Coordinated Post-Transcriptional Regulation of the Chemokine System: Messages from CCL2

Ronaldo P. Panganiban,¹ Becky M. Vonakis,² Faoud T. Ishmael,¹ and Cristiana Stellato^{2,3}

The molecular cross-talk between epithelium and immune cells in the airway mucosa is a key regulator of homeostatic immune surveillance and is crucially involved in the development of chronic lung inflammatory diseases. The patterns of gene expression that follow the sensitization process occurring in allergic asthma and chronic rhinosinusitis and those present in the neutrophilic response of other chronic inflammatory lung diseases such as chronic obstructive pulmonary disease (COPD) are tightly regulated in their specificity. Studies exploring the global transcript profiles associated with determinants of post-transcriptional gene regulation (PTR) such as RNA-binding proteins (RBP) and microRNAs identified several of these factors as being crucially involved in controlling the expression of chemokines upon airway epithelial cell stimulation with cytokines prototypic of Th1- or Th2-driven responses. These studies also uncovered the participation of these pathways to glucocorticoids' inhibitory effect on the epithelial chemokine network. Unmasking the molecular mechanisms of chemokine PTR may likely uncover novel therapeutic strategies for the blockade of proinflammatory pathways that are pathogenetic for asthma, COPD, and other lung inflammatory diseases.

Introduction

THE SUPERFAMILY of chemokines critically regulates the cellular trafficking occurring in both homeostatic and diseased states, such as inflammatory and neoplastic processes. The functions of these small proteins go well beyond the chemotactic activity that chiefly defines them, and nearly all cell types have been found to possess chemokine receptors, which make them susceptible to their wide range of regulatory instructions (Charo and Ransohoff 2006; White and others 2013). The regulation of innate and adaptive immune responses through control of the activation, phenotype, and trafficking of circulating leukocytes remains a defining and pivotal function of the chemokine superfamily, which is divided, based on the number and spacing of conserved cysteine residues, in the CXC, CC, CX3C, and C subfamilies. The CXC and CC subclasses, though with overlaps, segregate their control over different cell populations, with CXC members acting on effector function and diseases that are characterized by neutrophilic Th1-driven responses; CC chemokines instead exert potent effects on leukocyte trafficking in Th2-dependent, eosinophil-rich pathologic processes. Among CC chemokines, *CCL2*/monocyte chemoattractant protein-1 (MCP-1) acts as a potent agonist for monocytes, basophils, and dendritic cells and participates in phenotypic polarization of memory T cells toward a

Th2 phenotype (Gu and others 2000; Daly and Rollins 2003). On these bases, understanding the mechanisms of aberrant expression or function of chemokines may hold the key to major breakthroughs in the therapeutic management of inflammatory, autoimmune, and neoplastic diseases. However, chemokine biology offers major challenges to this aim, due to the predominant functional redundancy among members of each subclass and to the complexity of the control of their expression, spanning from transcriptional to post-translational and extracellular matrix-dependent mechanisms that collectively converge in defining functional specificity. Therefore, the antagonism of single chemokine receptors has shown only partial success as a therapeutic strategy, leading to a shift in research toward more specific downstream regulatory pathways modulating the chemokine network (White and others 2013). Among these pathways, the factors involved in post-transcriptional gene regulation (PTR), such as RNA-binding proteins (RBPs) and microRNAs (miRNAs), are increasingly scrutinized as potential targets, or conveyers, of therapies with immunomodulatory/anti-inflammatory action. These molecules—in tight integration with the upstream transcriptional control—coordinately regulate functionally related genes containing common untranslated sequence elements for regulations (USER), recognized by RBPs, and “seed regions” specific for miRNAs (Baltimore and others 2008; Anderson 2010).

¹Department of Biochemistry and Molecular Biology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania.

²Division of Allergy and Clinical Immunology, Johns Hopkins University, Baltimore, Maryland.

³Department of Medicine and Surgery, University of Salerno, Baronissi, Italy.

Collectively, these factors engage in sequence-specific, dynamic interactions and associate with ribonucleoprotein (RNP) complexes that may act in cooperative or antagonistic regulation of the targeted mRNA. Inflammatory signaling affects the RNP composition, through phosphorylation and other translational modifications of the RBP participating in the RNP, and directs the bound targets toward cytoplasmic sites of translation or decay, modulating the rate of these processes and determining the timing and amplitude of protein output (Anderson 2010). Genes involved in immune and inflammatory responses, including chemokines, are highly enriched in USER and, thus, are preferred targets of such mechanisms (Anderson 2008; Palanisamy and others 2012). Therefore, interventions aimed at modulating aberrant RNP complexes may convey a more targeted action toward the functionally related genes that are regulated through common post-transcriptional pathways (Keene 2007).

While post-transcriptional regulation of chemokines has been described elsewhere in detail (Fan and others 2005; Tauler and Mulshine 2009; Hamilton and others 2010), the next paragraphs will review studies that focused on the understanding of chemokines' global PTR using a ribonomic approach (Keene 2007), where genome wide-scale analysis identified chemokine transcripts clustered by association with a common RBP. The CC chemokine *CCL2/MCP-1* has been found to be a target of diverse post-transcriptional pathways in ribonomic-based studies (Ishmael and others 2011; Fan and others 2011), and its post-transcriptional regulation will be reviewed to highlight how mediators of PTR could modulate chemokine expression within pro- or anti-inflammatory contexts, acting through different regulatory elements present in the targeted transcripts.

Coordinate PTR of Functionally Related Genes: Focus on the Chemokine System

The factors and pathways conveying PTR are major determinants of inflammatory and immune responses, where the rise, fall, and duration of protein output in the expression of dangerous and protective genes are tightly regulated (Anderson 2010). Increasing experimental evidence indicates that such mechanisms are, indeed, critical in determining either the successful resolution of the immune response or its chronic overexpression (Anderson 2008). The process of mRNA turnover and translation is regulated by an interaction of cis-regulatory elements (Bakheet and others 2006), which are present more frequently in the untranslated regions (UTR) of the mature cytoplasmic mRNA, with RBP and miRNAs. These molecules are targeted by the same signaling pathways—chiefly mitogen-activated protein kinase (MAPK)—that modulate transcriptional regulation, providing a complex signaling system within the cell which underscores the integrative nature of the nuclear and cytoplasmic regulatory events (Kracht and Saklatvala 2002; Clark and others 2003, 2008).

Regulation of mRNA stability can have drastic effects on the level of gene expression. Small changes in mRNA half life, in the range of 2- to 4-fold changes, can lead to more than a 1,000-fold difference in mRNA levels (Ross 1995). Additional regulation at the level of protein translation enables the potential of profound effects on gene expression products. A major experimental evidence of such importance is constituted by the phenotypes of the mouse models

in which the PTR of the tumor necrosis factor- α (TNF- α) gene has been altered by disrupting the main RNP components regulating the mRNA metabolism, either by deletion of the cis-regulatory elements present in the mRNA 3' UTR (Kontoyiannis and others 1999) or by ablation of tristetraprolin (TTP) (Taylor and others 1996), an RBP promoting mRNA degradation. The two mouse models present largely overlapping, profound inflammatory phenotypes in which chronic inflammatory syndromes developed via the persistence of highly stable TNF- α mRNA and, consequently, increased protein levels.

The relevance of post-transcriptional mechanisms, among all the regulatory mechanisms determining the expression of a specific gene product, is growing in ranking, thanks to the discovery and characterization of post-transcriptional regulons (Keene 2001, 2007). Functionally related genes, such as cytokines and chemokines, may be co-ordinately regulated post transcriptionally in response to a certain stimulus via a specific RBP through common USER present in their sequences. The involvement of a common regulatory element can be hypothesized, for example, on the basis of data such as those obtained in THP-1 monocyte cells, in which lipopolysaccharide (LPS) challenge induced up-regulation of multiple chemokines (*CXCL1*, *CXCL2*, *CXCL3*, *CXCL8*, *CCL2*, *CCL3*, and *CCL4*) in association with an increase in their mRNA stability (Frevel and others 2003). Importantly, this process was found to be dependent on the p38 MAPK pathway, highlighting the integration of PTR with p38 MAPK-driven transcriptional mechanisms of gene regulation (Kracht and Saklatvala 2002). Using the ribonomic analysis, it is possible to understand whether under specific experimental conditions (such as the LPS stimulation of the previous study), a particular RBP is, indeed, orchestrating the levels of specific transcripts, grouped according to the process triggered by the stimulation, and recognized by the RBP through an interaction of its RNA-recognition motifs with the specific USER sequences. Technically, immunoprecipitation of mRNPs (RIP-ChIP) is performed using specific antibodies against an RBP, and the associated mRNA—in comparison with transcript pools obtained with an irrelevant control antibody—are identified by gene arrays (Tenenbaum and others 2002; Keene and others 2006). With this approach, the profiles of transcripts associated with several RBPs that are particularly relevant in immunity-like Hu-antigen R (HuR), T-cell intracellular antigen 1 (TIA), and TTP have now been defined in several experimental conditions (Lopez de Silanes and others 2004, 2005; Lai and others 2006). Further methodological developments, through the photo-activatable ribonucleoside-enhanced cross-linking and immunoprecipitation (CLIP) and other CLIP protocols (Hafner and others 2010), are currently expanding the map of interaction sites among RBP, miRNA, and transcripts and will likely contribute in the near future to additional knowledge of PTR networks for chemokine expression.

Inflammatory signals originated by the epithelium, in the context of innate and adaptive immune responses, critically orchestrate the recruitment and activation of leukocytes at sites of mucosal inflammation through the expression of cytokine and chemokine genes, whose profile is highly influenced by the immunomodulatory cytokines present in the specific inflammatory context: though with overlaps especially in the area of monocyte-activating CC chemokines, a predominant Th1-skewed stimulation, with its high levels of

interferon (IFN)- γ , favors the overexpression of CXC chemokines; while Th2, interleukin (IL)-4/IL-13-driven responses lead to eosinophil recruitment through increased levels of eosinophilic chemokines (CCL11, CCL13 and others) (Stellato 2007). These cytokines, acting with strong synergistic effect with macrophage-derived proinflammatory cytokines such as TNF- α , modulate epithelial gene expression at transcriptional and post-transcriptional levels (Matsukura and others 1999; Stellato and others 1999; Stellato and Beck 2000). This paradigm is particularly defined in airway epithelium, whose chemokine profile is a major determinant of the Th2-driven eosinophilic inflammation present in allergic airway diseases, such as asthma, and correlates with clinical readouts of disease activity (Fan and others 2005). The overwhelming majority of the epithelial-derived genes described in these settings represent transcripts bearing adenylylate/uridylylate (AU)-rich elements (AREs) and other USER sequences, making the occurrence of stimulus-dependent coordinated PTR very likely to be occurring. Post-transcriptional pathways have been, in fact, recognized in the regulation of several chemokines, and the factors mediating these effects are increasingly characterized (Fan and others 2005; Hamilton and others 2010).

One of the RBPs more extensively studied in chemokine PTR is HuR, the sole ubiquitous member of the Hu family of neuronal RBPs (Hinman and Lou 2008). HuR binds to ARE present in the 3' UTR of numerous inflammatory transcripts. It is functionally defined as a positive regulator of RNA-stability and/or translation, though mouse models for this factor suggest a more diverse functional spectrum with complex indirect effects (Katsanou and others 2005; Papadaki and others 2009). HuR is a well-recognized regulator of inflammatory genes, such as *TNF- α* , *IL-3*, *IL-6*, *IL-8*, *GM-CSF*, *COX-2*, *VEGF*, *TGF- β* , *iNOS*, *CD154* (the CD40 ligand), and the β -adrenergic receptor (Ma and others 1996; Levy and others 1998; Ford and others 1999; Blaxall and others 2000a, 2000b; Rodriguez-Pascual and others 2000; Dean and others 2001; Dixon and others 2001; Ming and others 2001; Nabors and others 2001, 2003; Goldberg-Cohen and others 2002; Sakai and others 2003). Among the cytokine genes clustered on chromosome 5q and therefore relevant for asthma pathogenesis and other Th2-driven, chronic inflammatory responses, *IL-3*, *GM-CSF*, *IL-4*, and *IL-13* and the transcription factor *GATA-3* are established targets of HuR (Ma and others 1996; Ford and others 1999; Yarovsky and others 2006; Casolaro and others 2008; Stellato and others 2011).

The role of HuR in the regulation of CC chemokines was initiated by studies showing that the treatment of human airway epithelial cells with IL-4 and TNF- α , a stimulation that induces the expression of several eosinophilic chemokines such as *CCL5/RANTES*, *CCL11/eotaxin-1*, and *CCL13/MCP-4* (Stellato and others 1999), triggered HuR activation (identified as an increase in cytoplasmic levels of HuR). This led to an association of HuR with CCL11 mRNA, coupled with an increase of CCL11 mRNA stability and protein levels on transient overexpression of HuR (Atasoy and others 2003). Based on these findings and on the established role of HuR as a modulator of many inflammatory genes that are relevant for chronic allergic responses and epithelial activation, Fan and others (2011) utilized a ribonomics approach to test the role of HuR as a common regulatory factor of the chemokine-rich expression profile induced by TNF- α and IFN- γ , a cytokine

challenge which polarizes epithelial gene expression (Stellato and others 1999; Schleimer and others 2007). This study identified a transcript pool containing a considerable cluster of chemokines and of signaling molecules. In particular, a group of CCR2 ligands—the chemokines *CCL2/MCP-1*, *CCL8/MCP-2*, and *CCL13/MCP-4*—and the neutrophilic chemokines *CXCL1/Gro- α* and *CXCL2/Gro- β* were among the most enriched HuR-associated mRNAs. After single gene validation of HuR association, sequence analysis indicated that these transcripts displayed in their 3' UTR diverse putative ARE-containing HuR binding sites [previously identified computationally by Lopez de Silanes and others (2004)] (Fig. 1A). Using biotinylated, full-length chemokine 3' UTR and coding regions as probes for biotin pull-down experiments, transcript association with HuR was found to occur for the targets that selectively bind the ARE-containing 3' UTR regions (Fig. 1B). Interestingly, only CCL2/MCP-1 and CCL8/MCP-2 displayed a stimulus-dependent increase in mRNA turnover and responded to transient HuR overexpression with concordant changes in mRNA levels in both primary human airway epithelial cells and the airway epithelial cell line BEAS-2B. Of notice, while on cytokine challenge CCL2 mRNA was found to be mostly cytoplasmatic, as it was HuR in activated cells, CXCL1 mRNA was detected predominantly in the nuclear RNA extract. This may indicate a more critical role for HuR in a stimulus-dependent increase of CCL2 expression, with the promotion of mRNA stability as well as nuclear export, whereas for CXCL1—and possibly for the other HuR-associated chemokines with unchanged mRNA stability—additional signaling may need to be coupled to HuR association, affecting other factors participating in the RNP complexes in which HuR was detected. HuR also exerts effects on translation (Katsanou and others 2005; Prechtel and others 2006; Hinman and Lou 2008), and levels of targeted chemokines could reflect this additional layer of regulation by HuR (Anderson 2009). Translation of CCL2 and other targets could be also influenced by HuR indirectly, by relieving miRNA-mediated translational repression, as in the case of HuR and CAT-1 mRNA (Bhattacharyya and others 2006). Taken together, these data point at the complex composition of transcript-specific RNP complexes as a potential mechanism of PTR specificity for chemokine expression.

The RBP TTP is a zinc finger protein that accelerates the mRNA decay turnover (Anderson 2008) through multiple mechanisms, including the recruitment of deadenylases and exoribonucleases to the RNP and its compartmentalization to sites of mRNA degradation (Sandler and Stoecklin 2008). TTP exerts its regulatory function on many HuR targets by binding to distinct, but partially overlapping AREs that are conserved in their 3' UTR (Carballo and others 2000; Lai and others 2006; Ishmael and others 2008). Indeed, CCL2 and CXCL1 have been found to be regulated by TTP, as well as by miRNAs (124a and 155, respectively) (Kedersha and others 2000, 2002; Ishmael and others 2008; Liang and others 2009). Since TTP and HuR share similar binding sites, the interplay between these two RBPs has been the subject of multiple studies (Young and others 2009; Lin and others 2011; Roff and others 2013). It appears that under inflammatory conditions, the effects of HuR become dominant in the PTR process; while under conditions limiting the inflammatory cascade, TTP drives the RNP function toward acceleration of mRNA degradation. The study by Fan and others, identifying an UAUUUU sequence, which binds TTP (Brewer and others 2004), in the portion of CCL2

FIG. 1. Association of Hu-antigen R (HuR) with chemokines mRNA through the 3' untranslated region (UTR). (A) Putative HuR binding sites [according to Lopez de Silanes and others (2004)] in chemokine transcripts associated with HuR. (B) Western blot analysis showing HuR detection after biotin pull-down of BEAS-2B cell lysates with the biotinylated transcripts spanning either the coding regions (C) or the 3' UTR (U) of the indicated chemokines and GAPDH (representative of $n=3$). (C) Biotin pull-down assay using either the CCL2 mRNA 3' UTR full length (nt 374–749) or segments (A–C) containing different putative HuR sites (indicated as 1 to 4 in the figure, sequences listed in the box). Underlined is the sequence 1, which is included in the biotinylated probe A that retained HuR binding. Modified from Fan and others (2011), reprinted with permission.

3' UTR associated with HuR [(Fig. 1C), discussed in the next paragraph], supports the hypothesis that an interplay between these RBP may play a role in modulating chemokine levels. These events could occur in a condition of TNF- α and IFN- γ overexpression in the airway mucosa, for example, during a respiratory viral infection. The mecha-

nisms that regulate these effects have not been fully characterized and are currently under research scrutiny. Along with RBP remodeling at a single cis-element site, it is important to underscore how different regulatory sequences can drive, for a specific chemokine, its post-transcriptional fate. In the case of keratinocyte chemoattractant

(KC), the murine CXCL1, the chemokine is controlled by a stimulus- and lineage-specific PTR mechanism that is mediated by different sequences: In macrophages, TLR-mediated KC mRNA degradation is dependent on TTP binding to multiple AUUUA pentamers present in the 3' UTR; in non-myeloid cells such as epithelial cells, KC mRNA remains short lived after deletion of the 7 AUUUA pentamers and retains the ability to become stable on stimulation with TNF- α and IL-17, pointing in the latter case at the presence of a different, as yet identified determinant of mRNA stability (Novotny and others 2005; Datta and others 2008, 2010; Hamilton and others 2010).

It is becoming increasingly obvious that endogenous anti-inflammatory stimuli utilize PTR as a part of the integrated pathways governing self-limitation of inflammatory reactions (Murray and Smale 2012), indicating new targetable areas for therapeutic strategies. This became first apparent with studies on the role of PTR in the mechanism of action of the anti-inflammatory cytokine, IL-10 that has been shown to affect the mRNA stability of KC as well as of CCL3, CCL4, and CXCL8 through ARE/TTP-dependent and independent pathways (Wang and others 1994; Berkman and others 1995; Kim and others 1998; Kishore and others 1999). Importantly, PTR has also been implicated in the anti-inflammatory effects of glucocorticoids (GC) (Stellato 2004). Mediators known to be regulated by GC via changes in mRNA stability include TNF- α , GM-CSF, COX-2, IL-4R α , IL-6, inducible NO synthase, vascular endothelial growth factor, the chemokines CCL2, CCL7, CCL11, CCL13, and many others (Stellato and others 1999; Stellato 2004; Smoak and Cidlowski 2006, 2007). GC-mediated effect on PTR can occur indirectly, as in the case of GC-mediated induction of MAP-kinase phosphate 1 (MKP-1), a phosphatase that inhibits p38-driven activation of inflammatory genes (Clark 2007; Clark and others 2008). Since signaling through the p38 MAPK pathway exerts, via different mechanisms, a strong positive effect on mRNA stability and translation, GCs, on the other hand, exerts a negative effect through MAPK phosphatase 1 (MKP-1/DUSP1) up-regulation; thus, GCs blunt a major pathway favoring overexpression of inflammatory genes by changes in their PTR (Clark and Lasa 2003). Direct effects of GC on PTR have been extensively studied in models of chemokine expression in airway epithelium, given that the inhibition of epithelial-derived genes is a major mechanism of the efficacy of topical GC in the therapy of inflammatory respiratory diseases (Stellato 2007; Ishmael and others 2008, 2011). The GC effects on chemokine mRNA destabilization occur via ARE-dependent and ARE-independent mechanisms, as is discussed in greater detail next.

Post-Transcriptional Regulation of CCL2

CCL2 is a central chemokine in the inflammatory response. It serves as a chemoattractant for monocytes and macrophages, and plays key roles in many immune processes. The CCL2 receptor is expressed in antigen-presenting cells and T cells (Deshmane and others 2009), and CCL2 has been implicated in asthma pathogenesis (Rose and others 2003), also by affecting the differentiation of T cells toward a Th2 phenotype (Gu and others 2000; Luther and Cyster 2001). In addition, CCL2 has been shown to play roles in inflammatory bowel disease, rheumatoid arthritis, cardiovascular diseases, and cancer (Daly and Rollins 2003; Deshmane and others 2009). Mouse knockout models for CCL2 and its receptor,

CCR2 indicate a nonredundant role of this pathway in monocyte recruitment in the majority of tissues (Charo and Peters 2003; Daly and Rollins 2003). As such, elucidation of the mechanisms that regulate CCL2 expression and sensitivity to anti-inflammatory therapy has implications for understanding the pathogenesis of important chronic diseases, the generation of novel therapeutics, and the development of research models to understand how chemokines are regulated in general.

Zhai and others (2008) showed that combined stimulation with TNF- α and IL-4 of the pulmonary epithelial BEAS-2B cell line results in a 2-fold increase in the CCL2 mRNA half life and a concomitant increase in CCL2 protein production, and that the increased CCL2 mRNA stability occurs independently of its 3' UTR. Since HuR has been reported to associate to transcripts outside its canonical ARE-containing sites, as in the case of its binding within the coding region of IL-4 mRNA (Yarovinsky and others 2006), it is possible that this cytokine stimulation may drive CCL2 post-transcriptionally through different cis-elements. However, mapping experiments using biotin-labeled full-length CCL2 3' UTR and coding region in the study by Fan and others (2011) found HuR binding to be absent within the coding region, and occurring to a specific ARE placed, among the 4 putative HuR sites identified in the CCL2 3' UTR, between nucleotide 374 and 49 of the 3' UTR (Fig. 1C). The region consists of an hexamer with flanking U, a sequence also known to bind TTP (Brewer and others 2004), which may be the functional region of cytokine-mediated displacement of TTP by HuR (Al-Ahmadi and others 2009).

The observation that cytokine stimulation utilizes coordinate post-transcriptional mechanism to regulate chemokines and other inflammation-related factors led to the investigation of GCs' ability to target PTR mechanisms as a part of their anti-inflammatory mechanism of action, which constitutes the basis of topical GC therapy in airway inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD) (Stellato 2007). Indeed, GCs are potent inhibitors of CCL2 expression, and PTR via the action of TTP and the GC receptor (GR) have been implicated in this process. First, the treatment of airway cells with GCs induces production of TTP (Smoak and Cidlowski 2006; Ishmael and others 2008) and increases TTP binding to its canonical mRNA target, TNF- α (Smoak and Cidlowski 2006). Ishmael and others (2008) examined the role of TTP in GC mechanism of action, and found that GC-mediated changes in gene expression were severely blunted in TTP^{-/-} mouse embryonic fibroblasts (MEFs) compared with wild-type (WT) littermate-derived cells. Importantly, several members of the CC and CXC chemokine families: CCL2, CCL7, CXCL1, CXCL5, and CXCL7 were among the genes whose down-regulation by cell treatment with the GC budesonide was significantly lost in TTP-knockout MEF. Immunoprecipitation and biotin pull-down experiments confirmed 3' UTR-dependent association of TTP with the chemokine transcripts. Although a detailed deletional analysis was not performed in this study, it is very likely that TTP would bind through the AREs which all these transcripts bear, either as AUUUA pentamers or as UUAUUUAUU nonamers, both of which are compatible with TTP binding (Stoecklin and others 2008). Lastly, GC-induced acceleration of CCL2 and CCL7 mRNA decay present in GC-treated WT cells was lost in TTP^{-/-} cells,

indicating a functional and nonredundant role of TTP for GC-mediated CCL2 and CCL7 PTR. The key findings of the study were replicated in human airway epithelial cells in which TTP was silenced, demonstrating in a human cellular model that TTP played an important role in mediating the effects of GCs on epithelial-derived CCL2 and other relevant GC-sensitive genes.

Second, GC-induced acceleration of CCL2 and CCL7 mRNA turnover was found to be regulated in additional studies by other cis-regulatory elements, identified within the 5' UTR of the transcripts. Poon and others (1999) first described that extracts from GC-treated rat smooth muscle cells exhibited rapid CCL2 mRNA degradation compared with untreated controls. A subsequent study by Dhawan and others (2007) indicated by RNA electromobility shift assay that the GR can interact with CCL2 mRNA, and using a cell-free mRNA stability assay—in which cytoplasmic lysates are incubated with a labeled synthetic transcript in order to follow its degradation over time—it showed that antibody-mediated GR neutralization abolished the lysates' ability to induce CCL2 mRNA degradation, and that this process relied on the presence of the transcript 5' UTR (Dhawan and others 2007). These data highlighted a novel, cytoplasmic function for the GR as well as the existence of additional, non-ARE determinants of CCL2 mRNA stability.

To explore the role of this mechanism in a human cellular model of GC response, the study by Ishmael and others

(2011) started from the demonstration that endogenous CCL2 and CCL7 mRNA, but not CCL5, associated with GR on cell treatment with budesonide in human airway epithelial cells and in the epithelial cell line BEAS-2B. Using the same cell-free mRNA stability assay of the study by Dhawan and others (2007), GR neutralization in epithelial cell lysates prevented GC-induced increase in mRNA decay of synthetic CCL2 and CCL7 mRNA. Furthermore, the GR-RNA interaction, identified by RNP-IP and biotin pull-down experiments, occurred within the 5' UTR region in the human CCL2 mRNA as well, was mapped through the cross-linking of purified recombinant GR with truncated 5' UTR regions, and was localized to a 15-mer fragment spanning nt 44–60 (Fig. 2 A, B).

Transcription factors, such as the GR, and RBPs share functional features such as nucleocytoplasmic shuttling and binding to conserved nucleic acid sequences; some regulatory proteins, structurally similar to the GR due to the presence of zinc fingers, can bind to both DNA and RNA, as in the case of NF-90 that regulates IL-2 transcription as well as mRNA turnover through site-specific interactions (Shi and others 2007). Since both transcription factors and RBPs can coordinate the expression of multiple genes by an interaction with specific cis-regulatory sequences, validation of the biological relevance of this novel role of GR in human lung epithelial cells came by the identification, employing RIP-ChIP analysis, of approximately 500

FIG. 2. The glucocorticoid (GC) receptor (GR) binds to CCL2 mRNA via a G/C-rich Motif present in the 5' UTR. **(A)** Above: schematic of CCL2 mRNA, with nucleotide numbers corresponding to UTR and coding region (CR). Below: detection of GR by Western blot after biotin pull-down assay from unstimulated BEAS-2B cell lysate, using biotinylated transcripts encompassing the CCL2 full-length RNA (FL), the 5' and 3' UTRs, or the CR. The RNA-binding protein (RBP) HuR is detected as a positive control for the association with CCL2 3' UTR. **(B)** UV cross-linking of purified GR protein to 15-nt fragments of the CCL2 5' UTR, followed by detection by Western blot of the covalently linked GR via mobility shift of the RNA-bound GR protein (GR-RNA). **(C)** Right: Graphic logo representing the probability matrix of the GR motif, showing the relative frequency of each nucleotide for each position within the motif sequence. Left: Secondary structure of the GR motif comprising the nucleotides with the highest frequency for each position within the motif shown in A. **(D)** Biotin pull-down assay showing the association of GR from unstimulated BEAS-2B cell lysates with the GR motif shown in (C) (sequence shown next), compared with GR association with the full-length 5' UTR of CCL2, CCL7, and CCL5 mRNA (the latter as negative control). Modified from Ishmael and others (2011), reprinted with permission.

transcripts that are significantly associated with the GR. Furthermore, a specific Guanidine/Cytidine (GC)-rich motif, identified by computational analysis and validated for its association with GR, was identified in the 5' UTRs of 7,889 predicted mRNA targets (Fig. 2C, D).

Although many as yet unanswered questions remain about the molecular mechanisms driving the cytoplasmic function of the GR and its relative weight in the global gene regulatory function of GCs, these data indicate that the GR can mediate GC action beyond its nuclear functions in transcriptional gene control. Chemokines, with CCL2 being a prominent example, represent a class of inflammatory and immunomodulatory molecules that rely heavily on the complex, combinatorial nature of PTR to achieve optimal timing and appropriate levels of expression. Figure 3 summarizes the regulatory elements identified by the studies mentioned earlier.

The post-transcriptional regulation of CCL2 also involves miRNA-dependent mechanisms. miRNAs are small, noncoding RNA molecules of emerging importance in post-transcriptional regulation, acting predominantly as translational repressors (Baltimore and others 2008). This function is accomplished by recruitment of the RNA-induced silencing complex to the miRNA-bound mRNA and its consequent destabilization and/or reduction of the translation efficiency. Down-regulation of miRNA-mediated gene silencing was found by Zhai and others (2008) to play a role in cytokine-mediated CCL2 expression in the airway epithelial cell line BEAS-2B. Using [³⁵S]-protein labeling for pulse-chase experiments and let-7 miRNA luciferase reporter constructs, Zhai and others identified a cytokine-induced increase in global translation in cells where CCL2 expression and mRNA stability were up-regulated, which was paralleled by impairment of miRNA-mediated gene silencing. Decreased translational repression was also indirectly suggested by a reduced number of P-bodies, which are cytoplasmic storage sites for untranslated mRNAs, in cytokine-stimulated cells and in pulmonary epithelial cells isolated from ovalbumin-challenged mice. Therefore, a coordinated post-transcriptional response supports epithelial CCL2 overexpression after cytokine stimulation, involving the increase in CCL2 mRNA stability and the up-regulation of global translation, coupled with down-regulation of miRNA-induced gene silencing and decreased P-body formation.

Involvement of miRNA-based regulation of CCL2 expression is increasingly found in human diseases in which this chemokine orchestrates inflammatory cell recruitment. miRNA expression profiling of synoviocytes obtained from rheumatoid arthritis patients demonstrated decreased expression of miR-124a (Nakamachi and others 2009). Predictive algorithm analyses revealed that CCL2 contains a putative miR-124a binding site (seed sequence). Indeed, overexpression of miR-124a in synoviocytes led to decreased CCL2 synthesis. This finding was further corroborated using a luciferase reporter construct containing the CCL2 3' UTR, which showed a decrease in luciferase activity in the presence of miR-124a overexpression. Using a comparable experimental approach, Arner and others found that miR-126 expression is significantly down-regulated in white adipose tissue obtained from obese women compared with nonobese women. They show that miR-126 directly binds to CCL2 and that overexpression of this miRNA results in decreased CCL2 expression (Arner and others

FIG. 3. Model of CCL2 post-transcriptional regulation. The CCL2 mRNA (numbers indicate nucleotides defining 5'/3' UTRs and coding region) undergoes post-transcriptional regulation in response to inflammatory or anti-inflammatory stimuli. Pro-inflammatory and immunomodulatory cytokines such as tumor necrosis factor- α and IFN- γ promote the binding of HuR to adenylate/uridylate (AU)-rich elements at the CCL2 3'-UTR. Similarly, in autoimmune and chronic inflammatory diseases, there is documented down-regulation of the expression of microRNAs (miRNAs) that target and repress CCL2 expression. Both mechanisms result in the stabilization of the CCL2 mRNA, which, ultimately, leads to increased CCL2 production. Conversely, anti-inflammatory stimuli such as GCs promote the association of the CCL2 transcript with mRNA-destabilizing RBPs such as tristetraprolin (TTP) or with the GR itself, leading to decreased levels of CCL2 expression. The balance between functionally opposite post-transcriptional mechanisms is likely critical in determining the timing and amplitude of CCL2 expression and, therefore, in supporting its many functions in immunity. In dark gray, stimuli and factors involved (through their activation in the case of HuR or their repression, in the case of miRNAs) in inflammation-mediated up-regulation of CCL2 expression; in light gray, stimuli and factors involved in down-regulation of CCL2 expression in the experimental models discussed in the text.

2012). Moreover, they demonstrated that miR-193b also affects CCL2, though indirectly, by targeting the transcription factors MAX and EST1, which are positive regulators of CCL2 expression (Arner and others 2012).

Clinical Implications of Post-Transcriptional Regulation of Chemokines

Substantial insights about novel cellular processes have been gained from the study of post-transcriptional regulation. However, the emerging role of chemokine PTR in human disease pathogenesis and its potential for novel clinical management strategies has been only recently evaluated. Currently, the majority of these studies are in the

field of cancer biology and immune regulation. In cancer research, miRNA-mediated chemokine PTR has been identified in different types of cancer: Zhang and others (2013) identified, through miRNA expression profiling analysis, that miR-126 and miR-126* levels were significantly decreased in tumorigenic human breast cancer cell lines derived from the nontumorigenic MCF10 cell line, compared with the MCF10 cell line itself. Using predictive algorithms and luciferase gene reporter assays, these investigators demonstrated that miR-126 and miR-126* target an important pathway known to promote breast cancer metastasis (Karnoub and others 2007; Qian and others 2011), and showed that decreased CXCL12 production by 4T1 mouse mammary tumor cells in the tumor microenvironment resulted in decreased mesenchymal stem cell recruitment and CCL2 production (Zhang and others 2013).

HuR-mediated chemokine PTR has also been implicated in cancer pathogenesis. Nabors and others (2001) detected a significant increase in HuR expression by an immunohistochemical analysis of samples from high-malignancy grade brain tumors, such as glioblastoma multiforme and medulloblastomas, compared with tumors with a lower grade of malignancy such as pilocytic astrocytomas and meningiomas. The authors further proposed that HuR promotes tumorigenesis by stabilizing the CXCL8 mRNA and the transcripts of other pro-angiogenic factors such as VEGF and COX2 (Nabors and others 2001).

Clinical studies investigating the role of PTR in inflammatory diseases are also emerging. Differential expression of miRNAs has been found in exhaled breath condensates and in serum of patients with asthma and COPD compared with healthy individuals (Panganiban and others 2012; Pinkerton and others 2013). Interestingly, miR-1248, miR-1291, miR-570-3p, and Let-7a were found to be differentially expressed in patients with asthma compared with patients with COPD and healthy subjects, suggesting that specific miRNA profiles could be associated with different mechanisms of airway inflammation. Collectively, these 4 miRNAs regulate numerous cytokines and chemokines that are important in allergic inflammation and asthma, including IL-5, IL-13, CCL2, CCL4, and CCL8 (Panganiban and others 2012; Pinkerton and others 2013). Importantly, some of these miRNAs can also regulate other components of the PTR machinery: miR-570-3p is capable of repressing HuR expression, thus indirectly influencing CCL2 and numerous other chemokines and cytokines targeted by this RBP (F.T. Ishmael, pers. commun.). Along the same lines, miR-374a is differentially expressed in serum and CD4⁺ T cells of asthmatic subjects compared with healthy subjects (F.T. Ishmael, pers. commun.); the putative targets of this miRNA include the chemokines CCL2, CCL8, CXCL5, and CCL11 as well as IL-6 and TTP.

Utilizing Post-Transcriptional Regulation of Chemokines as a Therapeutic Target

Factors involved in PTR, such as RBPs, miRNAs and the signaling pathways controlling their function, may represent important targets for the generation of novel anti-inflammatory agents for chronic inflammatory and allergic diseases with relative greater specificity than the current, gold-standard GC therapy (Lu and Rothenberg 2013). The strategies of miRNA-based therapeutics are based on the administration of

miRNA mimics in case of aberrantly down-regulated miRNAs, mirrored by the inhibition of overexpressed miRNAs with antisense oligonucleotides, or antagomirs (Mattes and others 2007). Implementation of these approaches is complex, and the development of miRNA-based therapeutics is currently facing several important, rate-limiting issues. Among the most pressing ones are the ability to deliver the miRNA mimics or antagomirs to the appropriate subcellular compartment of their target tissue. Moreover, since a single miRNA can target multiple mRNA transcripts, the adverse off-target effects of miRNA-based therapeutics should be thoroughly studied (Love and others 2008).

Pharmacologic targeting of RBPs can be more readily utilized as a strategy for the generation of anti-tumor agents and anti-inflammatory agents. The signaling pathways that modulate the activities of HuR and TTP are increasingly characterized, and the development of RBP-based therapeutic strategies targeting HuR and TTP mainly targets the signaling pathways controlling their functional activation. Stimulus-induced activation of the AMP-activated protein kinase signaling pathway results in reduced cytoplasmic HuR levels (Wang and others 2002, 2004) and the inhibition of HuR function, while inhibition of the p38 MAPK signaling pathway would overall up-regulate TTP activity (Clark and others 2003).

Apocyanin is a naturally occurring methoxyphenolic compound derived from the medicinal plant, *Picorhiza kurroa*. This compound has long been used as a traditional medicinal treatment for numerous diseases, including asthma (Stefanska and Pawliczak 2008). Houser and others (2012) investigated the anti-inflammatory properties of apocyanin and other methoxyphenols in airway epithelial cells and found that the chemokines CCL2, CCL5, CXCL1, CXCL8, CXCL10 as well as IL-6, ICAM-1, MIF, and Serpin E1 were inhibited by these compounds. The authors investigated the mechanism of CCL2 down-regulation and found that apocyanin inhibited the binding of HuR to the CCL2 3' UTR, which resulted in the repression of CCL2 expression (Houser and others 2012). Nebulization of apocyanin has been shown to have anti-inflammatory effects in asthmatic subjects, and it may represent a novel class of anti-inflammatory agents (Stefanska and others 2012). The mechanisms of action and effects on HuR are still under investigation, but current findings suggest that the targeting of HuR is a novel strategy for anti-inflammatory action.

Additional in-depth preclinical and pilot clinical studies in larger patient populations are required to bridge the bench-to-bedside gap in new therapies based on the modulation of miRNA and RBP biology. Recent advances in chemokine PTR, however, convincingly demonstrate the potential efficacy of targeting miRNA- and RBP-mediated functions in developing novel diagnostic, prognostic, and therapeutic strategies for the clinical management of neoplastic and inflammatory diseases.

Author Disclosure Statement

No competing financial interests exist.

References

- Al-Ahmadi W, Al-Ghamdi M, Al-Haj L, Al-Saif M, Khabar KS. 2009. Alternative polyadenylation variants of the RNA

- binding protein, HuR: abundance, role of AU-rich elements and auto-regulation. *Nucleic Acids Res* 37(11):3612–3624.
- Anderson P. 2008. Post-transcriptional control of cytokine production. *Nat Immunol* 9(4):353–359.
- Anderson P. 2009. Intrinsic mRNA stability helps compose the inflammatory symphony. *Nat Immunol* 10(3):233–234.
- Anderson P. 2010. Post-transcriptional regulons coordinate the initiation and resolution of inflammation. *Nat Rev Immunol* 10(1):24–35.
- Arner E, Mejhert N, Kulyté A, Balwierz PJ, Pachkov M, Cormont M, Lorente-Cebrián S, Ehlund A, Laurencikienė J, Hedén P, Dahlman-Wright K, Tanti JF, Hayashizaki Y, Rydén M, Dahlman I, van Nimwegen E, Daub CO, Arner P. 2012. Adipose tissue microRNAs as regulators of CCL2 production in human obesity. *Diabetes* 61(8):1986–1993.
- Atasoy U, Curry SL, López de Silanes I, Shyu AB, Casolaro V, Gorospe M, Stellato C. 2003. Regulation of eotaxin gene expression by TNF and IL-4 through messenger RNA stabilization: involvement of the RNA-binding protein HuR. *J Immunol* 171(8):4369–4378.
- Bakheet T, Williams BR, Khabar KS. 2006. ARED 3.0: the large and diverse AU-rich transcriptome. *Nucleic Acids Res* 34(Database issue):D111–D114.
- Baltimore D, Boldin MP, O'Connell RM, Rao DS, Taganov KD. 2008. MicroRNAs: new regulators of immune cell development and function. *Nat Immunol* 9(8):839–845.
- Berkman N, John M, Roesems G, Jose P, Barnes P, Chung K. 1995. Inhibition of macrophage inflammatory protein-1 alpha expression by IL-10. Differential sensitivities in human blood monocytes and alveolar macrophages. *J Immunol* 155(9):4412–4418.
- Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W. 2006. Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell* 125(6):1111–1124.
- Blaxall BC, Dwyer-Nield LD, Bauer AK, Bohlmeier TJ, Malkinson AM, Port JD. 2000a. Differential expression and localization of the mRNA binding proteins, AU-rich element mRNA binding protein (AUF1) and Hu antigen R (HuR), in neoplastic lung tissue. *Mol Carcinog* 28(2):76–83.
- Blaxall BC, Pellett AC, Wu SC, Pende A, Port JD. 2000b. Purification and characterization of beta-adrenergic receptor mRNA-binding proteins. *J Biol Chem* 275(6):4290–4297.
- Brewer BY, Malicka J, Blackshear PJ, Wilson GM. 2004. RNA sequence elements required for high affinity binding by the zinc finger domain of tristetraprolin: conformational changes coupled to the bipartite nature of AU-rich mRNA-destabilizing motifs. *J Biol Chem* 279(27):27870–27877.
- Carballo E, Lai WS, Blackshear PJ. 2000. Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. *Blood* 95(6):1891–1899.
- Casolaro V, Fang X, Tancowny B, Fan J, Wu F, Srikantan S, Asaki SY, De Fanis U, Huang SK, Gorospe M, Atasoy UX, Stellato C. 2008. Posttranscriptional regulation of IL-13 in T cells: role of the RNA-binding protein HuR. *J Allergy Clin Immunol* 121(4):853–859 e4.
- Charo IF, Peters W. 2003. Chemokine receptor 2 (CCR2) in atherosclerosis, infectious diseases, and regulation of T-cell polarization. *Microcirculation* 10(3–4):259–264.
- Charo IF, Ransohoff RM. 2006. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* 354(6):610–621.
- Clark AR. 2007. Anti-inflammatory functions of glucocorticoid-induced genes. *Mol Cell Endocrinol* 275(1–2):79–97.
- Clark AR, Dean JL, Saklatvala J. 2003. Post-transcriptional regulation of gene expression by mitogen-activated protein kinase p38. *FEBS Lett* 546(1):37–44.
- Clark AR, Lasa M. 2003. Crosstalk between glucocorticoids and mitogen-activated protein kinase signalling pathways. *Curr Opin Pharmacol* 3(4):404–411.
- Clark AR, Martins JR, Tchen CR. 2008. Role of dual specificity phosphatases in biological responses to glucocorticoids. *J Biol Chem* 283(38):25765–25769.
- Daly C, Rollins BJ. 2003. Monocyte chemoattractant protein-1 (CCL2) in inflammatory disease and adaptive immunity: therapeutic opportunities and controversies. *Microcirculation* 10(3–4):247–257.
- Datta S, Biswas R, Novotny M, Pavicic PG, Jr., Herjan T, Mandal P, Hamilton TA. 2008. Tristetraprolin regulates CXCL1 (KC) mRNA stability. *J Immunol* 180(4):2545–2552.
- Datta S, Novotny M, Pavicic PG, Jr., Zhao C, Herjan T, Hartupée J, Hamilton T. 2010. IL-17 regulates CXCL1 mRNA stability via an AUUUA/tristetraprolin-independent sequence. *J Immunol* 184(3):1484–1491.
- Dean JL, Wait R, Mahtani KR, Sully G, Clark AR, Saklatvala J. 2001. The 3' untranslated region of tumor necrosis factor alpha mRNA is a target of the mRNA-stabilizing factor HuR. *Mol Cell Biol* 21(3):721–730.
- Deshmane SL, Kremlev S, Amini S, Sawaya BE. 2009. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* 29(6):313–326.
- Dhawan L, Liu B, Blaxall BC, Taubman MB. 2007. A novel role for the glucocorticoid receptor in the regulation of monocyte chemoattractant protein-1 mRNA stability 10.1074/jbc.M605925200. *J Biol Chem* 282(14):10146–10152.
- Dixon DA, Tolley ND, King PH, Nabors LB, McIntyre TM, Zimmerman GA, Prescott SM. 2001. Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. *J Clin Invest* 108(11):1657–1665.
- Fan J, Heller NM, Gorospe M, Atasoy U, Stellato C. 2005. The role of post-transcriptional regulation in chemokine gene expression in inflammation and allergy. *Eur Respir J* 26(5):933–947.
- Fan J, Ishmael FT, Fang X, Myers A, Cheadle C, Huang SK, Atasoy U, Gorospe M, Stellato C. 2011. Chemokine transcripts as targets of the RNA-binding protein HuR in human airway epithelium. *J Immunol* 186(4):2482–2494.
- Ford LP, Watson J, Keene JD, Wilusz J. 1999. ELAV proteins stabilize deadenylated intermediates in a novel *in vitro* mRNA deadenylation/degradation system. *Genes Dev* 13(2):188–201.
- Frevel MA, Bakheet T, Silva AM, Hissong JG, Khabar KS, Williams BR. 2003. p38 Mitogen-activated protein kinase-dependent and -independent signaling of mRNA stability of AU-rich element-containing transcripts. *Mol Cell Biol* 23(2):425–436.
- Goldberg-Cohen I, Furneaux H, Levy AP. 2002. A 40-bp RNA element that mediates stabilization of vascular endothelial growth factor mRNA by HuR. *J Biol Chem* 277(16):13635–13640.
- Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. 2000. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature* 404(6776):407–411.
- Hafner M, Landthaler M, Burger L, Khorshid M, Hausser J, Berninger P, Rothballer A, Ascano M, Jr., Jungkamp AC, Munschauer M, Ulrich A, Wardle GS, Dewell S, Zavolan M, Tuschl T. 2010. Transcriptome-wide identification of

- RNA-binding protein and microRNA target sites by PAR-CLIP. *Cell* 141(1):129–141.
- Hamilton T, Novotny M, Pavicic PJ, Jr., Herjan T, Hartup J, Sun D, Zhao C, Datta S. 2010. Diversity in post-transcriptional control of neutrophil chemoattractant cytokine gene expression. *Cytokine* 52(1–2):116–122.
- Hinman MN, Lou H. 2008. Diverse molecular functions of Hu proteins. *Cell Mol Life Sci* 65(20):3168–3181.
- Houser KR, Johnson DK, Ishmael FT. 2012. Anti-inflammatory effects of methoxyphenolic compounds on human airway cells. *J Inflamm (Lond)* 9:6.
- Ishmael FT, Fang X, Galdiero MR, Atasoy U, Rigby WF, Gorospe M, Cheadle C, Stellato C. 2008. Role of the RNA-binding protein tristetraprolin in glucocorticoid-mediated gene regulation. *J Immunol* 180(12):8342–8353.
- Ishmael FT, Fang X, Houser KR, Pearce K, Abdelmohsen K, Zhan M, Gorospe M, Stellato C. 2011. The human glucocorticoid receptor as an RNA-binding protein: global analysis of glucocorticoid receptor-associated transcripts and identification of a target RNA motif. *J Immunol* 186(2):1189–1198.
- Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. 2007. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 449(7162):557–563.
- Katsanou V, Papadaki O, Milatos S, Blackshear PJ, Anderson P, Kollias G, Kontoyiannis DL. 2005. HuR as a negative post-transcriptional modulator in inflammation. *Mol Cell* 19(6):777–789.
- Kedersha N, Chen S, Gilks N, Li W, Miller IJ, Stahl J, Anderson P. 2002. Evidence that ternary complex (eIF2-GTP-tRNA(i)(Met))-deficient preinitiation complexes are core constituents of mammalian stress granules. *Mol Biol Cell* 13(1):195–210.
- Kedersha N, Cho MR, Li W, Yacono PW, Chen S, Gilks N, Golan DE, Anderson P. 2000. Dynamic shuttling of TIA-1 accompanies the recruitment of mRNA to mammalian stress granules. *J Cell Biol* 151(6):1257–1268.
- Keene JD. 2001. Ribonucleoprotein infrastructure regulating the flow of genetic information between the genome and the proteome. *Proc Natl Acad Sci U S A* 98(13):7018–7024.
- Keene JD. 2007. RNA regulons: coordination of post-transcriptional events. *Nat Rev Genet* 8(7):533–543.
- Keene JD, Komisarow JM, Friedersdorf MB. 2006. RIP-Chip: the isolation and identification of mRNAs, microRNAs and protein components of ribonucleoprotein complexes from cell extracts. *Nat Protoc* 1(1):302–307.
- Kim HS, Armstrong D, Hamilton TA, Tebo JM. 1998. IL-10 suppresses LPS-induced KC mRNA expression via a translation-dependent decrease in mRNA stability. *J Leukoc Biol* 64(1):33–39.
- Kishore R, Tebo JM, Kolosov M, Hamilton TA. 1999. Cutting edge: clustered AU-rich elements are the target of IL-10-mediated mRNA destabilization in mouse macrophages. *J Immunol* 162(5):2457–2461.
- Kontoyiannis D, Pasparakis M, Pizarro TT, Cominelli F, Kollias G. 1999. Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity* 10(3):387–398.
- Kracht M, Saklatvala J. 2002. Transcriptional and post-transcriptional control of gene expression in inflammation. *Cytokine* 20(3):91–106.
- Lai WS, Parker JS, Grissom SF, Stumpo DJ, Blackshear PJ. 2006. Novel mRNA targets for tristetraprolin (TTP) identified by global analysis of stabilized transcripts in TTP-deficient fibroblasts. *Mol Cell Biol* 26(24):9196–9208.
- Levy NS, Chung S, Furneaux H, Levy AP. 1998. Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR. *J Biol Chem* 273(11):6417–6423.
- Liang J, Lei T, Song Y, Yanes N, Qi Y, Fu M. 2009. RNA-destabilizing factor tristetraprolin negatively regulates NF-kappaB signaling. *J Biol Chem* 284(43):29383–29390.
- Lin FY, Tsai YT, Lee CY, Lin CY, Lin YW, Li CY, Shih CM, Huang CY, Chang NC, Tsai JC, Chen TL, Tsai CS. 2011. TNF-alpha-decreased thrombomodulin expression in monocytes is inhibited by propofol through regulation of tristetraprolin and human antigen R activities. *Shock* 36(3):279–288.
- Lopez de Silanes I, Galban S, Martindale JL, Yang X, Mazan-Mamczarz K, Indig FE, Falco G, Zhan M, Gorospe M. 2005. Identification and functional outcome of mRNAs associated with RNA-binding protein TIA-1. *Mol Cell Biol* 25(21):9520–9531.
- Lopez de Silanes I, Zhan M, Lal A, Yang X, Gorospe M. 2004. Identification of a target RNA motif for RNA-binding protein HuR. *Proc Natl Acad Sci U S A* 101(9):2987–2992.
- Love TM, Moffett HF, Novina CD. 2008. Not miR-ly small RNAs: big potential for microRNAs in therapy. *J Allergy Clin Immunol* 121(2):309–319.
- Lu TX, Rothenberg ME. 2013. Diagnostic, functional, and therapeutic roles of microRNA in allergic diseases. *J Allergy Clin Immunol* 132(1):3–13; quiz 14.
- Luther SA, Cyster JG. 2001. Chemokines as regulators of T cell differentiation. *Nat Immunol* 2(2):102–107.
- Ma WJ, Cheng S, Campbell C, Wright A, Furneaux H. 1996. Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein. *J Biol Chem* 271(14):8144–8151.
- Matsukura S, Stellato C, Plitt JR, Bickel C, Miura K, Georas SN, Casolaro V, Schleimer RP. 1999. Activation of eotaxin gene transcription by NF-kappa B and STAT6 in human airway epithelial cells. *J Immunol* 163(12):6876–6883.
- Mattes J, Yang M, Foster PS. 2007. Regulation of microRNA by antagomirs: a new class of pharmacological antagonists for the specific regulation of gene function? *Am J Respir Cell Mol Biol* 36(1):8–12.
- Ming XF, Stoecklin G, Lu M, Looser R, Moroni C. 2001. Parallel and independent regulation of interleukin-3 mRNA turnover by phosphatidylinositol 3-kinase and p38 mitogen-activated protein kinase. *Mol Cell Biol* 21(17):5778–5789.
- Murray PJ, Smale ST. 2012. Restraint of inflammatory signaling by interdependent strata of negative regulatory pathways. *Nat Immunol* 13(10):916–924.
- Nabors LB, Gillespie GY, Harkins L, King PH. 2001. HuR, a RNA stability factor, is expressed in malignant brain tumors and binds to adenine- and uridine-rich elements within the 3' untranslated regions of cytokine and angiogenic factor mRNAs. *Cancer Res* 61(5):2154–2161.
- Nabors LB, Suswam E, Huang Y, Yang X, Johnson MJ, King PH. 2003. Tumor necrosis factor alpha induces angiogenic factor up-regulation in malignant glioma cells: a role for RNA stabilization and HuR. *Cancer Res* 63(14):4181–4187.
- Nakamachi Y, Kawano S, Takenokuchi M, Nishimura K, Sakai Y, Chin T, Saura R, Kurosaka M, Kumagai S. 2009. MicroRNA-124a is a key regulator of proliferation and monocyte chemoattractant protein 1 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Arthritis Rheum* 60(5):1294–1304.
- Novotny M, Datta S, Biswas R, Hamilton T. 2005. Functionally independent AU-rich sequence motifs regulate KC (CXCL1) mRNA. *J Biol Chem* 280(34):30166–30174.

- Palanisamy V, Jakymiw A, Van Tubergen EA, D'Silva NJ, Kirkwood KL. 2012. Control of cytokine mRNA expression by RNA-binding proteins and microRNAs. *J Dent Res* 91(7):651–658.
- Panganiban RP, Pinkerton MH, Maru SY, Jefferson SJ, Roff AN, Ishmael FT. 2012. Differential microRNA expression in asthma and the role of miR-1248 in regulation of IL-5. *Am J Clin Exp Immunol* 1(2):154–165.
- Papadaki O, Milatos S, Grammenoudi S, Mukherjee N, Keene JD, Kontoyiannis DL. 2009. Control of thymic T cell maturation, deletion and egress by the RNA-binding protein HuR. *J Immunol* 182(11):6779–6788.
- Pinkerton M, Chinchilli V, Banta E, Craig T, August A, Bascom R, Cantorna M, Harvill E, Ishmael FT. 2013. Differential expression of microRNAs in exhaled breath condensates of patients with asthma, patients with chronic obstructive pulmonary disease, and healthy adults. *J Allergy Clin Immunol* 132(1):217–219.
- Poon M, Liu B, Taubman MB. 1999. Identification of a novel dexamethasone-sensitive RNA-stabilizing region on rat monocyte chemoattractant protein 1 mRNA. *Mol Cell Biol* 19(10):6471–6478.
- Prechtel AT, Chemnitz J, Schirmer S, Ehlers C, Langbein-Detsch I, Stulke J, Dabauvalle M-C, Kehlenbach RH, Hauber J. 2006. Expression of CD83 is regulated by HuR via a novel cis-active coding region RNA element. *J Biol Chem* 281(16):10912–10925.
- Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, Kaiser EA, Snyder LA, Pollard JW. 2011. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature* 475(7355):222–225.
- Rodriguez-Pascual F, Hausding M, Ihrig-Biedert I, Furneaux H, Levy AP, Forstermann U, Kleinert H. 2000. Complex contribution of the 3'-untranslated region to the expressional regulation of the human inducible nitric-oxide synthase gene. Involvement of the RNA-binding protein HuR. *J Biol Chem* 275(34):26040–26049.
- Roff AN, Panganiban RP, Bond JS, Ishmael FT. 2013. Post-transcriptional regulation of meprin alpha by the RNA-binding proteins Hu antigen R (HuR) and tristetraprolin (TTP). *J Biol Chem* 288(7):4733–4743.
- Rose CE, Jr., Sung SS, Fu SM. 2003. Significant involvement of CCL2 (MCP-1) in inflammatory disorders of the lung. *Microcirculation* 10(3–4):273–288.
- Ross J. 1995. mRNA stability in mammalian cells. *Microbiol Rev* 59(3):423–450.
- Sakai K, Kitagawa Y, Saiki M, Saiki S, Hirose G. 2003. Binding of the ELAV-like protein in murine autoimmune T-cells to the nonameric AU-rich element in the 3' untranslated region of CD154 mRNA. *Mol Immunol* 39(14):879–883.
- Sandler H, Stoecklin G. 2008. Control of mRNA decay by phosphorylation of tristetraprolin. *Biochem Soc Trans* 36(Pt 3):491–496.
- Schleimer RP, Kato A, Kern R, Kuperman D, Avila PC. 2007. Epithelium: at the interface of innate and adaptive immune responses. *J Allergy Clin Immunol* 120(6):1279–1284.
- Shi L, Godfrey WR, Lin J, Zhao G, Kao PN. 2007. NF90 regulates inducible IL-2 gene expression in T cells. *J Exp Med* 204(5):971–977.
- Smoak K, Cidlowski JA. 2006. Glucocorticoids regulate tristetraprolin synthesis and posttranscriptionally regulate tumor necrosis factor alpha inflammatory signaling. *Mol Cell Biol* 26(23):9126–9135.
- Stefanska J, Pawliczak R. 2008. Apocynin: molecular aptitudes. *Mediators Inflamm* 2008:106507.
- Stefanska J, Sarniak A, Wlodarczyk A, Sokolowska M, Pniewska E, Doniec Z, Nowak D, Pawliczak R. 2012. Apocynin reduces reactive oxygen species concentrations in exhaled breath condensate in asthmatics. *Exp Lung Res* 38(2):90–99.
- Stellato C. 2004. Post-transcriptional and nongenomic effects of glucocorticoids. *Proc Am Thorac Soc* 1:255–263.
- Stellato C. 2007. Glucocorticoid actions on airway epithelial responses in immunity: functional outcomes and molecular targets. *J Allergy Clin Immunol* 120(6):1247–1263; quiz 1264–1265.
- Stellato C, Beck LA. 2000. Expression of eosinophil-specific chemokines by human epithelial cells. *Chem Immunol* 76:156–176.
- Stellato C, Gubin MM, Magee JD, Fang X, Fan J, Tartar DM, Chen J, Dahm GM, Calaluce R, Mori F, Jackson GA, Casolaro V, Franklin CL, Atasoy U. 2011. Coordinate regulation of GATA-3 and Th2 cytokine gene expression by the RNA-binding protein HuR. *J Immunol* 187(1):441–449.
- Stellato C, Matsukura S, Fal A, White J, Beck LA, Proud D, Schleimer RP. 1999. Differential regulation of epithelial-derived C-C chemokine expression by IL-4 and the glucocorticoid budesonide. *J Immunol* 163(10):5624–5632.
- Stoecklin G, Tenenbaum SA, Mayo T, Chittur SV, George AD, Baroni TE, Blackshear PJ, Anderson P. 2008. Genome-wide analysis identifies interleukin-10 mRNA as target of tristetraprolin. *J Biol Chem* 283(17):11689–11699.
- Tauler J, Mulshine JL. 2009. Lung cancer and inflammation: interaction of chemokines and hnRNPs. *Curr Opin Pharmacol* 9(4):384–388.
- Taylor GA, Carballo E, Lee DM, Lai WS, Thompson MJ, Patel DD, Schenkman DI, Gilkeson GS, Broxmeyer HE, Haynes BF, Blackshear PJ. 1996. A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. *Immunity* 4(5):445–454.
- Tenenbaum SA, Lager PJ, Carson CC, Keene JD. 2002. Ribonomics: identifying mRNA subsets in mRNP complexes using antibodies to RNA-binding proteins and genomic arrays. *Methods* 26(2):191–198.
- Wang P, Wu P, Anthes JC, Siegel MI, Egan RW, Billah MM. 1994. Interleukin-10 inhibits interleukin-8 production in human neutrophils. *Blood* 83(9):2678–2683.
- Wang W, Fan J, Yang X, Furer-Galban S, Lopez de Silanes I, von Kobbe C, Guo J, Georas SN, Foufelle F, Hardie DG, Carling D, Gorospe M. 2002. AMP-activated kinase regulates cytoplasmic HuR. *Mol Cell Biol* 22(10):3425–3436.
- Wang W, Yang X, Kawai T, Lopez de Silanes I, Mazan-Mamczarz K, Chen P, Chook YM, Quensel C, Kohler M, Gorospe M. 2004. AMP-activated protein kinase-regulated phosphorylation and acetylation of importin alpha1: involvement in the nuclear import of RNA-binding protein HuR. *J Biol Chem* 279(46):48376–48388.
- White GE, Iqbal AJ, Greaves DR. 2013. CC chemokine receptors and chronic inflammation—therapeutic opportunities and pharmacological challenges. *Pharmacol Rev* 65(1):47–89.
- Yarovinsky TO, Butler NS, Monick MM, Hunninghake GW. 2006. Early Exposure to IL-4 Stabilizes IL-4 mRNA in CD4+ T Cells via RNA-Binding Protein HuR. *J Immunol* 177(7):4426–4435.
- Young LE, Sanduja S, Bemis-Standoli K, Pena EA, Price RL, Dixon DA. 2009. The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. *Gastroenterology* 136(5):1669–1679.

- Zhai Y, Zhong Z, Chen CY, Xia Z, Song L, Blackburn MR, Shyu AB. 2008. Coordinated changes in mRNA turnover, translation, and RNA processing bodies in bronchial epithelial cells following inflammatory stimulation. *Mol Cell Biol* 28(24):7414–7426.
- Zhang Y, Yang P, Sun T, Li D, Xu X, Rui Y, Li C, Chong M, Ibrahim T, Mercatali L, Amadori D, Lu X, Xie D, Li QJ, Wang XF. 2013. miR-126 and miR-126* repress recruitment of mesenchymal stem cells and inflammatory monocytes to inhibit breast cancer metastasis. *Nat Cell Biol* 15(3):284–294.

Address correspondence to:

Dr. Cristiana Stellato
Department of Medicine and Surgery
University of Salerno
Via Salvador Allende
84081 Baronissi (SA)
Italy

E-mail: cstellato@unisa.it

Received 19 December 2013/Accepted 20 December 2013