Expression of the calcium-binding proteins MRP8 and MRP14 in monocytes is regulated by a calcium-induced suppressor mechanism

Johannes ROTH,* Matthias GOEBELER, Verena WROCKLAGE, Christian van den BOS and Clemens SORG
Institute of Experimental Dermatology, University of Münster, von-Esmarch-Strasse 56, D-48149 Münster, Germany

MRP8 and MRP14 are two calcium-binding proteins of the S-100 family the expression of which is restricted to distinct stages of monocytic differentiation. Heteromeric MRP8/MRP14 complexes have been shown to represent their biologically active forms. However, it is not as yet clear whether biochemical modification of complexes, or regulation on the transcriptional level, are responsible for the control of MRP8/MRP14 expression. Employing Western-blot analysis and metabolic labelling we have demonstrated that patterns and metabolism of MRP8/MRP14 complexes do not change during up- or down-regulation of MRP8/MRP14. By Northern-blot analysis it was shown that MRP8/MRP14 are regulated at the transcriptional level rather than by biochemical modification of the complexes. Elevation of intracellular calcium levels by A23187, as well as by thapsigargin, was found to lead to specific down-regulation of MRP8/MRP14 mRNA which is in contrast with data reported for inflammatory factors such as interleukin-1 or tumour necrosis factor α. Concomitant application of actinomycin D and calcium ionophore indicated that this suppressive effect is mediated by decreased synthesis rather than increased degradation of MRP8/MRP14 mRNA. Finally, we demonstrated that calcium-mediated down-regulation of MRP8–MRP14 can be antagonized by cycloheximide, suggesting that a calcium-induced repressor protein is responsible for suppression of MRP8–MRP14 at the transcriptional level. Our data indicate that the function of MRP8–MRP14 is restricted to events associated with early stages of myelomonocytic activation.

INTRODUCTION

MRP8 and MRP14 are two calcium-binding proteins which are expressed by granulocytes and monocytes ([1–3] for reviews). Both molecules belong to the S-100 family which comprises low-molecular-mass proteins such as for example S-100α/β, calcyclin, intestinal calcium-binding protein and p11, all of which are characterized by two calcium-binding EF-hands [1,4]. Members of this family show a tissue-specific distribution and are supposed to be involved in calcium-dependent intracellular processes such as activation of calcium-dependent enzymes, cytoskeletal–membrane interactions, regulation of cellular differentiation and cell-cycle progression [4–8].

MRP8 and MRP14, which have also been referred to as L1 heavy and light chains [9,10], cystic fibrosis antigen [11,12], p8 and p14 [13,14], or calgranulin A and B [15] show molecular masses of 10 and 14 kDa respectively [1]. They assemble into non-covalently associated complexes of 25, 35 and 48 kDa in a calcium-dependent manner [16]. The complexed forms of MRP8 and MRP14 have been supposed to represent their biologically active state [16–20]. Elevation of intracellular calcium levels leads to the translocation of these proteins from the cytoplasm to the plasma membrane, as well as to intermediate filaments which have been shown to correlate with phagocyte activation [21–23].

MRP8 and MRP14 are expressed by granulocytes and monocytes, but are absent in lymphocytes [1,2,24,25]. In many inflammatory disorders infiltrating monocytes are positive for these proteins [1,2,14,24,26,27]. MRP8 and MRP14 represent a major portion of the total cellular protein content in early differentiation stages of monocytes, whereas mature macrophages do not contain these calcium-binding proteins at all [1,2,25,28]. Thus, expression of MRP8 and MRP14 by monocytes reflects distinct stages of cellular differentiation which appear to differ in their modes of calcium-dependent signalling. However, mechanisms being responsible for down-regulation of MRP8 and MRP14 are currently not understood.

We therefore initiated the present study to analyse events regulating the expression of these proteins. We now report that elevation of the intracellular calcium concentration leads to the suppression of MRP8 and MRP14 mRNA levels, suggesting that the function of these proteins is restricted to processes occurring before or during activation of monocytes.

EXPERIMENTAL

Cells and cell culture

Human peripheral blood monocytes were isolated from buffy coats by Ficoll–Paque and Percoll (Pharmacia, Freiburg, Germany) density-gradient centrifugation. Purity of monocytes was $> 90\%$, as demonstrated by flow cytometry employing monoclonal antibodies raised against CD14, CD15 and CD16 (Dianova, Hamburg, Germany). Monocytes were cultured in Teflon bags using McCoy's 5A medium containing 2 mM $CaCl_2$ and 20% (v/v) human serum for 0–12 days as previously reported [24]. In some experiments cells were incubated with 0.01–10 μM calcium ionophore A23187 (Sigma, Deisenhofen, Germany), 300 nM thapsigargin (Sigma), 5 mM $CaCl_2$ or 2 mM EGTA. To suppress *de novo* synthesis of mRNA or protein, actinomycin D (1 μg/ml; Sigma) or cycloheximide (10 μg/ml; Sigma) were added to the medium. Cell viability was assayed by Trypan Blue exclusion staining and determination of lactate dehydrogenase activity in the medium at the end of exposure

Abbreviations used: BS^3, bis-(sulphosuccinimidyl) suberate; IL-1, interleukin-1; M-CSF, macrophage colony-stimulating factor; rh, recombinant human; TNF-α, tumour necrosis factor α; NP-40, Nonidet P-40; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

* To whom correspondence should be addressed.

periods as described [29]. Viability was found to be over 90% in all experiments; lactate dehydrogenase activity in the medium did not differ significantly between control and treated cells.

Cross-linking of MRP8–MRP14

Cross-linking of MRP8/MRP14 complexes was carried out employing bis-(sulphosuccinimidyl) suberate (BS^3) (Pierce, Cologne, Germany) according to Staros [30]. Briefly, monocytes were harvested after 0–12 days of culture and lysed in PBS and 1% (v/v) Nonidet P-40 (NP-40). Lysates were diluted to a protein concentration of 1 mg/ml and a 10 mM stock solution of BS^3 was added to a final concentration of 1.5 mM. Samples were incubated for 45 min at 20 °C and the cross-linking reaction was subsequently stopped by addition of 0.17 (v/v) of a stock solution of 50 mM ethanolamine, 20 mM *N*-ethylmaleimide in 50 mM sodium phosphate, pH 7.4, and stored at −80 °C before Western-blot analysis.

Immunostaining of MRP8–MRP14 on Western blots

Samples cross-linked by BS^3 were separated on SDS/PAGE (15% polyacrylamide) under reducing conditions [31] and subsequently semi-dry-blotted [32] on to polyvinylidene difluoride membranes (Immobilon P, Millipore, Eschborn, Germany). Non-specific binding of antibodies was prevented by immersion of membranes in 1% (w/v) milk powder in PBS for 16 h at 4 °C. Primary antibodies diluted in 100 mM Tris/HCl (pH 7) containing 150 mM NaCl and 1% (w/v) BSA were allowed to react with their antigens for 1 h at 20 °C. After several washes, bound antibodies were detected by goat anti-(rabbit IgG) alkaline phosphatase-conjugated second-stage antibodies (Dianova). Blots were developed using 0.15 mg/ml 5-bromo-4-chloro-3-indolyl phosphate (Sigma) and 0.3 mg/ml Nitroblue Tetrazolium chloride (Sigma) in 0.1 M Tris/HCl (pH 9.5) containing 0.1 M NaCl, 50 mM $MgCl_2$ and 0.05% Tween 20.

Metabolic labelling and immunoprecipitation

Monocytes of culture day 1 were harvested, washed three times, and preincubated in modified Eagle's medium without methionine (Gibco, Egginstein, Germany) at a density of 1×10^8 cells/ml. Monocytes were then pulse-labelled by adding 100 μCi/ml [^{35}S]methionine (Amersham, Braunschweig, Germany) to the same medium for 4 h. Medium was removed and after washing cells three times was replaced by McCoy's 5A medium supplemented with 20% (v/v) human serum. Some cells (3×10^7) were harvested directly after the pulse phase, 6×10^7 cells were cultured for another 24 h and 1×10^8 for 48 h. After these chase phases cells were washed three times and lysed in PBS with 1% (v/v) NP-40 and 2 mM phenylmethanesulphonyl fluoride (Sigma). MRP8–MRP14 complexes were stabilized by adding 1.5 mM BS^3 and processed as described above. Lysates were preabsorbed by incubation for 1 h with 100 μg/ml rabbit IgG (Calbiochem, Bad Soden, Germany), followed by incubation for 1 h after addition of 100 μl/ml Protein G–Sepharose Fast Flow (Pharmacia). After centrifugation for 10 min at 13000 *g*, 1 μg/ml non-specific mouse IgG_1 or 1 μg/ml monoclonal antibody 27E10, which is directed against the MRP8–MRP14 heterodimer [21], were added to the supernatants and incubated for 2 h. Protein G–Sepharose Fast Flow (30 μl/ml) was then added and samples incubated for 1 h. Sepharose was collected by centrifugation and the bound antigen was eluted with SDS/PAGE sample buffer after washing three times in PBS containing 1% (v/v) NP-40 and undergoing an additional washing step with 50 mM ammonium bicarbonate, pH 7. Samples were separated by SDS/PAGE, gels were subsequently treated with Amplify (Amersham), dried and exposed to X-ray films. The relative amounts of MRP8 and MRP14 monomers and their heterodimer were determined densitometrically by scanning of autoradiography bands with a Fast Scan supplied with Image Quant software (Molecular Dynamics, Sunny Vale, CA, U.S.A.).

Northern-blot analysis

Total RNA was prepared according to the guanidine hydrochloride method [33]. Human monocytes, freshly isolated or cultured for 1–12 days, were lysed in 4 M guanidine isothiocyanate and RNA isolated by centrifugation through 5.7 M CsCl at 100000 *g* for 21 h at 20 °C. Denatured RNA (20 μg), quantified by u.v. absorption at 260 nm, was separated on a 1% (w/v) agarose/formaldehyde gel and subsequently vacuum-blotted on Biodyne B membranes (Pall, Dreieich, Germany). Filters were u.v.-cross-linked and hybridized with cDNA probes specific for MRP8 (279 nucleotides), MRP14 (342 nucleotides) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which were labelled with ^{32}P by a random-primer method (Multiprime DNA labelling system; Amersham). Membranes were reprobed with the cDNAs described above after stripping the blots in between using 0.1% SDS at 95 °C. Autoradiography was performed using Kodak X-Omat AR films. Specific mRNA was quantified densitometrically by scanning of autoradiography bands. Data obtained from MRP8 or MRP14 mRNA bands were normalized to the corresponding GAPDH mRNA bands.

RESULTS

Expression and complex formation of MRP8–MRP14 during maturation of human monocytes

First we investigated whether modulation of MRP8–MRP14 during monocytic maturation occurs at the transcriptional or post-transcriptional level. Human peripheral blood monocytes cultured in Teflon bags were harvested at days 0, 1, 3, 5, 8 and 12 and processed by Northern- and Western-blot analysis. Monocytes showed parallel amounts of MRP8 and MRP14 mRNA, with maximal levels at day 1 and declining values thereafter. Between days 8 and 12, mRNA for MRP8 and MRP14 could not be detected any more (Figure 1). A similar time course of MRP8/MRP14 expression was observed at the protein level. After covalent cross-linking with BS^3 and Western blotting MRP8/MRP14 complexes of 25, 35 and 48 kDa were seen the pattern of which did not change qualitatively during culture (Figure 2). These protein bands were the same as those described in detail by Teigelkamp et al. [16]. Labelling of monocytes with [^{35}S]methionine, subsequent immunoprecipitation with monoclonal antibody 27E10 and densitometric analysis of autoradiographic bands demonstrated that MRP8 and MRP14 are translated at similar rates (MRP8, A 0.9 ± 0.2; MRP14, A 1.1 ± 0.2; mean ± S.D., $n = 3$, normalized to methionine content). Since [^{35}S]methionine incorporation persisted for a relatively long period we chose a pulse phase of 4 h, followed by chase periods of 0, 24 and 48 h, to study the metabolic fate of MRP8–MRP14 complexes. At the end of the chase phases monocytes were lysed and protein complexes stabilized by cross-linking with BS^3 before immunoprecipitation and SDS/PAGE. Densitometric analysis of autographs revealed that the ratio of

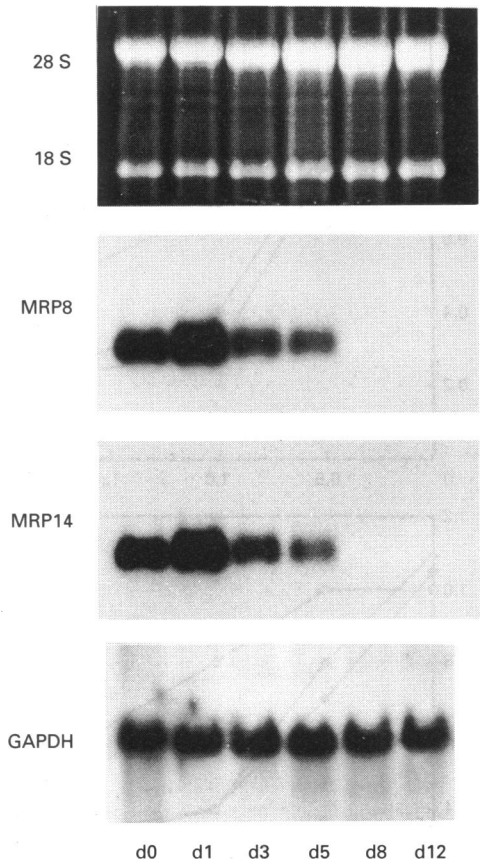

Figure 1 Northern-blot analysis of MRP8 and MRP14 mRNA expression during culture of human peripheral blood monocytes

Cells were cultured for 0 to 12 days and RNA subsequently isolated as described in the Materials and methods section. Total RNA (20 μg) samples were separated on an agarose/formaldehyde gel and filters successively hybridized with MRP8, MRP14 and GAPDH cDNA. Equal loading of the gel was controlled by ethidium bromide staining.

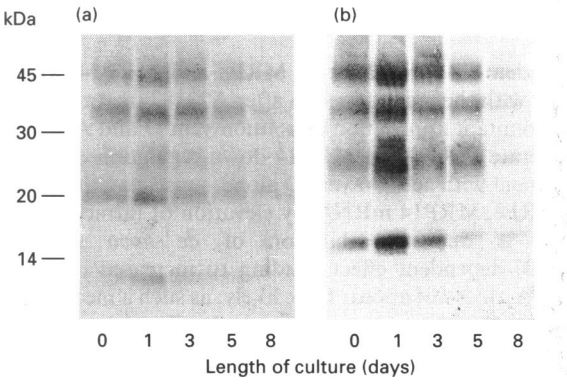

Figure 2 Complex pattern of MRP8 and MRP14 during culture of monocytes

Lysates of monocytes obtained after 0–8 days of culture were cross-linked by BS^3 as described in the Materials and methods section. Each sample (50 μg) was separated on SDS/PAGE (15% acrylamide), blotted and stained with (**a**) anti-MRP8 (left-hand side) and (**b**) anti-MRP14 sera (right-hand side).

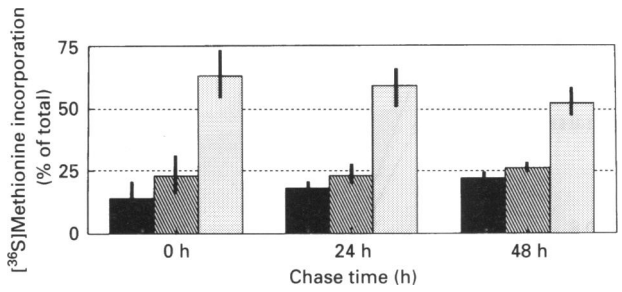

Figure 3 [^{35}S]Methionine incorporation into MRP8 and MRP14 monomers and complexes during pulse–chase labelling

Monocytes of culture day 1 were pulsed with [^{35}S]methionine for 4 h. Cells were harvested immediately after the pulse phase or after a chase period of 24 h and 48 h respectively. After covalent cross-linking with BS^3 MRP8 and MRP14 were immunoprecipitated with monoclonal antibody 27E10 or mouse IgG_1 as control as described in the Materials and methods section. The relative distribution of [^{35}S]methionine incorporation in MRP monomers (■, MRP8; ▧, MRP14) and heterodimers (▨, MRP8–MRP14) was determined by densitometric analysis of autoradiographs. Data (mean ± S.E.M.) from three independent experiments are shown.

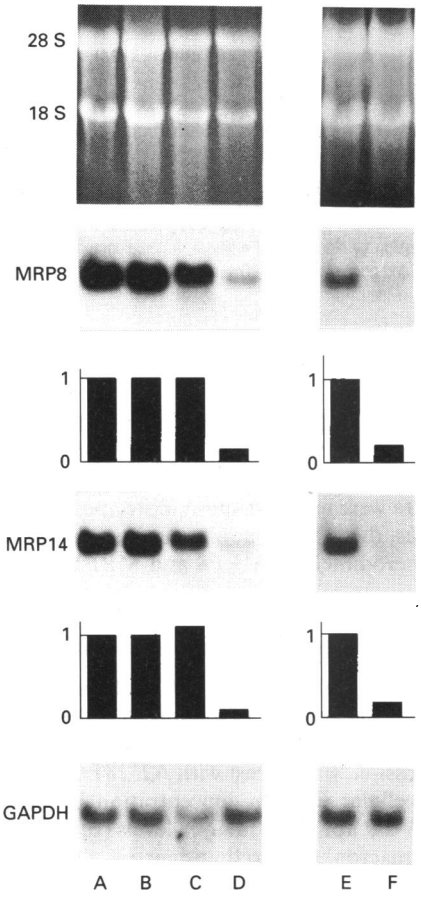

Figure 4 Influence of intracellular and extracellular calcium concentrations on MRP8 and MRP14 mRNA levels

Monocytes cultured for 1 day in McCoy's medium containing 2 mM $CaCl_2$ were treated for 3 h with (A) medium as control; (B) 5 mM $CaCl_2$; (C) 2 mM EGTA; (D) 10 μM A23187; (E) medium as control; (F) 300 nM thapsigargin and processed for Northern-blot analysis as described in the Materials and methods section. In addition, densities of MRP8 and MRP14 bands were determined by laser densitometry, normalized to GAPDH mRNA bands and presented as arbitrary units. Results were essentially similar in three independent experiments.

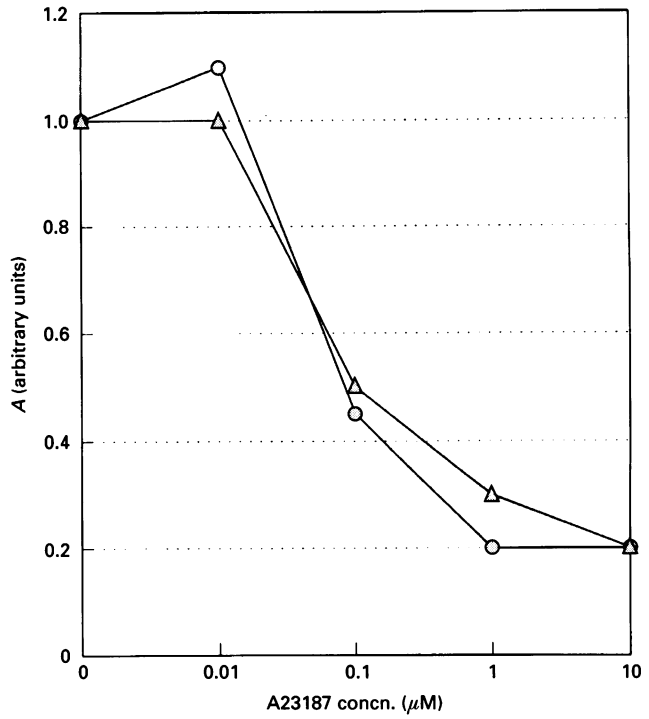

Figure 5 Influence of variable concentrations of A23187 on MRP8 and MRP14 mRNA levels

Monocytes on culture day 1 were incubated for 3 h with 0, 0.01, 0.1, 1.0 and 10.0 μM A23187 in McCoy's medium containing 2 mM $CaCl_2$ and studied for MRP8 (△) and MRP14 (○) mRNA expression by Northern-blot analysis. Autoradiographic bands were quantified by laser densitometry and normalized to GAPDH mRNA bands. Data from one of three independent experiments are provided.

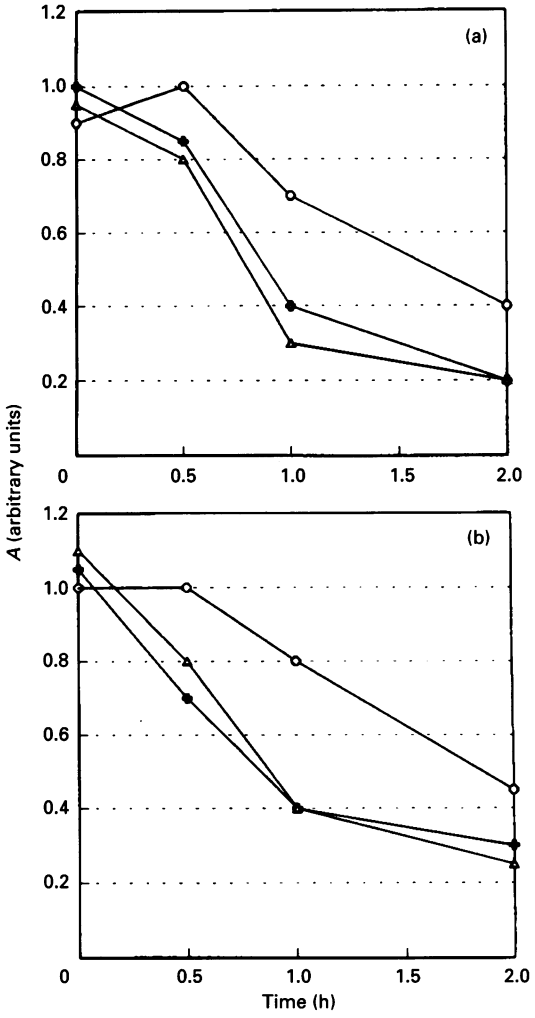

Figure 6 Influence of the mRNA synthesis inhibitor actinomycin D on (a) MRP8 and (b) MRP14 mRNA levels

Monocytes of culture day 1 were incubated with either 1 μg/ml actinomycin D (△) or 10 μM A23187 alone (○) or concomitantly treated with both agents (✣) for 0, 0.5, 1 or 2 h respectively. Autoradiographic bands were quantified by laser densitometry and normalized to the 18S rRNA. Data were essentially similar in three independent experiments.

MRP8 monomers:MRP14 monomers:MRP8–MRP14 heterodimers was independent of the duration of the chase periods (Figure 3). Essentially similar results were obtained when polyclonal antisera were used for immunoprecipitation of MRP8 and MRP14. Thus, the [^{35}S]methionine turnover is similar for monomers and heterodimers of MRP8 and MRP14.

Regulation of MRP8 and MRP14 expression at the mRNA level

Elevation of intracellular calcium levels in macrophages by treatment with the calcium ionophore A23187 was found to be associated with a marked down-regulation of MRP8 and MRP14 mRNA expression. In contrast with A23187 treatment, variation of the extracellular calcium concentration by 5 mM $CaCl_2$ or 2 mM EGTA did not affect mRNA levels (Figure 4, lanes A–D). Exposure of macrophages to thapsigargin, which causes calcium release exclusively from intracellular stores by inhibition of the endoplasmic calcium-ATPase [34], led to down-regulation of MRP8 and MRP14 mRNA as well (Figure 4, lanes E and F).

A23187 revealed a dose–response relationship with maximal effects at $\geqslant 1$ μM and still a pronounced suppression with 100 nM calcium ionophore (Figure 5). To analyse further whether A23187-induced elevation of intracellular calcium acts either via inhibition of MRP8/MRP14 mRNA synthesis or via accelerated degradation of their mRNA, time-dependent effects of actinomycin D and A23187 were compared. Application of 1 μg/ml actinomycin D to cultures of monocytes led to a faster time-dependent disappearance of MRP8 and MRP14 mRNA compared with the reduction seen after A23187 treatment (Figure 6). Concomitant application of actinomycin D and A23187 did not accelerate MRP8 and MRP14 down-regulation compared with treatment with actinomycin D alone, suggesting that suppression of MRP8/MRP14 mRNA by elevation of intracellular calcium levels is due to inhibition of *de novo* synthesis. An A23187-dependent effect, leading to increased degradation of mRNA, does not appear to be likely, as such a mechanism would enhance the effects of actinomycin D. The delayed response of MRP8/MRP14 mRNA to A23187, compared with the action of actinomycin D, may rather be explained by indirect mechanisms, e.g. induction of a specific suppressor protein. We therefore analysed the effect of the protein synthesis inhibitor cycloheximide (10 μg/ml) on A23187-mediated down-regulation of MRP8/MRP14 mRNA. Indeed, compared with transcription of GAPDH, cycloheximide was found to be capable of almost completely antagonizing MRP8/MRP14 mRNA suppression by A23187 (Figure 7), indicating dependency on protein synthesis.

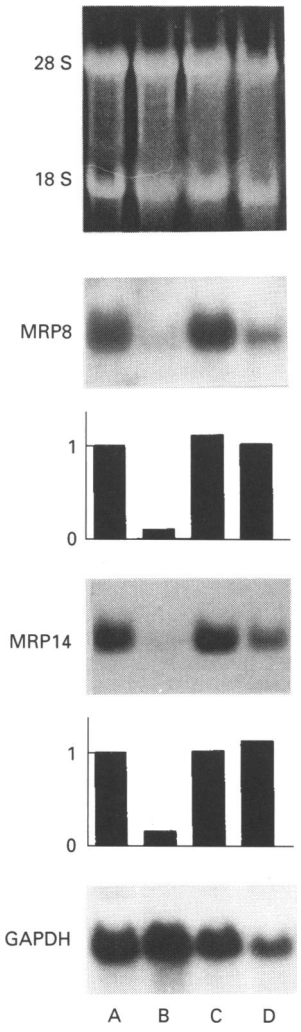

Figure 7 Influence of the protein synthesis inhibitor cycloheximide on MRP8 and MRP14 mRNA levels

Monocytes cultured for 1 day in McCoy's medium containing 2 mM $CaCl_2$ were treated with lane A, medium as control; lane B, 10 μM A23187; lane C, 10 μg/ml cycloheximide; and lane D, both 10 μM A23187 and 10 μg/ml cycloheximide for 3 h. Data are presented as described in Figure 4.

In addition, neutralization of A23187-mediated suppression of MRP8/MRP14 mRNA levels by cycloheximide appears to exclude that potential toxic effects are responsible for down-regulation by A23187.

DISCUSSION

Differentiation of monocytes is associated with changes in morphological, biochemical and functional properties [35]. One striking event is the down-regulation of the two calcium-binding proteins MRP8 and MRP14 during monocytic maturation [1,2,24,28]. The molecular processes leading to this suppression are not yet clear.

In the present study MRP8 and MRP14 mRNA levels were found to decline during culture of human peripheral blood monocytes. Time kinetics thus paralleled those seen earlier after immunocytochemical staining of cytospin preparations [2,24], indicating that MRP8 and MRP14 expression is regulated at the transcriptional level. It has previously been proposed that MRP8/MRP14 complex formation is a prerequisite for their biological function [16–20]; changes in their complex patterns may therefore also be involved in modulating MRP8/MRP14 function during differentiation. Besides quantitative changes at the protein level, which paralleled mRNA expression, no qualitative alterations of the MRP8/MRP14 complex pattern were detected. Furthermore, pulse–chase experiments revealed that the different protein bands of MRP8 and MRP14 monomers and complexes do not represent a sequence of successive metabolical stages; as the pattern of MRP8/MRP14 protein bands did not change throughout a chase period of 48 h. This is in accordance with the assumption that complex formation is a calcium-dependent process as previously demonstrated [16] and not modulated by events associated with differentiation. Our data thus indicate that suppression on the transcriptional level, rather than biochemical modification, appears to be responsible for the control of MRP8 and MRP14 function.

Elevation of intracellular calcium levels by the calcium ionophore A23187 significantly down-regulated the mRNA of MRP8 and MRP14. Since A23187 may lead to equilibration of extra- and intra-cellular calcium concentrations, resulting in a non-physiological calcium overload suppression of MRP8/MRP14, transcription could be due to a more or less toxic effect. However, mRNA levels of GAPDH were not affected by A23187 treatment. Furthermore, the suppressive concentrations of A23187 were already effective at 100 nM and led to maximal down-regulation of MRP8/MRP14 at $\geqslant 1$ μM. The concentrations of A23187 employed in our study were thus at a range comparable with those ionophore concentrations which had been described earlier to be effective for induction of inflammatory cytokines such as IL-1, TNF-α, platelet-activating factor, JE/MIP-1 [36–39], colony-stimulating factors as macrophage colony-stimulating factor [40], oncogenes such as c-*fos* [41], and for modulation of arachidonic acid metabolism [42,43] in myelomonocytic cells. In addition, exposure of macrophages to the calcium-ATPase inhibitor thapsigargin, which releases calcium exclusively from intracellular stores [34], caused down-regulation of MRP8/MRP14 mRNA as well. Concomitant application of A23187 with actinomycin D did not accelerate suppression of MRP8 and MRP14 compared with treatment with the mRNA synthesis inhibitor alone. Thus A23187 seems to interfere with the synthesis, rather than the degradation, of MRP8 and MRP14 mRNA. Down-regulation of MRP8 and MRP14 mRNA as it is mediated by the ionophore requires substantially more time than transcriptional suppression by actinomycin D, thus indicating that calcium acts via an indirect mechanism. Concomitant application of cycloheximide antagonizes the inhibitory effect of A23187 on MRP8 and MRP14 transcription, suggesting that protein synthesis is necessary for suppression of MRP8 and MRP14 mRNA. Thus, *de novo* synthesis of repressor protein(s) seems to be responsible for calcium-dependent regulation of MRP8 and MRP14 expression. Interestingly, in a recent study binding of nuclear factors to the 5′ promotor region of MRP8 and MRP14 genes has been shown which correlates with vitamin D_3-dependent induction of MRP8 and MRP14 transcription in HL-60 cells [44]. However, these factors have not been characterized at the molecular level so far.

Down-regulation of MRP8 and MRP14 mRNA by A23187 treatment appears to be a rather specific event during inflammatory activation as elevation of intracellular calcium levels leads to the induction of mRNA, for , e.g. TNF-α, JE and c-*fos* [37,39,41].

Expression of other S-100-like proteins has been shown to be tightly regulated by various mechanisms [8,45,46]. However,

there is so far only one member of this protein family, CaN19 (a protein preferentially expressed by mammary epithelial cells during G_1/S phases), which has been described to be down-regulated at the mRNA level in a calcium-dependent manner [47]. The physiological role of CaN19, however, is yet not clear.

Regarding MRP8 and MRP14, calcium-induced down-regulation may be of functional relevance since these proteins comprise a major portion of the total cellular protein content in distinct differentiation stages of monocytes. Elevation of intracellular calcium levels has been shown to lead to assembly of non-covalently linked complexes of MRP8 and MRP14 [16], which are supposed to represent the biologically active forms [16–20]. Furthermore, calcium-dependent translocation of these proteins to membrane structures [21,22] and intermediate filaments [23] refers to a modulatory role of MRP8 and MRP14 in the network of cytoskeleton–membrane interactions. Thus, the same initial stimulus, i.e. a rise in intracellular calcium, is not only responsible for biochemical alterations of these proteins, but also for their transcriptional down-regulation. MRP8 and MRP14 therefore do not appear to be just calcium buffers maintaining homoeostasis of calcium in monocytes, but seem to represent proteins interfering with cellular functions in a more specific manner. Calcium is known to be involved in events associated with monocytic activation [48]. Accordingly, calcium-induced translocation of MRP8 and MRP14 to membrane structures has been shown to coincide *in vitro* with secretion of proinflammatory cytokines by macrophages [21] and enhanced superoxide release [22]. Thus, our data suggest that the biological role of MRP8 and MRP14 is restricted to processes occurring before or during calcium-dependent activation. Monocytic activation appears to be associated with marked changes regarding their mode of calcium-dependent signalling, which is reflected by down-regulation of MRP8 and MRP14. Further analysis of this calcium-induced repressor mechanism will help to elucidate the role of MRP8/MRP14 in the complex sequence of events during monocyte activation and differentiation.

We gratefully thank D. Kortevoß and C. Wulff for excellent technical assistance. This study was supported by a grant from the Bundesministerium für Forschung und Technologie, Bonn, Germany.

REFERENCES

1 Odink, K., Cerletti, N., Brüggen, J., Clerc, R. G., Tarcsay, L., Zwadlo, G., Gerhards, G., Schlegel, R. and Sorg, C. (1987) Nature (London) **330**, 80–82
2 Zwadlo, G., Brüggen, J., Gerhards, G., Schlegel, R. and Sorg, C. (1988) Clin. Exp. Immunol. **772**, 510–515
3 Hessian, P. A., Edgeworth, J. and Hogg, N. (1993) J. Leukocyte Biol. **53**, 197–204
4 Kligman, D. and Hilt, D. C. (1988) Trends Biochem. Sci. **13**, 437–443
5 Baudier, J. and Cole, R. D. (1988) J. Biol. Chem. **263**, 5876–5883
6 Zokas, L. and Glenney, J. R., Jr. (1987) J. Cell Biol. **105**, 2111–2121
7 Greenberg, M. E. and Edelman, G. M. (1983) Cell **33**, 767–779
8 Calabretta, B., Battini, R., Kaczmarek, L., de Riel, J. K. and Baserga, R. (1986) J. Biol. Chem. **261**, 12628–12632
9 Andersson, K. B., Sletten, K., Berner Berntzen, H., Fagerhol, M. K., Dale, I., Brandtzaeg, P. and Jellum, E. (1988) Nature (London) **332**, 688
10 Dale, I., Brandtzaeg, P., Fagerhol, M. K. and Scott, H. (1984) Am. J. Clin. Pathol. **84**, 24–34
11 Brüggen, J., Tarcsay, L., Cerletti, N., Oding, K., Rutishauser, M., Holländer, G. and Sorg, C. (1988) (London) **331**, 570
12 Dorin, J. R., Novak, M., Hill, R. E., Brock, D. J. H., Secher, D. S. and van Heyningen, V. (1987) Nature (London) **326**, 614–617
13 Edgeworth, J., Gorman, M., Bennett, R., Freemont, P. and Hogg, N. (1991) J. Biol. Chem. **266**, 7706–7713
14 Hogg, N., Allen, C. and Edgewort, J. (1989) Eur. J. Immunol. **19**, 1053–1061
15 Wilkinson, M. M., Busuttil, A., Hayward, C., Brock, D. J. H., Dorin, J. R. and van Heyningen, V. (1988) J. Cell Sci. **91**, 221–230
16 Teigelkamp, S., Bhardwaj, R., Roth, J., Meinardus-Hager, G., Karas, M. and Sorg, C. (1991) J. Biol. Chem. **266**, 13462–13467
17 Roth, J., Teigelkamp, S., Wilke, M., Grün, L., Tümmler, B. and Sorg, C. (1992) Immunobiology **186**, 304–314
18 Murao, S., Collart, F. R. and Huberman, E. (1988) J. Biol. Chem. **264**, 8356–8460
19 Murao, S., Collart, F. and Huberman, E. (1990) Cell Growth Different. **1**, 447–454
20 Steinbakk, M., Naess-Andresen, C.-F., Lingaas, E., Dale, I., Brandtzaeg, P. and Fagerhol, M. K. (1990) Lancet **336**, 763–765
21 Bhardwaj, R., Zotz, C., Zwadlo-Klarwasser, G., Roth, J., Goebeler, M., Mahnke, K., Falk, M., Meinardus-Hager, G. and Sorg, C. (1992) Eur. J. Immunol. **22**, 1891–1897
22 Lemarchand, P., Vaglio, M., Mauël, J. and Markert, M. (1992) J. Biol. Chem. **267**, 19379–19382
23 Roth, J., Burwinkel, F., van den Bos, C., Goebeler, M., Vollmer, E. and Sorg, C. (1993) Blood **82**, 1875–1883
24 Zwadlo, G., Schlegel, R. and Sorg, C. (1986) J. Immunol. **137**, 512–518
25 Lagasse, E. and Weissman, I. L. (1992) Blood **79**, 1907–1915
26 Roth, J., Sunderkötter, C., Goebeler, M., Gutwald, J. and Sorg, C. (1992) Int. Arch. Allergy Immunol. **98**, 140–145
27 Sunderkötter, C., Beil, W., Roth, J. and Sorg, C. (1991) Am. J. Pathol. **138**, 931–939
28 Goebeler, M., Roth, J., Henseleit, U., Sunderkötter, C. and Sorg, C. (1993) J. Leukocyte Biol. **53**, 11–18
29 Vassaualt, A. (1974) in Methods of Enzymatic Analysis, vol. III, (Bergmeyer, H. U., ed.), pp. 607–610, Academic Press, New York
30 Staros, J. V. (1982) Biochemistry **21**, 3950–3955
31 Laemmli, U. K. (1970) Nature (London) **227**, 680–685
32 Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354
33 Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. and Rutter, W. J. (1979) Biochemistry **18**, 5294–5299
34 Thastrup, O., Cullen, P. J., Drobak, B. K., Hanley, M. R. and Dawson, A. P. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 2466–2470
35 Johnston, R. B., Jr. (1988) N. Engl. J. Med. **318**, 745–752
36 Matsushima, K. and Oppenheim, J. J. (1985) Cell. Immunol. **90**, 226–233
37 Bethea, J. R., Gillespie, G. Y. and Benveniste, E. N. (1992) J. Cell Physiol. **152**, 264–273
38 Camussi, G., Bussolino, F., Salvidio, G. and Baglioni, C. (1987) J. Exp. Med. **166**, 1390–1404
39 Tannenbaum, C. S. and Hamilton, T. A. (1989) J. Immunol. **142**, 1274–1280
40 Sater, R. A., Cripe, L. D., Taylor, J. D. and Niedel, J. E. (1991) J. Immunol. **147**, 633–637
41 Radzioch, D. and Varesio, L. (1991) Mol. Cell. Biol. **11**, 2718–2722
42 Shibata, Y., McCaffrey, P. G., Minowada, J., Volkman, A. and Oghiso, Y. (1992) J. Leukocyte Biol. **51**, 32–38
43 Mackenzie, R., Coles, G. A., Topley, N., Powell, W. S. and Williams, J. D. (1992) Immunology **76**, 648–654
44 Kuwayama, A., Kuruto, R., Horie, N., Takeishi, K. and Nozawa, R. (1993) Blood **81**, 3116–3121
45 Freeman, M. R. and Sueoka, N. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 5808–5812
46 Barraclough, R., Savin, J., Dube, S. K. and Rudland, P. S. (1987) J. Mol. Biol. **198**, 13–20
47 Lee, S. W., Tumasetto, C., Swisshelm, K., Keyomarsi, K. and Sager, R. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 2504–2508
48 Hamilton, T. A. and Adams, D. O. (1987) Immunol. Today **8**, 151–158

Received 25 October 1993/18 February 1994; accepted 23 February 1994