. In fact, using the Tet-regulated system, IL-10 did not change, in the absence of LPS, the rate of KC mRNA decay. The regulation of KC mRNA stability by LPS and the antagonising effect of IL-10 were both found to be ARE dependent. Therefore, the interference of IL-10 with the LPS-sensitive pathway might entail the regulation of ARE-mediated events, such as changes in the expression or function of RNA-binding proteins, or regulation of upstream signalling pathways mediating the mRNA-stabilising effects of LPS.

POST-TRANSCRIPTIONAL REGULATION OF CHEMOKINES: FUTURE CHALLENGES

The studies described so far indicate a growing attention to the post-transcriptional regulation of chemokines, and an increased appreciation for the impact of these mechanisms in determining the magnitude of the inflammatory response.

Numerous issues still remain to be addressed. First, the RNA motifs present in the regulatory regions of chemokine transcripts, such as the 5′- and 3′-UTRs, need to be identified, and their ability to mediate changes in stabilisation of endogenous transcripts and reporter constructs, as well as to affect translation, need to be verified. Definition of these motifs will be critical in identifying the RNA-binding proteins that probably interact with such sequences. Recently, knowledge about the relationship between RNA-binding proteins and their targets, and of the functional outcome of this association, has been greatly advanced by the experimental approach (pioneered by J. Keene) defined as ''ribonomics'' [130]. This approach is based on the hypothesis that a group of biologically related mRNA molecules sharing a recognition motif, such as the AREs, could be regulated by the interaction with a common RNA-binding protein, thus creating subsets of transcripts whose fate is determined post-transcriptionally in a coordinate fashion, as post-transcriptional operons [23, 24]. To identify the subsets of mRNAs that associate with a given RNA-binding protein *in vivo* under specific conditions, the experimental approach utilises the IP of mRNPs using specific antibodies for an RNA-binding protein, and reveals the identity of the associated mRNAs by hybridisation assays. In a recent array study, a pool of transcripts associated with HuR has been identified using this approach in the human colonic cancer cell line RKO [28]. A combined computational analysis of the primary sequences and secondary structures of the HuR mRNA targets has allowed the identification of a 17–20-base-long uracil-rich RNA motif. This binding motif was found in the vast majority of the transcripts previously known to be regulated by HuR, including CCL11. Using this methodology, it was possible to predict, and subsequently to validate, novel targets of HuR among transcripts in the gene database.

As the knowledge regarding the recognition motifs for more RNA-binding protein increases, identification of these critical regions in chemokine mRNAs will be useful in identifying regulatory molecules and pathways that are potentially relevant for chemokine expression and, therefore, targetable by therapy.

Secondly, the signalling pathways that regulate post-transcriptional control of chemokines have only recently started to be identified, and the studies conducted so far have already uncovered their complexity. As discussed previously, ARE-mediated regulation can be mediated by different pathways, sometimes in a stimulus- or cell-specific fashion. For example, stabilisation of CXCL3 mRNA is sensitive to p38 MAPK inhibition in the THP-1 monocytic cell line when induced by LPS [65], but not in HEK 293 cells when induced by IL-1 [31]. It also needs to be established whether a certain signalling pathway controls RNA turnover or translation. The MAPKAP kinase (MK2), for example, regulates ARE-mediated stability of IL-6 and IL-8 mRNA, while controlling ARE-dependent translational control of TNF-α [51, 90].

Thirdly, the biology of the RNA-binding proteins mediating post-transcriptional control of chemokines needs to be unravelled. A rapid, transient increase in the expression of ARE-bearing inflammatory genes following immune activation is regulated post-transcriptionally through the integrated activity of RNA-binding proteins of opposite function [107]. In Jurkat cells, for example, RNA electromobility shift assays showed that the mRNA-stabilising protein HuR binds initially to the GM-CSF 3′-UTR in response to activation by mitogens, and, subsequently, binding of mRNA decay-promoting factors, such as TTP, follows [107, 131]. Indeed, induction of HuR precedes that of TTP after T-cell activation [131] and both proteins partially overlap in binding to a subset of ARE-containing 3′-UTRs, such as those of TNF-α, GM-CSF, IL-3 and c-fos [104, 131].

The establishment of HuR as a mediator of cytokine-induced stabilisation of the CCL11 mRNA suggests that similar pathways may occur in the regulation of chemokines

and opens the way to a broader investigation of the regulatory factors involved in this process, either those binding to the RNA or other proteins that, without direct association with the mRNA, may associate with the RNA-binding proteins and facilitate, or inhibit, the mechanisms responsible for chemokine mRNA transport, turnover or translation (fig. 1).

Several recent studies have revealed an increased expression and activation of HuR in different types of cancers. In these settings, HuR was found to bind to and coordinate post-transcriptional regulation of angiogenic and growth-related factors in cancer, providing validation of the post-transcriptional operon model in human disease [40–42]. Immune responses and inflammation are also characterised by proliferative responses and by remodelling processes that include angiogenesis [132], making such models applicable to these biological processes as well. It can be envisioned that aberrant mRNA stabilisation of early-response genes following stimulation with proinflammatory mediators may contribute to the establishment of chronic inflammatory conditions. In fact, the RNA-binding proteins governing this process, such as HuR, may no longer act in transient fashion, but rather remain constantly activated, as a result of being themselves targets of signalling pathways initiated by inflammatory mediators, thus prolonging transcript stability and chronically sustaining an inflammatory response.

Increased awareness of the function of these RNA-binding molecules, and of the potential role of their altered function in diseases, has led to more systematic studies, both in human and mouse, on their tissue distribution and subcellular localisation, as well as on the stimuli and signalling pathways that regulate their function [113, 115, 116, 133–138]. The task ahead should be to focus on studies searching for alterations of RNA-binding protein expression, function and signalling during inflammatory and allergic processes, where chemokines and other proinflammatory molecules that are regulated through their function play a major role. Furthermore, it will be necessary to correlate such findings with the increased expression and changes in the half-life, or translation, of their target mRNAs [139]. As RNA-binding factors appear to post-transcriptionally regulate the coordinate expression of multiple genes that participate in a biological process, identification of their aberrant level or function may became an important therapeutic target, as modulation of their activity may profoundly affect the protein levels of multiple targets, and, therefore, convey a powerful anti-inflammatory action.

CONCLUSION

The amplitude of an inflammatory reaction can be greatly affected by post-transcriptional regulatory mechanisms that influence the levels of many key genes participating in that response, including chemokines. It is becoming increasingly clear that perturbations of these mechanisms can be pathogenetic [32, 46, 47, 140]. The molecular basis of these processes is now the focus of intense research, aiming at a deeper understanding of the signalling pathways, the regulatory sequences and the binding factors ultimately conveying the changes in mRNA turnover and translation. The definition of the cell and stimulus specificity of these mechanisms will also be crucial for considering these novel, important pathways as targets for anti-inflammatory therapies.

ACKNOWLEDGEMENTS

C. Stellato is indebted to V. Casolaro, S.N. Georas and R.P. Schleimer for helpful discussions on the topic of the review, and to L.A. Beck for kind help with the table regarding chemokine nomenclature.

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