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*J Immunol* 1999; 163:5228-5234; ;
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Primary Peripheral Blood Eosinophils Rapidly Degrade Transfected Granulocyte-Macrophage Colony-Stimulating Factor mRNA

Stéphane Esnault and James S. Malter

Despite increasing interest, very little information exists regarding gene regulatory mechanisms employed by eosinophils. This largely stems from the difficulty in transfecting these primary cells. In this study, we demonstrate that peripheral blood eosinophils (PBEos) can be successfully transfected with both GM-CSF cDNA and mRNA and reporter constructs by particle-mediated gene transfer. The transfection efficiency was 1.2% based on green fluorescent protein-positive cells. Promoter studies revealed CMV-driven expression vectors were initially active but rapidly quenched, while viral long terminal repeats had greater activity, indicating that certain viral constructs may be relatively poor to direct the production of transgenic proteins in PBEos. Exogenous GM-CSF mRNA was readily delivered and detected by Northern blot, permitting determination of its $t_{1/2}$ in the absence of transcriptional poisons. These data show PBEos rapidly degraded GM-CSF mRNA with a $t_{1/2}$ of 8 min. Mutant GM-CSF mRNAs, lacking the AUUUA motifs, were more stable, but were still rapidly degraded, suggesting the existence of accessory, destabilizing elements. We were able to measure minute amounts of intracellular GM-CSF after the transfection of mutant GM-CSF mRNA, but extracellular cytokine was below the sensitivity of our ELISA. However, the presence of secreted GM-CSF was established by in vitro, survival bioassay. In conclusion, the existence of this new technology should allow detailed studies of eosinophil-specific transcriptional and posttranscriptional regulation. The Journal of Immunology, 1999, 163: 5228–5234.

Eosinophils are critical effector cells in allergic diseases including asthma. Upon allergen challenge, they are recruited from the peripheral blood, and move through the endothelium and pulmonary interstitium into the airways (1–3). Pulmonary-derived eosinophils are resistant to apoptosis (4), exhibit enhanced numbers of cell surface cytokine receptors (5), and exhibit enhanced functional and secretory capacity (1, 6). The migration and differentiation of peripheral blood eosinophils (PBEos) are driven by cytokine and chemokine gradients (7, 8). In particular, GM-CSF participates in the recruitment, survival, and activation of eosinophils both in vitro and in vivo (9, 10). GM-CSF is expressed by activated immune cells, fibroblasts, and eosinophils themselves, suggesting it functions as both an autocrine and paracrine factor (11–13).

Despite its importance, the underlying molecular mechanisms that control GM-CSF production by PBEos remain essentially unknown. In resting T lymphocytes, cytokine mRNAs such as GM-CSF are rapidly degraded, preventing mRNA accumulation and translation. Within a few hours of activation with phorbol ester or mitogenic anti-CD3 and anti-CD28 Abs, GM-CSF mRNA was rapidly degraded GM-CSF mRNA with a $t_{1/2}$ of 8 min. Mutant GM-CSF mRNAs, lacking the AUUUA motifs, were more stable, but were still rapidly degraded, suggesting the existence of accessory, destabilizing elements. We were able to measure minute amounts of intracellular GM-CSF after the transfection of mutant GM-CSF mRNA, but extracellular cytokine was below the sensitivity of our ELISA. However, the presence of secreted GM-CSF was established by in vitro, survival bioassay. In conclusion, the existence of this new technology should allow detailed studies of eosinophil-specific transcriptional and posttranscriptional regulation.

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Received for publication May 18, 1999. Accepted for publication August 27, 1999.

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This work was supported by the National Institutes of Health (Project 5 of Specialized Center of Research (SCOR) Asthma P50HL56396 to J.S.M.).

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Abbreviations used in this paper: PBEos, peripheral blood eosinophils; ARE, adenosine-uridine-rich element; EGFP, enhanced green fluorescent protein; LTR, long terminal repeat; PMGT, particle-mediated gene transfer; UTR, untranslated region.

1 J. Ruth, S. Esnault, J. Jarzembskowsi, and J. Malter, Submitted for publication.
of GM-CSF mRNA $t_{1/2}$ in resting and activated cells. Exogenous mRNA was translated, and despite very low levels of extracellular GM-CSF, transfected PBEos showed significantly prolonged in vitro survival.

Materials and Methods

Reagents and cell cultures

The AML14.3D10 cell line was generously provided by Cassandra Paul (Wright State University, Dayton, OH). The cells were maintained in RPMI 1640 medium, 8% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, 5 $\times$ 10^{-5} M 2-ME, and 50 $\mu$g/ml gentamicin, all from Life Technologies (Grand Island, NY). The K562 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA) and was maintained in the same medium as AML14.3D10 cell line.

Subjects and eosinophil separation

Peripheral blood was obtained by venipuncture from patients with allergic rhinitis or asthma. Informed consent was acquired according to a protocol approved by the University of Wisconsin Human Subjects Committee.

Eosinophils were purified using a negative immunomagnetic procedure, as previously described (29). Briefly, heparinized whole blood was centrifuged (700 $g$, 20 min) over a Percoll density gradient (density 1.090 g/ml; Pharmacia Biotech, Piscataway, NJ) to separate mononuclear cells from granulocytes. After removal of the mononuclear cell band, RBC were lysed by twice incubating (for 30 s) with sterile, deionized water. The remaining white blood cells were incubated with anti-CD16-coated microbeads (100 ml per 10^6 cells) for 40 min, and were then passed through steel mesh columns that had been previously washed with 2% newborn calf serum. The cells in the eluent were stained (Diff Quik; Baxter, Miami, FL), and 400 cells were examined microscopically. The cells were used only if >99% of the cells were eosinophils. The few contaminating cells were either neutrophils or mononuclear cells. After isolation, PBEos were maintained in RPMI 1640 medium, 10% FCS, and 50 $\mu$g/ml gentamicin, at 37°C in a 5% CO₂ environment.

Plasmid constructions

cDNA coding for human GM-CSF was obtained from the ATCC. Using overlap extension PCR, the adenosine-uridine-rich elements (AREs) of wild-type GM-CSF (GM-AUUUA) were selectively replaced with four tandem AUGUA sequences (GM-AUGUA) (27). The full-length mutant GM-AUGUA cDNA was ligated into an expression vector with a CMV promoter and 3' SV40 polyadenylation signal, as previously described (27). Plasmids for in vitro, wild-type, or mutant GM-CSF mRNA synthesis have been described previously (28), and contained complete 5'-UTRs, coding, and 3'-UTRs, except as noted above for GM-AUGUA. In addition, all in vitro transcripts were capped and terminated with a 90-base polyadenylate tail. After production, mRNA was phenol/chloroform extracted and precipitated at -20°C. The quality and the quantity of synthesized mRNAs were verified by agarose gel electrophoresis and by absorbance at 260 nm.

An LTR-driven plasmid coding for enhanced green fluorescent protein (EGFP) was generously provided by N. S. Kumar and M. Zaboikin (University of Wisconsin, Madison, WI).

cDNA and mRNA transfection

PMGT of expression vectors or in vitro transcribed mRNAs into cultured cells was performed using the Accell Gene-Gun (PowderJect, Madison, WI), as previously described (26, 28, 30). Briefly, mRNAs in aqueous solution were precipitated at -20°C for 1 h with 1 vol of 2-propanol and 0.10 vol of 5 M ammonium acetate onto 1-μm gold beads (1 μg/mg of gold beads) at a concentration of 5 μg of mRNA/ng of gold beads. A total of 80–95% of the input mRNA was typically loaded onto the beads. Successive transfections of 2 × 10^6 cells were pooled and washed twice in culture medium to remove any extracellular mRNAs. The transfected PBEos were placed in culture at 1 × 10^6 cells/ml. cDNAs were loaded onto beads at 1 μg/ml of gold beads with 0.2 M CaCl₂ and 10% PEG4000. Transfected K562 and AML14.3D10 cell lines were placed in culture at 1 × 10^6 cells/ml.

Northern blotting

At indicated times, cells were pelleted and lysed in TRIsreagent (Molecular Research Center, Cincinnati, OH), and total RNA was quantitatively isolated and analyzed by Northern blotting with a radiolabeled, antisense GM-CSF riboprobe, as described previously (19). GM-CSF mRNA signals were normalized to those for GAPDH or actin mRNA to accommodate any differences in the extraction, gel loading, and transfer of total RNA. After stringent washing at 70°C for 5 min with 0.1× SSC, 0.1% SDS, the blots were quantitated by PhosphorImaging (Molecular Dynamics, model 445SI).

Measurement of GM-CSF proteins in conditioned medium and cell lysates

Cells were lysed by incubation for 20 min in 25 mM Tris-HCl (pH 8), 0.1 mM EDTA, 20 mg/ml Pefabloc (Boehringer Mannheim, Indianapolis, IN), and 0.5% Nonidet P-40. Cytoplasmic or media samples were centrifuged at 12,000 $\times$ g for 2 min, and supernatants were stored at -80°C until assayed with commercial ELISA kits (Cytoscreen; Biosource, Camarillo, CA) (sensitivity 1 pg/ml), according to the manufacturer's recommended procedure.

Results

Successful transfection of PBEos by PMGT

To identify gene regulatory mechanisms employed by PBEos for the controlled production of GM-CSF, we sought an easy and reliable transfection method for the introduction of cDNA or mRNA. PMGT has been successfully employed for the introduction of genes into normal cells and tissues that are resistant to other transfection methods (26). Thus, we evaluated transfection efficiency and cell death after PMGT with different sizes of gold beads (0.6–5 $\mu$m in diameter) and particle velocity. The latter was varied by adjusting the propulsive gas pressure. Optimal conditions were obtained with 1-μm-diameter beads and 450 PSI, which resulted in <10% cell death (not shown). These were identical to previously optimized conditions for the transfection of primary lymphocytes (28). To assess transfection efficiency, we delivered an LTR-driven, EGFP expression plasmid into PBEos. Five hours after transfection, cells were pelleted and fixed onto cover slips, and positive cells were counted by fluorescence microscopy. In repeat experiments, 10–15 cells per 1000 were unambiguously positive (see Fig. 1) for an average transfection efficiency of 1.2%. Despite the very high purity of the initial PBEos population (>99% based on morphological examination after preparation), it remained possible that a contaminating cell population was transfected. However, as shown in Fig. 1, many of the fluorescent cells clearly had bilobed nuclei, heavy granularity, and cell diameters characteristic of PBEos.

We next investigated whether expression constructs were active in PBEos. Positive results could lead to the successful expression...
of transgenic proteins by PBEos, which heretofore has been impossible. To put these studies in context, we compared transgene mRNA and protein expression by PBEos with the eosinophil-like AML14.3D10 cell line and widely used human erythroleukemia line, K562. Identical amounts of a CMV-driven, GM-CSF expression vector (pCMV-GM) (28), which also contains a 3’ SV40 polyadenylation signal, were loaded onto gold beads (1 μg DNA/1 mg gold beads). In a previous study, PBMC showed peak steady state levels of GM-CSF mRNA at 12 h posttransfection (19). Herein, transfected K562 cells expressed high levels of GM-CSF mRNA for >48 h, which peaked at ~4 h (Fig. 2A, K562). Transfected AM14.3D10 accumulated substantially less GM-CSF mRNA than K562 cells. Expression peaked at 1 h after transfection and rapidly decreased over the next 4 h (Fig. 2A, AML14.3D10). In both cell lines, GM-CSF mRNA levels and transfected plasmid content (as determined by PCR; data not shown) decreased proportionally. In PBEos, GM-CSF mRNA accumulation was much more abbreviated than even in AML14.3D10 cells, with maximal levels immediately after transfection, which decreased by 50% after 40 min (Fig. 2A, PBEos). Despite rapidly decreasing mRNA levels, pCMV-GM could be easily detected by PCR for at least 24 h (data not shown). Thus, compared with the two cell lines, CMV-driven transcription was rapidly suppressed in PBEos despite the continued presence of plasmid. GM-CSF secretion (Fig. 2B) generally mirrored intracellular mRNA levels, with the highest levels secreted by K562 cells, much less by AML14.3D10 cells, and undetectable amounts by PBEos.

**GM-CSF mRNA is very unstable in transfected PBEos**

Due to prior data from T lymphocytes, we anticipated GM-CSF gene regulation in PBEos would involve a substantial component of posttranscriptional control, especially at the level of mRNA stability (15). Like most cytokines, GM-CSF mRNA accumulation is antagonized in resting cells by rapid cytoplasmic decay mediated by the 3′-UTR, AREs (16, 28). Thus, PBEos were transfected either with wild-type GM-CSF mRNA or a mutant version containing AUGUA repeats instead of AREs. All mRNAs were capped at the 5′ end and terminated with a 90-base polyadenylate tail (28). The decay rate was followed by Northern blotting at various times after transfection. GM-CSF protein was also measured to verify exogenous GM-CSF mRNAs were translated. As shown (Fig. 3, A and B), wild-type GM-CSF mRNA was readily detectable by Northern blotting immediately after transfection, but was rapidly degraded with a $t_{1/2}$ of 8 min. Mutant GM-CSF mRNA was almost 3-fold more stable than wild-type message ($t_{1/2} = 22$ min), demonstrating the potent destabilizing effect of the AUUUA motifs (Fig. 3, A and B). However, the rapidity of decay of the mutant mRNA suggests the existence of cryptic, destabilizing domains external to the AREs. Importantly, transfection with naked gold beads failed to induce detectable, endogenous GM-CSF mRNA (Fig. 3A, BK lanes). In addition, in vitro transcribed GM-CSF mRNAs are slightly smaller than their endogenous counterparts due to their somewhat shorter polyadenylate tail. Thus, on side by side Northern blots, a clear difference between them can be appreciated (not shown). Thus, the hybridization signals in Fig. 3 reflect only transfected, intracellular mRNA rather than endogenous message. Resting PBMC (28) and the eosinophil-like

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**FIGURE 2.** Steady state accumulation of mutant GM-CSF mRNAs in resting PBEos, AML14.3D10, or K562 cell lines. A. PBEos, AML14.3D10, or K562 cell lines were transfected with pCMV-GM or blank gold beads (denoted BK). At the indicated times after transfection, equal numbers of cells were harvested, and RNA was isolated for Northern blotting. Signals were visualized using a PhosphorImager after hybridization with $^{32}$P-labeled, antisense GM-CSF riboprobe or GAPDH cDNA probe. B. Transgenic GM-CSF protein in the culture medium was measured at the indicated time points using a human GM-CSF-specific ELISA. Values shown are representative of three experiments for PBEos and two experiments for AML14.3D10 or K562 cell lines.
exogenous GM-CSF mRNA was stabilized after 5-h ionomycin treatment. As shown in Fig. 4, ionophore stabilizes wild-type but not mutant GM-CSF mRNA. The stability of transfected mRNAs was modulated GM-CSF mRNA stability in PBEos. Cells were treated with IL-3, IFN-γ, and TNF-α, and TNF-α mRNAs (15, 32, 33). Previously, we observed that GM-CSF mRNA was stabilized in ionomycin-stimulated AML14.3D10 cells (22). Thus, we asked whether ionomycin modulated GM-CSF mRNA stability in PBEos. Cells were treated with ionomycin for 5 h before transfection with wild-type or mutant GM-CSF mRNAs. The stability of transfected mRNAs was then followed by Northern blotting. As shown in Fig. 4, A and B, exogenous GM-CSF mRNA was stabilized 3-fold to a t1/2 of 22 min. The t1/2 of the mutant lacking 3'-UTR AREs was unchanged (t1/2 = 18 min). In all cases, endogenous GM-CSF mRNA was undetectable by Northern blot due to the small amount of RNA loaded per lane. When we loaded larger amounts of total RNA and increased the PhosphorImager exposure time, endogenous mes-

**FIGURE 3.** GM-CSF mRNA is stabilized after 5-h ionomycin treatment. A, Experimental design. B, Radioactivity of PC3 (t1/2) of the respective mRNAs. AML14.3D10 cell line (22) degraded transfected wild-type and mutant GM-CSF mRNAs at rates nearly identical with that seen in PBEos. Therefore, it is likely that similar mechanisms are employed by these different cells to rapidly degrade GM-CSF mRNA.

Ionomycin stabilizes wild-type but not mutant GM-CSF mRNA

Activation of PBEos with a variety of stimulators including calcium ionophore (ionomycin) triggers GM-CSF mRNA accumulation and protein secretion (12, 31). In mast cells and T lymphocytes, similar agonists have been shown to stabilize GM-CSF, IL-3, IFN-γ, and TNF-α mRNAs (15, 32, 33). Previously, we observed that GM-CSF mRNA was stabilized in ionomycin-stimulated AML14.3D10 cells (22). Thus, we asked whether ionomycin modulated GM-CSF mRNA stability in PBEos. Cells were treated with ionomycin for 5 h before transfection with wild-type or mutant GM-CSF mRNAs. The stability of transfected mRNAs was then followed by Northern blotting. As shown in Fig. 4, A and B, exogenous GM-CSF mRNA was stabilized 3-fold to a t1/2 of 22 min. The t1/2 of the mutant lacking 3'-UTR AREs was unchanged (t1/2 = 18 min). In all cases, endogenous GM-CSF mRNA was undetectable by Northern blot due to the small amount of RNA loaded per lane. When we loaded larger amounts of total RNA and increased the PhosphorImager exposure time, endogenous message was detectable by Northern blot after 5-h ionomycin treatment in the absence of transfection (not shown). These data demonstrate that ionomycin stabilizes GM-CSF mRNA and that this effect requires the 3'-UTR ARES.

**Transfected mRNAs are translated**

GM-CSF mRNA most likely requires ongoing translation for normal decay (34). We have previously shown that GM-CSF mRNAs transacted into PBMC by PMGT were >90% polysome associated (28). Indeed, 2 h after transfection with mutant GM-CSF mRNA, supernatants contained 160 pg GM-CSF/ml/10^6 PBMC or 30 pg GM-CSF/ml/10^7 AML14.3D10, respectively (22, 28). Due to its rapid decay, PBMC or AML14.3D10 transacted with wild-type GM-CSF mRNA failed to secrete detectable GM-CSF (22, 28). Thus, at 1, 2, or 3 h posttransfection, PBEos supernatants were harvested and analyzed for GM-CSF by ELISA (sensitivity >1 pg/ml). Somewhat unexpectedly, we were unable to measure GM-CSF from PBEos after transfection with either wild-type or mutant GM-CSF mRNA (Table I). However, resting PBEos have been reported to accumulate cytokines, including IL-4 and IL-10, in cytoplasmic granules (35). Therefore, 1 h after transfection, PBEos were lysed, and total, cytoplasmic GM-CSF levels were measured by ELISA. Untreated PBEos or those transfected with blank gold beads had undetectable GM-CSF, while those transfected with mutant GM-CSF mRNA contained 7.94 pg GM-CSF/10^6 μg protein (Table I). While these levels of GM-CSF are low, they demonstrate that transfected mRNAs are translationally competent and at least some exogenous mRNA is polysome associated.

It remained possible, however, that the GM-CSF ELISA data were falsely positive due to the high concentration of irrelevant protein in the lysate. Thus, we sought an independent demonstration of GM-CSF production and secretion after mRNA transfection. It is well established that GM-CSF acts as a potent inhibitor of PBEos apoptosis (36). Thus, we examined whether transfected PBEos displayed prolonged in vitro survival. On posttransfection day 4, PBEos were stained with trypan blue and viability was determined. As shown in Fig. 5, only 16% of PBEos transfected with naked gold beads were viable after 4 days in culture. The addition of anti-GM-CSF-neutralizing Ab had a minimal and statistically insignificant additional effect. However, PBEos transfected with either wild-type or mutant GM-CSF mRNAs showed significantly increased survival, which was greatest after the transfection of mutant GM-CSF mRNA. In both cases, survival was completely attenuated by anti-GM-CSF Ab (Fig. 5), but unaffected by irrelevant or anti-IL-5 Abs (not shown). Thus, despite our inability to measure extracellular GM-CSF, adequate concentrations were present to promote survival.

**Discussion**

In this study, we have shown for the first time that PBEos can be successfully transfected with both cDNA and mRNA. Based on unequivocal EGFP fluorescence in cells with characteristic eosinophyl morphology, the transfection efficiency was 1.2%. This does not differ substantially from prior data in primary lymphocytes (26), but is less than we have observed with tumor cell lines (unpublished observations). As transfection requires cell membrane penetration by a 1-μm gold particle, efficiency is roughly proportional to cell diameter. Thus, lymphocytes and eosinophils are “hit” less frequently in PMGT than larger K562 cells or adherent fibroblasts. In an effort to increase transfection efficiency, we tried larger gold beads (up to 5 μm). However, efficiency decreased and cell death increased. One of the advantages of PMGT is that all transfected cells receive all nucleic acid loaded on that bead. Thus, cells can be transfected with beads loaded with several cDNAs/
mRNAs. If one of the transgenes codes for a marker such as EGFP or cell surface protein, positive cells could be enriched by cell sorting or magnetic bead purification.

CMV-driven expression vectors were relatively inactive in PBEos. Transgenic promoter activity can only be compared in different cell types in relative terms because of differences in the efficiency of vector delivery. Based on signal quantification of pCMV-GM-CSF detection by PCR, PBEos and the eosinophil-like AM14.3D10 cell line exhibited similar transfection efficiencies. Therefore, observed differences in GM-CSF mRNA expression after pCMV-GM-CSF transfection most likely reflected differential promoter activity as opposed to transfection efficiency or GM-CSF mRNA decay. We do not know the underlying mechanism for promoter quenching in PBEos, but the data suggest that CMV-driven constructs will be poor choices for transgene expression. Although we have incompletely characterized the longevity of LTR activity, the high levels of EGFP expression suggest that this may be the superior promoter.

Table I. GM-CSF production by PBEos transfected with GM-CSF mRNA

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<th>GM-CSF (pg/10 µg of protein/10⁶ cells)</th>
<th>Supernatant</th>
<th>Cell lysate</th>
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<tr>
<td>Wild type</td>
<td>&lt;min</td>
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<td>Mutant</td>
<td>7.94 ± 2.05</td>
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a PBEos were transfected with wild-type or mutant GM-CSF mRNA. After 1 h, the supernatants were collected and the cells were lysed. GM-CSF levels were analyzed by ELISA. The data are presented as mean ± SEM from three experiments.

FIGURE 4. Ionomycin stabilizes transgenic GM-CSF mRNA. Resting or ionomycin-activated eosinophils were transfected with either wild-type (A) or mutant (B) GM-CSF mRNA, or with naked beads alone (“B” lanes). At the indicated time points, equal numbers of cells were harvested and total RNA was quantitatively isolated and Northern blotted with 32P-labeled riboprobe for GM-CSF or cDNA probe for β-actin. Signals were visualized using a PhosphorImager. Data presented are representative of three experiments. Radioactive signals were quantified using a PhosphorImager. Transfected wild-type (C) or mutant (D) GM-CSF mRNA signals were normalized to β-actin mRNA and plotted vs time. Each point is the mean ± SD of three experiments. The vertical dashed lines denote the t1/2 of the respective mRNAs.

FIGURE 5. Eosinophil survival is prolonged after GM-CSF mRNA transfection. PBEos were transfected, as described in Materials and Methods, with either naked beads (blank) or beads loaded with wild-type (AUUUA) or mutant GM-CSF (AUGUA) mRNAs. The viability was determined after 4 days by trypan blue exclusion. Neutralizing anti-GM-CSF mAb (α-GM) was added immediately after transfection. Each value represents the mean and SD of three cultures from the same donor representative of three different donors.
GM-CSF mRNA regulation in PBEos has not been investigated. In this study, we show that transfected GM-CSF mRNA decayed exceptionally rapidly, with a t_{1/2} of 8 min. Similar data have been observed in primary resting lymphocytes (28). Rapid GM-CSF mRNA decay required intact 3′-UTR AREs, as a mutant without these elements was 3-fold more stable. The AREs were also required for ionophore-mediated stabilization. These results are similar to those seen in activated lymphocytes (33) as well as fibroblasts (37). However, an accessory-stabilizing element in the proximal 3′-UTR upstream of the AREs has been proposed (33). This element was involved in mRNA stabilization by ionophore-mediated cell signaling, an effect not observed in this study. The rapid decay of mutant GM-CSF mRNA was somewhat unexpected. Irrespective of the precise identity of the additional destabilizer, the transfection of mutant GM-CSF mRNAs with additional deletions will permit its identification.

Several groups have recently characterized ARE-specific mRNA-binding proteins (17–20). The overexpression of HuR was associated with the stabilization of ARE-containing mRNAs (21). We have recently identified a series of ARE-specific binding proteins in AML14.3D10 cells. These activities were significantly up-regulated upon ionophore treatment, but none had molecular mass consistent with HuR. Thus, one mechanism for ionophore-mediated GM-CSF mRNA stabilization may be ARE masking by specific proteins. We are currently evaluating cytoplasmic lysates from PBEos for such activities.

Consistent with the very low levels of coding mRNA, transfected PBEos secreted undetectable levels of GM-CSF. AML14.3D10 cells secreted ~80 pg GM-CSF/ml/10^6 cells, at least 40-fold greater than produced by primary PBEos. As IL-4 and IL-10 are stored in cytoplasmic granules (35), it remained possible that transgenic GM-CSF was similarly sequestered. Indeed, ELISA of cytoplasmic protein after mRNA transfection revealed small, but detectable GM-CSF. However, even after mRNA transfection, PBEos translated considerably less GM-CSF protein than AML14.3D10 cells. As the decay rates were nearly identical, these data suggest that GM-CSF mRNA may also be under translational control in PBEos.

Despite levels below the sensitivity of the ELISA, secreted transgenic GM-CSF was produced and capable of blocking the apoptosis of PBEos in vitro. Survival required extracellular GM-CSF, as it was prevented by anti-GM-CSF-neutralizing Ab. We cannot exclude the remote possibility that GM-CSF secretion was necessary but not sufficient for PBEos survival. This would contradict a growing literature showing recombiant GM-CSF or IL-5 blocks eosinophil apoptosis (36, 38, 39). We have confirmed these observations, although ~100–500 pg/ml of recombinant cytokine was necessary to achieve comparable survival, as shown in this study. Perhaps even more striking is that while only 1–2% of the cells were successfully transfected, 70% of the entire culture survived. Thus, small amounts of GM-CSF released in the immediate vicinity of nontransfected cells were sufficient to prevent apoptosis. It is possible that transgenic GM-CSF has greater biologic potency or t_{1/2} than recombinant protein produced by bacteria. We also suspect that continuous release may contribute to the effectiveness of transgenic GM-CSF, as the t_{1/2} of exogenous cytokine in culture is on the order of 2 h (not shown).

The successful transfection of normal PBEos using PMGT will enable a wide variety of possible studies, including the characterization of eosinophil-specific promoters as well as identification of mRNA instability determinants. In preliminary studies, we have introduced GM-CSF promoter constructs with luciferase reporters into PBEos (R. Horwitz and J. S. Malter, unpublished data). Despite the low transfection efficiency, adequate signals above background were observed. Thus, analysis of changes in gene expression and their underlying mechanisms as the eosinophil migrates from the periphery to the lung during asthma can now be evaluated.

Acknowledgments

We greatly appreciate the creative and helpful comments from the laboratory and the remainder of the SCOR-Asthma participants. We also thank the SCOR-Asthma Cell Core for providing eosinophils.

References


