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Review

GM-CSF Regulation in Eosinophils

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Abstract. Allergic asthma is characterized by a temporally and quantitatively inappropriate immunologic response. One of the hallmarks of this response is the accumulation of eosinophils in the airway and lung parenchyma, which results in broncho-constriction, lung damage and, ultimately, fibrosis. Granulocyte-macrophage colony-stimulating factor (GM-CSF) plays a pivotal role in this process by modulating eosinophil function and survival. In this review, we discuss the effects and molecular regulation of GM-CSF secretion by eosinophils. Recent data demonstrate that activated eosinophils release small amounts of anti-apoptotic GM-CSF by stabilizing its coding mRNA.

Key words: eosinophils; GM-CSF; mRNA; survival; asthma.

Introduction

The late-phase inflammatory reaction in asthma occurs between 6 to 9 h after allergen provocation and involves the pulmonary recruitment and activation of $CD4^+$ T cells, basophils, neutrophils, and macrophages, but mainly eosinophils[10, 26]. The biological effects of eosinophils include the release of toxic granule protein (major basic protein, eosinophil cationic protein and eosinophil-derived neurotoxin), oxygen free radicals, eicosanoids and cytokines that lead to bronchial smooth muscle contraction, vascular permeability and airway hyperresponsiveness.

The release of cytokines by either mast cells during the early-phase reaction[97] or memory $CD4^+$ Th2 lymphocytes[81] are the likely initial triggers for the subsequent recruitment of eosinophils, which characterizes the chronic phase of asthma. There is compelling evidence that Th2 lymphocytes regulate the immune response responsible for allergy and asthma. Their products, the so-called type 2 cytokines, such as interleukin 4 (IL-4), IL-5, IL-9, and IL-13, are upregulated in the blood, bronchoalveolar lavage (BAL) and airway biopsies from allergic and asthmatic patients with active disease[25, 27, 28, 82]. Of these cytokines, IL-4 and IL-5 likely play central roles in eosinophil function. IL-4 augments migration into the lungs by upregulating the expression of vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells, providing additional binding sites for eosinophil cell-surface VLA-4[85]. IL-5 affects the early eosinophilic response by initiating their differentiation, survival, adhesion and efflux from the bone marrow[23, 72]. These effects are likely transitory, as eosinophils lose IL-5 receptor expression within several hours of activation with IL-5, granulocyte-macrophage

Abbreviations used: BAL – bronchoalveolar lavage, BALEos – eosinophils from BAL, ERK – extracellular signal-regulated kinase, Fn – fibronectin, GM-CSF – granulocyte-macrophage colony-stimulating factor, $t_{1/2}$ – half-life, IL-5 – interleukin 5, JNK – c-Jun NH_2--terminal kinase, MAPK – mitogen-activated protein kinase, MAPKK – mitogen-activated protein kinase kinase, PBEos – peripheral blood eosinophils, PMGT – particle-mediated gene transfer, TNF-α – tumor necrosis factor α, YB-1 – Y box-binding protein 1.

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colony-stimulating factor (GM-CSF) or IL-3[54, 101]. After IL-5 priming, chemokines, such as eotaxin and RANTES, extracellular matrix (ECM) proteins, including fibronectin (Fn) and hyaluronic acid (HA), or other cytokines, such as IL-1, IL-9, tumor necrosis factor (TNF), IL-3 and GM-CSF mediate eosinophil functions. Among these effectors, GM-CSF plays a pivotal role in eosinophilia during the inflammatory stage. In addition to enhancing eosinophil recruitment, maturation and the expression of adhesion molecules and degranulation, GM-CSF also blocks eosinophil apoptosis[93]. As GM-CSF is expressed by eosinophils themselves[12, 14, 45], the effective concentrations are minute and can be provided by a subset of activated cells, which can maintain the entire population[32]. In this review we assess how eosinophils control GM-CSF release and the consequences of this cytokine on cellular function and survival.

GM-CSF in Asthma

Growth factors such as GM-CSF are essential mediators of diverse and critical cellular functions, including hematopoiesis in the bone marrow and peripheral immune cell activation. GM-CSF, a 23 kDa glycoprotein, is elevated in BAL fluid[13], bronchial biopsies[94], and sputum[69] from asthmatics after sustained release by activated immune cells, fibroblasts, epithelial cells and eosinophils themselves. The measurement of GM-CSF protein by ELISA after allergen challenge or during symptomatic asthma likely underestimates the actual concentrations, as 10^6 purified peripheral blood eosinophils catabolized 50 pg of recombinant human GM-CSF in 2 h *in vitro* (unpublished observation). These measurements ignore likely synergistic contributions by other activated inflammatory cells, including neutrophils, macrophages and lymphocytes. Catabolism probably reflects a contribution by receptor-mediated uptake as well as protease-mediated inactivation. GM-CSF mRNA accumulation also occurs in airway-based cells by *in situ* hybridizaton after allergen challenge[12]. In contrast to studies using allergen challenge[71], GM-CSF, rather than IL-5 or IL-3, is associated with eosinophil survival in the BAL fluid from symptomatic asthmatics[1, 73], suggesting there may be differences between experimental challenge and natural exacerbation. Functionally, GM-CSF primes, enhances the generation of superoxide, leukotriene and platelet-activating factor, increases eosinophil cytotoxicity, adhesion, and induces the sequential activation and deactivation of the low-affinity IgG Fc receptor[24, 66]. Moreover, GM-CSF induces HLA-DR, which permits eosinophils to present antigen to T lymphocytes[96, 103]. Interestingly, HLA-DR is also found on airway eosinophils[86]. Beside its pleiotropic functions on eosinophils, GM-CSF elicits multiple effects on other components of the immune system including dendritic cells, which are essential for antigen presentation to naive or primed T lymphocytes, which initiate or perpetuate chronic eosinophilic airway inflammation[7]. Furthermore, GM-CSF also has multiple effects on neutrophil function, including increasing 5--lipoxygenase levels, tyrosine phosphorylation, aggregation, superoxide anion production, degranulation, cytotoxicity, adhesion to pulmonary vascular endothelium, and synthesis and expression of complement receptors[42]. Finally, GM-CSF stimulates monocyte survival and differentiation into mature macrophages[112], which are the largest cell population in the airways of patients with allergic asthma[6].

GM-CSF is Regulated at the mRNA Level

Many transiently expressed genes, including immediate early response genes (IERG) such as those for cytokines, transcription factors and proto-oncogenes, have extremely unstable mRNAs in resting cells. Cell activation, entry into the cell cycle, response to injury or cell-surface receptor engagement are associated with rapid and substantial IERG mRNA stabilization. Indeed, it is often mRNA stabilization rather than transcriptional upregulation which accounts for cytoplasmic mRNA accumulation under these conditions. GM-CSF is a prototypical IERG, and not unexpectedly, shows substantial post-transcriptional regulation.

In resting T cells, basal GM-CSF mRNA transcription is usually present, and yet, cytoplasmic mRNA does not accumulate. Upon mitogen treatment with phorbol ester or mitogenic anti-CD3 and anti-CD28 antibodies, T cells stabilize GM-CSF mRNAs, accounting for most if not all of their cytoplasmic accumulation and ensuing cytokine secretion within a few hours of activation[53]. Both rapid decay and mitogen driven stabilization require the presence of 3'-untranslated-region (3' UTR) adenosine-uridine rich elements (AREs)[20, 29, 89]. Alteration or deletion of the AREs significantly enhanced the stability of mutant GM-CSF mRNAs[78, 106]. Chimeric globin mRNAs fused to the AREs from GM-CSF decayed at rates similar to wild-type GM-CSF[89], demonstrating these elements are sufficient. The underlying molecular mechanisms for ARE mRNA regulation is slowly becoming clearer. Multiple groups have shown the interaction of cytoplasmic proteins with the

AREs. These tend to be high-affinity events, which also recruit additional cofactors by protein-protein interactions. Occupancy of the ARE has been associated with both more or less rapid mRNA decay, implying the existence of both stabilizing and destabilizing protein activities. The human Elav-like protein family appears to block ARE mRNA decay[34, 48], while tristetraproline (TTP) has the opposite effect[16]. Interestingly, AUF-1/ /hnRNP D[55, 111] has been reported to have both activities, depending on the circumstances and cell type. As many different ARE mRNAs can be simultaneously co-expressed and differentially stabilized, there must exist mechanisms to exert mRNA specific control. While the mechanisms remain at present obscure, they likely involve the recruitment and participation of auxiliary or accessory proteins. Recently, STEITZ demonstrated that human family member (HuR) associated with four such activities which may modulate HuR's interaction with ARE mRNAs[36].

GM-CSF Regulation and Production in Eosinophils

GM-CSF mRNA decay

Over the past few years, we have analyzed the intracellular mechanisms regulating GM-CSF mRNA accumulation in resting or calcium ionophore-stimulated, human eosinophil-like cell lines (AML14.3D10), in resting or in TNF-α plus Fn-activated peripheral blood eosinophils (PBEos), or in *in vivo* activated bronchoalveolar eosinophils (BALEos), derived after allergen challenge. Endogenous GM-CSF mRNA levels even in activated eosinophils are very low and it is difficult to obtain large numbers ($>2 \times 10^7$ cells) in highly purified form. In addition, to avoid activation, primary cells are obtained by negative selection[87]. In response to these limitations, we have developed a system that models endogenous GM-CSF metabolism in eosinophils by studying the decay of exogenous GM-CSF mRNA after its transfection into PBEos or those derived from BAL[30, 31]. Previously, we showed that primary lymphocytes could be productively transfected by particle-mediated gene transfer (PMGT)[76]. Either expression vectors or *in vitro* transcribed mRNAs could be precipitated onto 1 μm gold beads and accelerated into cells. Once inside, the nucleic acids were released and rapidly transcribed or translated. While the efficiency was modest (1–3%), adequate levels of nucleic acid were delivered to evaluate exogenous mRNA decay rates by Northern blotting of the bulk transfected population. In addition, delivery of mRNA eliminated the need to block transcription with metabolic poisons, including actinomycin D, which have independent effects on mRNA decay[29]. In pilot experiments we discovered that, like lymphocytes, primary eosinophils could be transfected to reasonable levels (1–3%) by PMGT and, subsequently, analyzed by Northern blotting. We initially evaluated GM-CSF mRNA metabolism in the eosinophil-like cell line AML14.3D10. These cells exhibit eosinophil-like morphology and, based on Northern blotting, express undetectable amounts of GM-CSF mRNA. Exogenous GM-CSF mRNA delivered via PMGT decayed with a half-life ($t_{1/2}$) of 8–12 min[84], similar to that seen in lymphocytes. Upon stimulation with calcium ionophore, stability increased by 4 fold, which began within 1 h of ionophore addition and required intact, 3' UTR, AU-rich motifs. Of note, transcriptional inhibitors, typically used in the measurement of mRNA decay, themselves stabilized GM-CSF mRNA, probably through translational inhibition. Transfected GM-CSF mRNA decayed exceptionally rapidly with a $t_{1/2}$ of 8 min in resting PBEos as well, and rapid decay again required intact 3' UTR AREs, as a mutant version without them was 3 fold more stable. Wild-type but not mutant, GM-CSF mRNA was stabilized 3 fold in PBEos activated by ionophore. Thus, the decay rates of these two mRNAs were essentially the same in activated cells.

GM-CSF secretion by PBEos can be induced by IFN-γ[65], lipopolysaccharide (LPS)[95], Fn[5], or mAbs against CD40[70], CD9[43] or CD32[44]. Recently, β7 integrin ligation with plate bound Fn or anti-β7 antibodies increased PBEos survival in a GM-CSF-dependent manner[62]. Similarly, TNF-α has been shown to positively influence *in vitro* eosinophil survival[50], although the mechanism for this effect remains unclear. Both TNF-α and Fn were increased in BAL from asthmatic or allergen-challenged subjects[13, 59, 61, 98], suggesting these agonists might contribute to intrapulmonary eosinophil survival by stimulating GM-CSF production. Indeed, TNF-α in association with extracellular matrix Fn was capable of mediating T cell functions[3, 35]. We showed that PBEos treated *in vitro* with TNF-α plus Fn secreted GM-CSF and displayed enhanced survival. Cytokine production was preceded by GM-CSF mRNA stabilization. Interestingly, eosinophils obtained from BAL of allergen-challenged subjects also showed prolonged GM-CSF mRNA half-life. These data suggest that exposure to TNF-α plus Fn during or after PBEos migration into pulmonary tissues and airspaces stabilized GM-CSF mRNA, thus accounting for GM-CSF production.

The kinetics of GM-CSF mRNA stabilization in re-

sponse to ionophore has been reasonably well defined. GM-CSF mRNA was stabilized in human T lymphocytes activated for 5 h with anti-CD3 plus anti-CD28 antibodies[53], murine T lymphocytes treated with Ca^{2+} ionophore for 6 h[39], or in mast cells after 3 h of activation[108]. We reported stabilization of GM-CSF mRNA in peripheral blood mononuclear cells (PBMC) within 1 h after adding phorbol ester[84]. In AML14.3D10, ionomycin significantly stabilized wild-type GM-CSF mRNA within 1 h of activation, which increased 50% more after 2 h. Importantly, mRNA stabilization closely paralleled GM-CSF mRNA accumulation, suggesting a cause and effect relationship.

Despite many attempts, we, and others, have been unable to measure GM-CSF transcription in PBEos by nuclear run-off assay. Thus, we are unable to determine the relative contributions of transcriptional and post-transcriptional regulation to GM-CSF mRNA accumulation. GM-CSF mRNA transcription was unchanged despite substantially suppressed mRNA decay in T cells activated with mitogenic antibodies[53]. These authors ascribed increased GM-CSF protein levels entirely to mRNA stabilization. However, others have shown[40] that GM-CSF transcription can be enhanced by phorbol esters and Ca^{2+} ionophore. However, these effects were considerably less rapid (~8 h) than those discussed above.

Trans and cis elements involved in GM-CSF post-transcriptional regulation

GM-CSF mRNA stabilization is too rapid for *de novo* transcription and translation of new regulatory proteins. Rather, GM-CSF stability is probably the consequence of the modification of preexisting regulatory protein(s), as previously proposed[58, 108]. Given that ionophore activates calcium-dependent signaling pathways, alterations in the phosphorylation or redox status of regulatory proteins could explain modifications in GM-CSF mRNA stability. Indeed, the activity of several AUUUA specific mRNA-binding proteins was responsive to phorbol ester, plant lectin (PHA) or ionophore[37, 57].

The recognition of ARE containing mRNAs likely involves sequence specific binding proteins (also see above). Indeed, several labs have identified mRNA protein-binding activities in tumor cell lines[57, 113], normal lymphocytes[77], fibroblasts[68] and macrophages[52]. Establishing a physiologic role for these proteins has proven more difficult, although antisense-mediated depletion of HuR was associated with destabilization of vascular endothelial growth factor (VEGF) mRNA[51] and failure to stabilize p21 in response to UV damage[102]. Thus, it has been proposed that cytokine mRNA stabilization likely occurs when sequence specific binding proteins interact with, and possibly mask, the AREs from cellular ribonucleases.

In the AML14.3D10 cell line, using gel mobility shift analysis, we identified several cytoplasmic mRNA-binding proteins specific for the ARE[84]. These binding protein activities were increased in ionophore-treated AML14.3D10 cells concomitant with GM-CSF mRNA stabilization. These observations suggest that several mRNA-binding proteins may be involved in stabilizing GM-CSF mRNA in AML14.3D10 cells upon ionophore-mediated activation. We were able to resolve RNA-protein complexes of 55, 60, 85, 100 and 125 kDa from AML14.3D10 cells using full-length, radiolabeled GM-CSF RNA. NAKAMAKI et al.[68] showed that human embryonic lung fibroblasts contained five major ARE-binding protein complexes of 70, 45, 40, 38 and 32.5 kDa. Based on competition assays, all RNA-binding proteins were highly specific for the AUUUA repeats. At this time, we do not know how many distinct proteins generate the observed number of RNA-protein complexes. For example, it is possible that multimers of the 55 or 60 kDa activities produce the 100 and 125 kDa complexes, respectively. During ionophore-mediated activation, the activity of ARE specific binding proteins increased substantially. Within 2 h, the overall activity was increased by 2.5 fold, which was highly significant. The timing of this event coincided with GM-CSF mRNA stabilization, suggesting a cause and effect relationship. Recently, overexpression of HuR, an AUUUA specific RNA-binding protein, stabilized c-fos mRNA in transfected mouse L939 cells[34]. Heterogeneous nuclear ribonuclear proteins (hnRNP) C and L have been shown to stabilize amyloid protein precursor (APP)[79] and VEGF[90] mRNAs, respectively. The adenosine-uridine binding factor (AUBF) was phosphorylated in response to phorbol ester or ionophore-mediated activation of PBMC[58]. This modification was associated with increased binding activity towards AUUUA RNA ligands. Conversely, hnRNP C protein-binding activity was enhanced by dephosphorylation in activated cells[60]. Thus, post-translational modifications involving the addition or removal of phosphate possibly mediate the changes in AUUUA-binding activity identified in AML14.3D10 cells.

Recently, a truncated human cDNA coding for the multifunctional nucleic acid-binding protein Y box-binding protein 1 (YB-1) was identified in a screen for potential ARE-binding proteins (BHATTACHARYA and MALTER, unpublished). YB-1 is a member of the highly

conserved cold shock domain (CSD) family of proteins and consists of three domains: an N-terminal stretch of undetermined function, a CSD which mediates nucleic acid binding and contains a consensus RNP-1 RNA--binding motif, and a C-terminal domain of basic/acidic repeats which has been implicated in RNA binding as well as protein-protein interactions[4, 11, 91]. The full-length YB-1 was fused to the eleven amino acid protein translocation sequence from HIV Tat protein in the vector pTat-HA. Proteins containing Tat tags cross lipid membranes with high efficiency (>95%), permitting transduction of normal as well as transformed cells[99]. We have demonstrated that human YB-1 bound the ARE of GM-CSF RNA *in vitro* and interacted with GM-CSF mRNA in activated eosinophils[15]. Five RNA recognition motifs (RRMs) have been identified in YB-1 as necessary for both its specific and non-specific interactions with RNA. All of the other ARE-binding proteins characterized to date also contain multiple RRMs that define specificity in different contexts (for example see HuR[56], HuD[74], TTP[46] or AUF-1[113]). HuR and HuD both have consensus RNP-1 motifs that are identical to each other and similar to that found in YB-1. However, AUF-1 and TTP show little commonality at the amino acid sequence level with any of the other ARE-binding proteins. YB-1 partners with a multitude of other proteins both for DNA and RNA binding, including the transcription factor CTCF, hnRNP K, FMRP and nucleolin[17, 19, 22, 91].

The *cis* element(s) necessary for the regulated stability of GM-CSF mRNA remains controversial. We showed that the AUUUA motifs destabilize wild-type GM-CSF in resting or unstimulated cells and that their presence was necessary for ionophore-mediated stabilization[29, 30]. However, in murine T lymphocytes, ionophore slowed the decay of GM-CSF mRNAs when both the AUUUA motifs and an ancillary element located about 160 bases upstream of the ARE were present[39]. We have directly examined this issue by comparing the stability of wild-type and mutant human GM-CSF mRNAs which differ solely in the AUUUA motifs. As the mutant was unaffected by Ca^{2+} ionophore, we conclude that stabilization of GM-CSF mRNA by Ca^{2+} absolutely requires intact AUUUA motifs. Whether this represents a fundamental difference between eosinophils and T cells or humans and mice in terms of signaling cascades or regulatory apparatus remains unclear.

The decay rates for transfected wild-type GM-CSF mRNA were remarkably rapid ($t_{1/2} \approx 8$ min[29, 30]). This is consistent with prior observations demonstrating the potent destabilizing effects of AUUUA motifs in cytokine and protooncogene mRNAs[53, 78, 89]. However, mutant GM-CSF mRNA, while significantly more stable ($t_{1/2} \approx$ 20–25 min), also decayed rapidly, especially when compared with more stable mRNAs such as β-globin ($t_{1/2}$ = 17 h)[41]. These results suggest the presence of other, calcium insensitive, destabilizing elements within the 3' UTR or coding region of GM-CSF mRNA. Of note, the 3' UTR of GM-CSF is very highly conserved across multiple species (≈80%).

Intracellular signaling

Activators of protein kinase A (PKA) and protein kinase C (PKC) are important modulators of mRNA stability for a number of gene systems, including lactate dehydrogenase A[92], Na^+/glucose cotransporter[75], renin[21] and phosphoenolpyruvate carboxykinase[38]. Ca^{2+} ionophore has been reported to stabilize GM-CSF mRNA in murine T lymphocytes[39] and primary human eosinophils[30]. Whereas phorbol esters have modest to negligible effects on GM-CSF mRNA accumulation in AML14.3D10 cells (ESNAULT and MALTER, unpublished data) or on GM-CSF secretion from normal, peripheral blood eosinophils, they, however, rapidly, stabilized GM-CSF mRNA ($t_{1/2} \approx 3$ h) in T lymphocytes[9]. This suggests that lymphocytes and eosinophils respond differently to PKC or the other kinases, such as mitogen-activated protein kinases sensitive to phorbol esters.

Most of the studies analyzing the role of MAPK on mRNA stability have employed transformed cell lines (Jurkat, Hela, THP-1, fibroblast-like or mast cell-like) as models. These have generally implicated p38 or c-Jun-NH_2-terminal kinase (JNK) as critical kinases[18, 47, 63, 64, 80, 83, 107]. For example, JNK activation was required for IL-2 and IL-3 mRNA stabilization in T or mast cell lines[18, 63]. Extracellular signal-regulated kinase (ERK) has, however, been linked to macrophage inflammatory protein 2 (MIP-2) mRNA in fresh rat peritoneal neutrophils and nucleolin mRNA regulation in human PBMC (huPBMC)[104, 105, 110]. In TNF-α plus Fn-treated eosinophils, activation of ERK, but not p38, correlated with GM-CSF mRNA stability. Consistent with these results, eosinophils transduced with Tat-MEK1(E), which activates ERK, showed increased GM-CSF mRNA stability. This was not seen after transduction with TatMKK3b(E), which activates p38[33]. The intracellular pathways that connect extracellular activation with protein-binding activity and mRNA stabilization remain unknown.

GM-CSF-Dependent Eosinophil Survival

GM-CSF production is tightly correlated with eosinophilia *in vivo*[13] and has been associated with inhibition of airway eosinophil apoptosis[93]. Our laboratory showed that TNF-α plus Fn increased PBEos survival through a GM-CSF-dependent process (≈30% survival at 4 days *in vitro*). As seen previously[86], we found that, after allergen challenge, BALEos were highly resistant to apoptosis (≈60% survival at 4 days *in vitro*) while only 5% of unstimulated PBEos survived 4 days in culture. Interestingly, Fn alone mediated survival could be entirely inhibited by anti-GM-CSF antibody, demonstrating dependence on extracellular GM-CSF. In contrast, TNF-α induced survival, was more modest (15%), and GM-CSF-independent. These data suggest that TNF-α signaling may be modulated or redirected by co-stimulation of PBEos with Fn. These data are consistent with RT-PCR results showing substantial and reproducibly increased GM-CSF mRNA in PBEos stimulated with Fn, Fn plus TNF-α, but not TNF-α alone[31].

As the upregulation of GM-CSF mRNA and protein secretion appeared necessary for enhanced survival of PBEos, we hypothesized *in vitro* BALEos survival would be similarly dependent on GM-CSF. The viability of BALEos obtained 48 h after segmental allergen challenge was accessed after addition of anti-GM-CSF, anti-IL-5 or irrelevant antibody. The majority of BALEos were dead at 6 days in the presence of neutralizing anti-GM-CSF, while irrelevant antibody or anti-IL-5 had no effect. These data show that GM-CSF, rather than IL-5, is critical for long-term BALEos survival *in vitro*. In addition, BALEos become GM-CSF secretors during migration into the lung or after residence there. The accumulation of GM-CSF mRNA in BALEos and the requirement for GM-CSF to support their long-term survival *in vitro* suggest that GM-CSF plays an important functional role in initiating and/or maintaining pulmonary eosinophilia *in vivo*. This is supported by several studies showing that elevations of GM-CSF in BAL fluid after segmental challenge or asthma strongly correlated with eosinophil content[88, 109]. It is also likely that macrophages and fibroblasts contribute to BALEos survival by secreting GM-CSF.

While significant amounts of GM-CSF (pg/ml) are typically found in the airways of asthmatics, how much GM-CSF is necessary to enhance eosinophil survival is unclear. The transfection of GM-CSF mRNA into primary eosinophils led to detectable intracellular but not extracellular levels of GM-CSF protein[30]. Despite its very low concentration (<1pg/ml), cultures of PBEos transfected with GM-CSF mRNA were resistant to *in vitro* apoptosis. Based on antibody neutralization experiments, the survival activity was entirely mediated by GM-CSF without a significant contribution by IL-5 and required the extracellular presence of cytokine. These data are consistent with recent reports showing that anti-GM-CSF antibodies blocked *in vitro* eosinophil survival induced by anti-CD9[43], TNF-α[50] or anti-CD32[44], while anti-IL-5 antibodies had no effect. Small amounts of secreted GM-CSF (<1 pg/ml) from transfected eosinophils was as efficient as 100–500 pg/ml of exogenously added recombinant human GM-CSF (rhGM-CSF). The contrasting biologic potency of GM-CSF protein produced by transfected cells compared with rhGM-CSF was unexpected. Obvious differences reflect the producing cells (PBEos vs. *Escherichia coli*), delivery (continuous vs. bolus) and cell proximity (secreted vs. added to the culture media). AGLIETTA et al.[2] reported that frequent, low dose administration of glycosylated rhGM-CSF was more effective with fewer side effects than single bolus dosing. In addition, continuous exposure to GM-CSF is essential for maximal cell survival. Neutralizing GM-CSF antibody was able to inhibit PBEos survival even when added 48 h after the initiation of culture[32]. The apparent discrepancy between the short life time of the transfected mutant GM-CSF mRNA ($t_{1/2}$ = 22 min) and the low level of protein secretion but the continued presence of GM-CSF in the supernatant (48 h) may reflect eosinophil accumulation and storage of cytokines in granules[49, 67] with continuous and long term low level release. Recently, a very interesting study showed IL-4 release by vesicular transport and membrane fusion from human eosinophils[8].

GM-CSF levels are elevated in the BAL fluid of symptomatic asthmatics (typically ≈2–20 pg/ml)[13, 100]. Thus, the levels observed to be functional *in vitro* are likely to be physiologically relevent. Perhaps more importantly, BAL GM-CSF levels remain persistently elevated in asthmatics even several weeks after antigen challenge[88]. Such an environment could result from autocrine GM-CSF production by "reservoir" eosinophils as well as fibroblasts and macrophages and prevent eosinophil apotosis and promote airway damage.

Conclusions

In this review, we have discussed the importance of GM-CSF mRNA stabilization for GM-CSF production and eosinophil survival. The timing of autocrine GM-CSF exposure critically influences eosinophil survival *in vitro* and, possibly, *in vivo*. Cytokines cooperating with extracellular matrix proteins, including Fn, clearly

participate in the stabilization process, suggesting eosinophil migration from the periphery to the airways provides the proper milieu to prevent apoptosis. The intracellular signaling-modulating GM-CSF mRNA decay involves ERK1/2 and culminates in the activation of binding proteins such as YB-1. These data suggest targeting GM-CSF with pharmacologic interventions should reduce eosinophilia and, possibly, the late asthmatic response.

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References

1. ADACHI T., MOTOJIMA S., HIRATA A., FUKUTA T. and MAKINO S., (1995): Eosinophil viability-enhancing activity in sputum from patients with bronchial asthma. Am. J. Respir. Crit. Care Med., **151**, 618–623.
2. AGLIETTA M., MONZEGLIO C., PASQUINO P., CARNINO F., STERN A. C. and GAVOSTO F. (1993): Short-term administration of granulocyte-macrophage colony stimulating factor decreases hematopoietic toxicity of cytostatic drugs. Cancer, **72**, 2970–2973.
3. ALON R., CAHALON L., HERSHKOVIC R., ELBAZ D., REIZIS B., WALLACH D., AKIYAMA S. K., YAMADA K. M. and LIDER O. (1994): TNF-α binds to the N-terminal domain of fribronectin and augments the β1-integrin-mediated adhesion of CDA^+ T lymphocytes to the glycoprotein. J. Immunol., **152**, 1304–1313.
4. ANSARI S. A., SAFAK M., GALLIA G. L., SAWAYA B. E., AMINI S. and KHALILI K. (1999): Interaction of YB-1 with human immunodeficiency virus type 1 Tat and TAR RNA modulates viral promoter activity. J. Gen. Virol., **80**, 2629–2638.
5. ANWAR A. R., MOQDEL R., WALSH G. M., KAY A. B. and WARDLAW A. J. (1993): Adhesion to fibronectin prolongs eosinophil survival. J. Exp. Med., **177**, 839–843.
6. ARM J. P. and LEE T. H. (1992): The pathobiology of bronchial asthma. Adv. Immunol. **51**, 323–382.
7. BANCHEREAU J. and STEINMAN R. M. (1998): Dentritic cells and the control of immunity. Nature, **392**, 245–252.
8. BANDEIRA-MELO C., SUGIYAMA K., WOODS L. J. and WELLER P. F. (2001): Eotaxin elicits rapid vesicular transport-mediated release of preformed IL-4 from human eosinophils. J. Immunol., **166**, 4813–4817.
9. BICKEL M., COHEN R. B. and PLUZNIK D. H. (1990): Post-transcriptional regulation of granulocyte-macrophage colony-stimulating factor synthesis in murine T cells. J. Immunol., **145**, 840–845.
10. BOUSQUET J., CHANEZ P., LACOSTE J. Y., BARNÉON G., GHAVANIAN N., ENANDER I., VENGE P., AHLSTEDT S., SIMONY-LAFONTAINE J., GODARD P. and MICHEL F.-B. (1990): Eosinophilic inflammation in asthma. N. Engl. J. Med., **323**, 1033–1039.
11. BOUVET P., MATSUMOTO K. and WOLFFE A. P. (1995): Sequence-specific RNA recognition by the *Xenopus* Y-box proteins. An essential role for the cold shock domain. J. Biol. Chem., **47**, 28297–28303.
12. BROIDE D. H. and FIRESTEIN G. S. (1991): Endobronchial allergen challenge in asthma. Demonstration of cellular source of granulocyte macrophage colony-stimulating factor by *in situ* hybridization. J. Clin. Invest., **88**, 1048–1053.
13. BROIDE D. H., LOTZ M., CUOMO A. J., COBURN D. A., FEDERMAN E. C. and WASSERMAN S. I. (1992): Cytokines in symptomatic asthma airways. J. Allergy Clin. Immunol., **89**, 958–967.
14. BROIDE D. H., PAINE M. M. and FIRESTEIN G. S. (1992): Eosinophils express interleukin 5 and granulocyte-macrophage colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics. J. Clin. Invest., **90**, 1414–1424.
15. CAPOWSKI E. E., ESNAULT S., BHATTACHARYA S. and MALTER J. S. (2001): Y box-binding factor promotes eosinophil survival by stabilizing granulocyte-macrophage colony-stimulating factor mRNA. J. Immunol., **167**, 5970–5976.
16. CARBALLO E., LAI W. S. and BLACKSHEAR P. J. (2000): Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. Blood, **95**, 1891–1899.
17. CEMAN S., NELSON R. and WARREN S. T. (2000): Identification of mouse YB1/p50 as a component of the FMRP-associated mRNP particle. Biochem. Biophys. Res. Commun., **279**, 904–908.
18. CHEN C.-Y., DEL GATTO-KONCZAK F., WU Z. and KARIN M. (1998): Stabilization of interleukin-2 mRNA by the c-Jun NH2--terminal kinase pathway. Science, **280**, 1945–1949.
19. CHEN C.-Y., GHERZI R., ANDERSEN J. S., GAIETTA G., JÜRCHOTT K., ROYER H.-D., MANN M. and KARIN M. (2000): Nucleolin and YB-1 are required for JNK-mediated interleukin-2 mRNA stabilization during T-cell activation. Genes Dev., **14**, 1236–1248.
20. CHEN C. Y. and SHYU A. B. (1995): AU-rich elements: characterization and importance in mRNA degradation. Trends Biochem. Sci., **20**, 465–470.
21. CHEN M., SCHNERMANN J., SMART A. M., BROSIUS F. C., KILLEN P. D. and BRIGGS J. P. (1993): Cyclic AMP selectively increases renin mRNA stability in cultured juxtaglomerular granular cells. J. Biol. Chem., **268**, 24138–24144.
22. CHERNUKHIN I. V., SHAMSUDDIN S., ROBINSON A. F., CARNES A. F., PAUL A., EL-KADY A. I., LOBANENKOV V. L. and KLENOVA E. M. (2000): Physical and functional interaction between two pluripotent proteins, the Y-box DNA/RNA-binding factor, YB-1, and the multivalent zinc finger factor, CTCF. J. Biol. Chem., **275**, 29915–29921.
23. CLUTTERBUCK E. J., HIRST E. M. and SANDERSON C. J. (1989): Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF. Blood, **73**, 1504–1512.
24. DE ANDRES B., CARDABA B., DEL POZO V., MARTIN-OROZCO E., GALLARDO S., TRAMON P., PALOMINO P. and LAHOZ C. (1994): Modulation of the Fc gamma RII and Fc gamma RIII induced by GM-CSF, IFN-gamma and IL-4 on murine eosinophils. Immunology, **83**, 155–160.
25. DEL PRETE G. F., DE CARLI M., D'ELIOS M. M., MAESTRELLI P., RICCI M., FABBRI L. and ROMAGNANI S. (1993): Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. Eur. J. Immunol., **23**, 1445–1449.
26. DE MONCHY J. G., KAUFFMAN H. F., VENGE P., KOETER G. H., JANSEN H. M., SLUITER H. J. and DE VRIES K. (1985): Bron-

choalveolar eosinophils during allergen-induced late asthmatic reaction. Am. Rev. Respir. Dis., **131**, 373–376.
27. DURHAM S. R., YING S., VARNEY V. A., JACOBSON M. R., SUDDERICK R. M., MACKAY I. S., KAY A. B. and HAMID Q. A. (1992): Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: Relationship to tissue eosinophilia. J. Immunol., **148**, 2390–2394.
28. ESNAULT S., BENBERNOU N., LAVAUD F., SHIN H.-C., POTRON G. and GUENOUNOU M. (1996): Differential spontaneous expression of mRNA for IL-4, IL-10, IL-13, IL-2 and interferon--gamma (IFN-γ) in peripheral blood mononuclear cells (PBMC) from atopic patients. Clin. Exp. Immunol., **103**, 111–118.
29. ESNAULT S., JARZEMBOWSKI J. A. and MALTER J. S. (1998): Stabilization of granulocyte-macrophage colony-stimulating factor RNA in a human eosinophil-like cell line requires the AUUUA motifs. Proc. Assoc. Am. Physicians, **110**, 575–584.
30. ESNAULT S. and MALTER J. S. (1999): Primary peripheral blood eosinophils rapidly degrade transfected granulocyte-macrophage colony-stimulating factor mRNA. J. Immunol., **163**, 5228–5234.
31. ESNAULT S. and MALTER J. S. (2001): Granulocyte macrophage-colony-stimulating factor mRNA is stabilized in airway eosinophils and peripheral blood eosinophils activated by TNF--alpha plus fibronectin. J. Immunol., **166**, 4658–4663.
32. ESNAULT S. and MALTER J. S. (2001): Minute quantities of granulocyte-macrophage colony-stimulating factor prolong eosinophil survival. J. Interferon Cytokine Res., **21**, 117–124.
33. ESNAULT S. and MALTER J. S. (2002): Extracellular signal-regulated kinase mediates GM-CSF mRNA stabilization in TNF-α plus fibronectin-activated peripheral blood eosinophils. Blood (in press).
34. FAN X. C. and STEITZ J. A. (1998): Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the *in vivo* stability of ARE-containing mRNAs. EMBO J., **17**, 3448–3460.
35. FRANITZA S., HERSHKOVIZ R., KAM N., LICHTENSTEIN N., VADAY G. G., ALON R. and LIDER O. (2000): TNF-α associated with extracellular matrix fibronectin provides a stop signal for chemotactically migrating T cells. J. Immunol., **165**, 2738–2747.
36. GALLOUZI I. E., BRENNAN C. M. and STEITZ J. A. (2001). Protein ligands mediate the CRM1-dependent export of HuR in response to heat shock. RNA, **7**, 1348–1361.
37. GILLIS P. and MALTER J. S. (1989). The adenosine-uridine binding factor recognizes the AU-rich elements of cytokine, lymphokine, and oncogene mRNAs. J. Biol. Chem., **266**, 3172–3177.
38. HOD Y. and HANSON R. W. (1988): Cyclic AMP stabilizes the mRNA for phosphoenolpyruvate carboxykinase (GTP) against degradation. J. Biol. Chem., **263**, 7747–7752.
39. IWAI Y., AKAHANE K., PLUZNIK D. H. and COHEN R. B. (1993): Ca^{2+} ionophore A23187-dependent stabilization of granulocyte-macrophage colony-stimulating factor messenger RNA in murine thymoma EL-4 cells is mediated through two distinct regions in the 3' – untranslated region. J. Immunol., **150**, 4386–4394.
40. JENKINS F., COCKERILL P. N., BOHMANN D. and SHANNON M. F. (1995): Multiple signals are required for function of the human granulocyte-macrophage colony-stimulating factor gene promoter in T cells. J. Immunol., **155**, 1240–1251.
41. KABNICK K. S. and HOUSMAN D. E. (1988): Determinants that contribute to cytoplasmic stability of human c-fos and β-globin mRNAs are located at several sites in each mRNA. Mol. Cell. Biol., **8**, 3244–3250.
42. KHWAJA A. and LINCH D. C. (1994): Effects of granulocyte colony-stimulating factor and granulocyte-macrophage colony--stimulating factor on neutrophil formation and function. Curr. Opin. Hematol., **1**, 216–220.
43. KIM J.-T., GLEICH G. J. and KITA H. (1997): Roles of CD9 molecules in survival and activation of human eosinophils. J. Immunol., **159**, 926–933.
44. KIM J.-T., SCHIMMING A. W. and KITA H. (1999): Ligation of FcγRII (CD32) pivotally regulates survival of human eosinophils. J. Immunol., **162**, 4253–4259.
45. KITA H., OHNISHI T., OKUBO Y., WEILER D., ABRAMS J. S. and GLEICH G. J. (1991): Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. J. Exp. Med., **174**, 745–748.
46. LAI W. S., CARBALLO E., THORN J. M., KENNINGTON E. A. and BLACKSHEAR P. J. (2000): Interactions of CCCH zinc finger proteins with mRNA, binding of tristetraprolin-related zinc finger proteins to AU-rich elements and destabilization of mRNA. J. Biol. Chem., **275**, 17827–17837.
47. LASA M., BROOK M., SAKLATVALA J. and CLARK A. R. (2001): Dexamethasone destabilizes cyclooxygenase 2 mRNA by inhibiting mitogen-activated protein kinase p38. Mol. Cell. Biol., **21**, 771–780.
48. LAZAROVA D. L., SPENGLER B. A., BIEDLER J. L. and ROSS R. A. (1999): HuD, a neuronal-specific RNA-binding protein, is a putative regulator of N-myc pre-mRNA processing/stability in malignant human neuroblasts. Oncogene, **18**, 2703–2710.
49. LEVI-SCHAFFER F., LACY P., SEVERS N. J., NEWMAN T. M., NORTH J., GOMPERTS B., KAY A. B. and MOQBEL R. (1995): Association of granulocyte-macrophage colony-stimulating factor with the crystalloid granules of human eosinophils. Blood, **85**, 2579–2586.
50. LEVI-SCHAFFER F., TEMKIN V., MALAMUD V., FELD S. and ZILBERMAN Y. (1998): Mast cells enhance eosinophil survival *in vitro*: Role of TNF-α and granulocyte-macrophage colony-stimulating factor. J. Immunol., **160**, 5554–5562.
51. LEVY N. S., CHUNG S., FURNEAUX H. and LEVY A. P. (1998): Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR. J. Biol. Chem., **273**, 6417–6423.
52. LEWIS T., GUEYDAN C., HUEZ G., TOULME J. J. and KRUYS V. (1998): Mapping of a minimal AU-rich sequence required for lipopolysaccharide-induced binding of a 55-kDa protein on tumor necrosis factor-alpha mRNA. J. Biol. Chem., **273**, 13781–13786.
53. LINDSTEN T., JUNE C. H., LEDBETTER J. A., STELLA G. and THOMPSON C. B. (1989): Regulation of lymphokine messenger RNA stability by a surface-mediated T cell activation pathway. Science, **244**, 339–343.
54. LIU L. Y., SEDGWICK J. B., BATES M. R., VRIST R. F., JARJOUR N. N., BUSSE W. W., GERN J. E., KITA H. and KELLY E. A. B. Decreased expression of mIL-5a on airway eosinophils: IL-5 down-modulates its receptor via a protease-mediated process (submitted).
55. LOFLIN P., CHEN C. Y. and SHYU A. B. (1999): Unraveling a cytoplasmic role for hnRNP D in the *in vivo* mRNA destabilization directed by the AU-rich element. Genes Dev., **13**, 1884–1897.

56. MA W.-J., CHENG S., CAMPBELL C., WRIGTH A. and FURNEAUX H. (1996): Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein. J. Biol. Chem., **271**, 8144–8151.
57. MALTER J. S. (1989): Identification of an AUUUA-specific messenger RNA binding protein. Science, **246**, 664–666.
58. MALTER J. S. and HONG Y. (1991): A redox switch and phosphorylation are involved in the post-transcriptional up-regulation of the adenosine-uridine binding factor by phorbol ester and ionophore. J. Biol. Chem., **266**, 3167–3171.
59. MATTOLI S., MATTOSO V. L., SOLOPERTO M., ALLEGRA L. and FASOLI A. (1991): Cellular and biochemical characteristics of bronchoalveolar lavage fluid in symptomatic nonallergic asthma. J. Allergy Clin. Immunol., **87**, 794–802.
60. MAYRAND S. H., DWEN P. and PEDERSON T. (1993): Serine/ /threonine phosphorylation regulates binding of C hnRNP proteins to pre-mRNA. Proc. Natl. Acad. Sci. USA, **90**, 7764–7768.
61. MEERSCHAERT J., KELLY E. A. B., MOSHER D. F., BUSSE W. W. and JARJOUR N. N. (1999): Segmental antigen challenge increases fibronectin in brochoalveolar lavage fluid. Am. J. Respir. Crit. Care Med., **159**, 619–625.
62. MEERSCHAERT J., VRTIS R. F., SHIKAMA Y., SEDGWICK J. B., BUSSE W. W and MOSHER D. F. (1999): Engagement of $\alpha 4\beta 7$ integrins by monoclonal antibodies or ligands enhances survival of human eosinophils *in vitro*. J. Immunol., **163**, 6217–6227.
63. MING X.-F., KAISER M. and MORONI C. (1998): c-jun N-terminal kinase is involved in AUUUA-mediated interleukin-3 mRNA turnover in mast cells. EMBO J., **17**, 6039–6048.
64. MIYAZAWA K., MORI A., MIYATA H., AKAHANE M., AJISAWA Y. and OKUDAIRA H. (1998): Regulation of interleukin-1β-induced interleukin-6 gene expression in human fibroblast-like synoviocytes by p38 mitogen-activated protein kinase. J. Biol. Chem., **273**, 24832–24838.
65. MOQBEL R., HAMID Q., YING S., BARKANS J., HARTNELL A., TSICOPOULOS A., WARDLAW A. J. and KAY A. B. (1991): Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. J. Exp. Med., **174**, 749–752.
66. NAGATA M., SEDGWICK J. B. and BUSSE W. W. (1995): Differential effects of granulocyte-macrophage colony-stimulating factor on eosinophil and neutrophil superoxide anion generation. J. Immunol., **155**, 4948–4954.
67. NAKAJIMA H., GLEICH G. J. and KITA H. (1996): Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. J. Immunol., **156**, 4859–4866.
68. NAKAMAKI T., IMAMURA J., BREWER G., TSURUOKA N. and KOEFFLER H. P. (1995): Characterization of adenosine-uridine--rich RNA binding factors. J. Cell. Physiol., **165**, 484–492.
69. OH J. W., LEE H. B., KIM C. R., YUM M. K., KOH Y. J., MOON S. J., KANG J. O. and PARK I. K. (1999): Analysis of induced sputum to examine the effects of inhaled corticosteroid on airway inflammation in children with asthma. Ann. Allergy Asthma Immunol., **82**, 491–496.
70. OHKAWARA Y., LIM K. G., XING Z., GLIBETIC M., NAKANO K., DOLOVICH J., CROITORU K., WELLER P. F. and JORDANA M. (1996): CD40 expression by human peripheral blood eosinophils. J. Clin. Invest., **97**, 1761–1766.
71. OHNISHI T., KITA H., WEILER D., SEDGWICK J. B., CALHOUN W. J., BUSSE W. W., ABRAMS J. and GLEICH G. J. (1993): IL-5 is the predominant eosinophil-active cytokine in the antigen-induced pulmonary late phase reaction. Am. Rev. Respir. Dis., **147**, 901–907.
72. PALFRAMAN R. T., COLLINS P. D., SEVERS N. J., ROTHERY S., WILLIAMS T. J. and RANKIN S. M. (1998): Mechanisms of acute eosinophil mobilization from the bone marrow stimulated by interleukin 5: the role of specific adhesion molecules and phosphatidylinositol 3-kinase. J. Exp. Med., **188**, 1621–1632.
73. PARK C. S., CHOI Y. S., KI S. Y., MOON S. H., JEONG S., W. UH S.-T. and KIM Y. H. (1998): Granulocyte-macrophage colony-stimulating factor is the main cytokine enhancing survival of eosinophils in asthmatic airways. Eur. Respir. J., **12**, 872–878.
74. PARK S., MYSZKA D. G., YU M., LITTLER S. J. and LAIRD-OFFRINGA I. A. (2000): HuD RNA recognition motifs play distinct roles in the formation of stable complex with AU-rich RNA. Mol. Cell. Biol., **20**, 4765–4772.
75. PENG H. and LEVER J. E. (1995): Regulation of Na(+)-coupled glucose transport in LLC-PK1 cells. Message stabilization induced by cyclic AMP elevation is accompanied by binding of a M(r) = 48,000 protein to a uridine-rich domain in the 3'-untranslated region. J. Biol. Chem., **270**, 23996–4003.
76. RAJAGOPALAN L. E., BURKHOLDER J. K., TURNER J., CULP J., YANG N. S. and MALTER J. S. (1995): Granulocyte-macrophage colony-stimulating factor mRNA stabilization enhances transgenic expression in normal cells and tissues. Blood, **86**, 2551–2558.
77. RAJAGOPALAN L. E. and MALTER J. S. (1994): Modulation of granulocyte-macrophage colony-stimulating factor mRNA stability *in vitro* by adenosine-uridine binding factor. J. Biol. Chem., **269**, 23882–23888.
78. RAJAGOPALAN L. E. and MALTER J. S. (1996): Turnover and translation of *in vitro* synthesized messenger RNAs in transfected, normal cells. J. Biol. Chem., **271**, 19871–19876.
79. RAJAGOPALAN L. E., WESTMARK C. J., JARZEMBOWSKI J. A. and MALTER J. S. (1998): HnRNP C increases amyloid precursor protein (APP) production by stabilizing APP mRNA. Nucleics Acids Res., **26**, 3418–3423.
80. RIDLEY H. S., DEAN J. L. E., SARSFIELD S. J., BROOK M., CLARK A. R. and SAKLATVALA J. (1998): A p38 MAP kinase inhibitor regulates stability of interleukin-1-induced cyclooxygenase-2 mRNA. FEBS Lett., **439**, 75–80.
81. ROBINSON D. S., BENTLEY A. M., HARTNELL A., KAY A. B. and DURHAM S. R. (1993): Activated memory T helper cells in bronchoalveolar lavage fluid from patients with atopic asthma: relation to asthma symptoms, lung function, and bronchial responsiveness. Thorax, **48**, 26–32.
82. ROBINSON D. S., HAMID Q., YING S., TSICOPOULOS A., BARKANS J., BENTLEY A. M., CORRIGAN C., DURHAM S. R. and Kay A. B. (1992): Predominant Th2-like bronchoalveolar T--lymphocyte population in atopic asthma. N. Engl. J. Med., **326**, 298–304.
83. RUTAULT K., HAZZALIN C. A. and MAHADEVAN L. C. (2001): Combinaisons of ERK and p38 MAPK inhibitors ablate tumor necrosis factor-α (TNF-α) mRNA induction. J. Biol. Chem., **276**, 6666–6674.
84. RUTH J. H., ESNAULT S., JARZEMBOWSKI J. A. and MALTER J. S. (1999): Calcium ionophore upregulation of AUUUA-specific binding protein activity is contemporaneous with granulocyte--macrophage colony-stimulating factor messenger RNA stabilization in AML14.3D10 cells. Am. J. Respir. Cell. Mol. Biol., **21**, 621–628.
85. SCHLEIMER R. P., STERBINSKY S. A., KAISER J., BICKEL C. A., KLUNK D. A., TOMIOKA K., NEWMAN W., LUSCINSKAS F. W., GIMBRONE M. A. and MCINTYRE B. W. JR. (1992): IL-4 in-

duces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. J. Immunol., **148**, 1086–1092.
86. SEGWICK J. B., CALHOUN W. J., VRTIS R. F., BATES M. E., MCALLISTER P. K. and BUSSE W. W. (1992): Comparison of airway and blood eosinophil function after *in vivo* antigen challenge. J. Immunol., **149**, 3710–3708.
87. SEDGWICK J. B., SHIKAMA Y., NAGATA M., BRENER K. and BUSSE W. W. (1996): Effect of isolation protocol on eosinophil function: Percoll gradients versus immunomagnetic beads. J. Immunol. Methods, **198**, 15–24.
88. SHAVER J. R., ZANGRILLI J. G., CHO S.-K., CIRELLI R. A., POLLICE M., HASTIE A. T., FISH J. E. and PETERS S. P. (1997): Kinetics of the development and recovery of the lung from IgE-mediated inflammation: dissociation of pulmonary eosinophilia, lung injury, and eosinophil-active cytokines. Am. J. Respir. Crit. Care Med., **155**, 442–448.
89. SHAW G. and KAMEN R. (1986): A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell, **46**, 659–667.
90. SHIH S.-C. and CLAFFEY K. P. (1999): Regulation of human vascular endothelial growth factor mRNA stability in hypoxia by heterogeneous nuclear ribonucleoprotein L. J. Biol. Chem., **274**, 1359–1365.
91. SHNYREVA M., SCHULLERY D. S., SUZUKI H., HIGAKI Y. and BOMSZTYK K. (2000): Interaction of two multifunctional proteins. Heterogeneous nuclear ribonucleoprotein K and Y-box--binding protein. J. Biol. Chem., **275**, 15498–15503.
92. SHORT S., TIAN D., SHORT M. L. and JUNGMANN R. A. (2000): Structural determinants for post-transcriptional stabilization of lactate dehydrogenase A mRNA by the protein kinase C signal pathway. J. Biol. Chem., **275**, 12963–12969.
93. SIMON H.-U., YOUSEFI S., DIBBERT B., LEVI-SCHAFFER F. and BLASER K. (1997): Anti-apoptotic signals of granulocyte-macrophage colony-stimulating factor are transduced via Jak2 tyrosine kinase in eosinophils. Eur. J. Immunol., **27**, 3536–3539.
94. SOLOPERTO M., MATTOSO V. L., FASOLI A. and MATTOLI S. (1991): A bronchial epithelial cell-derived factor in asthma that promotes eosinophil activation and survival as GM-CSF. Am. J. Physiol., **260**, L530–538.
95. TAKANASHI S., NONAKA R., XING Z., O'BYRNE P., DOLOVICH J. and JORDANA M. (1994): Interleukin 10 inhibits lipopolysaccharide-induced survival and cytokine production by human peripheral blood eosinophils. J. Exp. Med., **180**, 711–715.
96. TAMURA N., ISHII N., NAKAZAWA M., NAGOYA M., YOSHINARI M., AMANO T., NAKAZIMA H. and MINAMI M. (1996): Requirement of CD80 and CD86 molecules for antigen presentation by eosinophils. Scand. J. Immunol., **44**, 229–238.
97. TURNER H. and KINET J. P. (1999): Signaling through the high-affinity IgE receptor Fc epsilon RI. Nature, **25**, B24–30.
98. VIRCHOW J.-C., WALKER C., HAFNER D., KORTSIK C., WERNER P., MATTHYS H. and KROEGEL C. (1995): T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. Am. J. Respir. Crit. Care Med., **151**, 960–968.
99. VOCERO-AKBANI A., LISSY N. A. and DOWDY S. F. (2000): Transduction of full-length tat fusion proteins directly into mammalian cells: analysis of T cell receptor activation-induced cell death. Methods Enzymol., **322**, 508–521.
100. WALKER C., BAUER W., BRAUN R. K., MENZ G., BRAUN P., SCHWARZ F., HANSEL T. T. and VILLIGER B. (1994): Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. Am. J. Respir. Crit. Care Med., **150**, 1038–1048.
101. WANG P., WU P., CHEEWATRAKOOLPONG B., MYERS J. G., EGAN R. W. and BILLAH M. M. (1998): Selective inhibition of IL-5 receptor α-chain gene transcription by IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor in human blood eosinophils. J. Immunol., **160**, 4427–4432.
102. WANG W., FURNEAUX H., CHENG H., CALDWELL M. C., HUTTER D., LIU Y., HOLBROOK N. and GOROSPE M. (2000): HuR regulates p21 mRNA stabilization by UV light. Mol. Cell. Biol., **20**, 760–769.
103. WELLER P. F., RAND T. H., BARRETT T., ELOVIC A., WONG D. T. and FINBERG R. W. (1993): Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 alpha expression. J. Immunol., **150**, 2554–2562.
104. WESTMARK C. J. and MALTER J. S. (2001): Extracellular-regulated kinase controls beta-amyloid precursor protein mRNA decay. Brain Res. Mol. Brain Res., **90**, 193–201.
105. WESTMARK C. J. and MALTER J. S. (2001): Up-regulation of nucleolin mRNA and protein in peripheral blood mononuclear cells by extracellular-regulated kinase. J. Biol. Chem., **276**, 1119–1126.
106. WILSON T. and TREISMAN R. (1988): Removal of poly(A) and consequent degradation of c-fos mRNA facilitated by 3' AU--rich sequences. Nature, **336**, 396–399.
107. WINZEN R., KRACHT M., RITTER B., WILHELM A., CHEN C.-Y., SHYU A. B., MULLER M., GAESTEL M., RESCH K. and HOLTMANN H. (1999): The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. EMBO J., **18**, 4969–4980.
108. WODNAR-FILIPOWICZ A. and MORONI C. (1990): Regulation of interleukin 3 mRNA expression in mast cells occurs at the post-transcriptional level and is mediated by calcium ions. Proc. Natl. Acad. Sci. USA, **87**, 777–781.
109. WOOLLEY K. L., ADELROTH E., WOOLLEY M. J., ELLIS R., JORDANA M. and O'BYRNE P. M. (1995): Effects of allergen challenge on eosinophils, eosinophil cationic protein, and granulocyte-macrophage colony-stimulating factor in mild asthma. Am. J. Respir. Crit. Care Med., **151**, 1915–1924.
110. XIAO Y.-Q., SOMEYA K.-I., MORITA H., TAKAHASHI K. and OHUCHI K. (1999): Involvement of p38 MAPK and ERK/ /MAPK pathways in staurosporine-induced production of macrophage inflammatory protein-2 in rat peritoneal neutrophils. Biochim. Biophys. Acta, **1450**, 155–163.
111. XU N., CHEN C. Y. and SHYU A. B. (2001): Versatile role for hnRNP D isoforms in the differential regulation of cytoplasmic mRNA turnover. Mol. Cell Biol., **21**, 6960–6971.
112. YOUNG D. A., LOWE L. D. and CLARK S. C. (1990): Comparison of the effects of IL-3, granulocyte-macrophage colony-stimulating factor, and macrophage colony-stimulating factor in supporting monocyte differentiation in culture: analysis of macrophage antibody-dependent cellular cytotoxicity. J. Immunol., **145**, 607–615.
113. ZHANG W., WAGNER B. J., EHRENMAN K., SCHAEFER A. W., DEMARIA C. T., CRATER D., DEHAVEN K., LONG L. and BREWER G. (1993): Purification, characterization, and cDNA cloning of an AU-rich element RNA-binding protein, AUF1. Mol. Cell. Biol., **13**, 7652–7665.

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