Pharmaceutical Research, Vol. 15, No. 2, 1998

Biochemical Characterization of a Glucocorticoid-Induced Membrane Protein (RM3/1) in Human Monocytes and Its Application as Model System for Ranking Glucocorticoid Potency

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Received July 31, 1997; accepted October 27, 1997

Purpose. Upon glucocorticoid stimulation, human mononuclear leucocytes express an antigen, RM3/1, which characterizes a subpopulation of human monocytes and macrophages evolving in late phase of inflammation. We investigated biochemical properties of the RM3/1 antigen and correlations between antigen expression and glucocorticoid potency.

Methods. Biochemical properties were analyzed after solubilization by immunoaffinity methods and SDS-PAGE.

Results. Induction of the RM3/1 antigen is a glucocorticoid receptor mediated process, in contrast, inflammatory mediators such as LPS or TPA were not able to upregulate RM3/1 expression. After SDS-PAGE, the antigen appeared as a 130 kDa (nonreduced)/150 kDa (reduced) glycoprotein with a 25 kDa N-linked glycoportion. The interdependence between antigen density and glucocorticoid efficacy was assessed by calculation of relative antigen expression induced by dexamethasone, fluticasone propionate, budesonide, triamcinolone acetonide, flunisolide, beclomethasone, prednisolone and triamcinolone. Relative antigen expression was significantly correlated with the relative receptor affinity of the glucocorticoid.

Conclusions. We described biochemical properties of the glucocorticoid-induced protein RM3/1. Though the precise role of the RM3/1 antigen in the antiinflammatory process is not fully understood yet, an useful application of the induced expression could already be demonstrated for pre-clinical screening of glucocorticoid potency.

KEY WORDS: glucocorticoids; RM3/1 antigen; human monocytes; glycoprotein; receptor affinity.

INTRODUCTION

Glucocorticoids exhibit antiinflammatory and immunosuppressive actions by induction or suppression of gene transcription (1,2). They can inhibit the transcription of inflammatory cytokines (3,4) or initiate the expression of antiinflamatory proteins (5–7). There are more proteins or mediators described which are repressed rather than induced by corticosteroids. Yet, those proteins which are expressed upon glucocorticoid stimulation might mediate antiinflammatory effects.

Observed glucocorticoid effects vary among different compounds. High clinical efficacy is generally related to a high specific binding affinity of the corticosteroid to its receptor and this is mainly determined by the lipophilicity of the glucocorticoid. Whether glucocorticoid-induced expression of potentially antiinflammatory proteins in monocytes also mirrors the potency of the substance needs to be elucidated.

Monocytes and macrophages are an interesting cell system to study glucocorticoid effects because they have a key position in the inflammatory process (8) and in modulation of responses of the immune system (9). Peripheral blood monocytes, which are precursors of tissue macrophages, form a heterogeneous population which exerts a great variety of physiological and pathological functions (10).

We previously described the generation of a monoclonal antibody (RM3/1) directed against a subpopulation of monocytes in human peripheral blood (11). The antigen is presented on the surface of 15–30% of freshly isolated monocytes of healthy donors and is induced by glucocorticoids *in vitro*. After 48 hrs incubation with dexamethasone more than 90% of the monocytes express the RM3/1 antigen. Injection of glucocorticoids into primates (12) or human volunteers (13) resulted in an increase of RM3/1 positive blood monocytes up to 80% within 6 hrs.

Monocytes expressing the RM3/1 antigen are also present in acute and chronic inflammation. During experimental gingivitis (14) and under allergic contact eczema (11) RM3/1 monocytes/macrophages appeared preferentially in the late inflammatory phase. Thus, it was concluded that this subpopulation of monocytes/macrophages could display anti-inflammatory properties. Indeed, the secretion of proteins with anti-inflammatory properties by RM3/1 positive macrophages were recently described (7,15).

Aim of the present study was the biochemical characterization of the RM3/1 antigen and to reveal a possible correlation between glucocorticoid-induced RM3/1 expression and the potency of glucocorticoids.

MATERIALS AND METHODS

Stimulants and Radiochemicals

Dexamethasone and prednisolone were purchased from Merck (Darmstadt, Germany), fluticasone-17-propionate, beclomethasone and RU486 were generous gifts from Glaxo Group Research (Greenford, England), budesonide was purchased from AB Draco (Lund, Sweden), triamcinolone and triamcinolone acetonide from Cyanamid (Wolfratshausen, Germany).

LPS from E. coli (type 055: B5), cycloheximide, actinomycine D, PMA (12-O-tetradodecanoylphorbol-13-acetate) and propidium iodine were purchased from Sigma (Deisenhofen, Germany). Radiolabelled ^{3}H-mannose, ^{3}H-glucosamin and ^{3}H-galactose were purchased from Amersham (Braunschweig, Germany).

Antibodies

The monoclonal antibody RM3/1 was previously generated and characterized by our group (11). The FITC (floures-

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cein-isothiocyanat) and alkaline phosphatase-labelled secondary antibody goat-anti mouse IgG_1 and the control mouse IgG_1 were purchased from Dianova (Hamburg, Germany).

Isolation of Blood Monocytes

Monocytes were isolated from pooled buffy coats (Blutbank Münster, Germany) by Ficoll-Paque (Pharmacia, Freiburg, Germany) and subsequent Percoll (Pharmacia) density gradient centrifugation. The monocytes purity was >90%. Monocytes were cultured at a density of 2×10^6 cells/ml in hydrophobic teflon bags (Heraeus, Hanau, Germany) in McCoy's5a medium (Biochrom, Berlin, Germany) supplemented with 15% fetal calf serum.

Stimulation of Monocytes

Monocytes for biochemical characterization of the RM3/1 antigen were cultivated for 2 days in presence of 10^{-7} M dexamethasone or 10^{-8} M fluticasone propionate. Stimulation with LPS was performed for 1 day at a concentration of 1 μg/ml, stimulation with cycloheximide and actinomycine D for 16 hrs at a concentration of 2 μg/ml and 0.75 μg/ml, respectively, stimulation with PMA for 1 hr at a concentration of 0.3 ng/ml.

FACS-analysis

For indirect immunofluorescence analysis, monocytes were washed with cold phosphate buffered saline (PBS, pH 7.4) and incubated with 1% bovine serum albumine (BSA) for 30 min at 4°C. Then cells were washed and incubated with optimal antibody concentration (5–10 μg/ml) for 45 min at 4°C. Mouse IgG_1 was included as isotype control. Subsequently, monocytes were washed with PBS and incubated with FITC-labeled secondary antibody goat-anti mouse IgG_1 in 1% BSA for 30 min at 4°C. Propidium iodine (1mM in PBS) was added for the last 2 min of incubation to determine cell viability and exclusion of dead cells. The fluorescence intensity of 10^4 vital cells was measured by FACS (fluorescence activated cell sorter)-analysis (FACscan, Becton Dickinson, Heidelberg, Germany). The parameters used were 488 nm excitation wavelength, 250 mW and logarithmic amplification. The antigen density and the number of RM3/1 positive cells corrected for isotype control were obtained from the main fluorescence channel at 510–530 nm using Lysis Software (Becton Dickinson).

Isolation of the RM3/1 Antigen

Cell lysis was performed by nitrogen decompression after 20 min (30 bar at 4°C). After centrifugation to separate cell nuclei, cell membrane pellet was solubilized in 0.5% Triton X-114 containing 1 mM PMSF for 15 min at 4°C. After 30 min at 37°C phase separation occured (16) and the lower, detergent-rich phase containing hydrophobic membrane proteins was collected.

Alternatively, in later experiments, cells were lysed and solubilized in a one-step procedure. Monocytes were incubated with 1–10 mM Pefabloc SC™ (Boehringer Mannheim, Germany) and octylthioglucopyranoside (Sigma) at a protein detergent ratio of 0.2 for 30 min at room temperature under gentle rotation. After centrifugation the supernatant was collected.

Immunoprecipitation and Affinity Chromatography

The detergent-rich phase was incubated for 1 hr at room temperature with 100 μg rabbit IgG (Pierce, Sankt Augustin, Germany) to block nonspecific binding to IgG antibodies. Rabbit IgG was removed by incubation with 30 μl IgG-binding protein G sepharose (Protein G Sepharose Fast Flow, Pharmacia LKB, Freiburg, Germany) for 1 hr at room temperature. Protein G sepharose was removed by centrifugation and supernatant was incubated with RM3/1 antibody for 16 hrs at 4°C under gentle shaking. Subsequently, 30 μl protein G sepharose was added and samples were incubated for another 1 hr at room temperature. The incubation mixture was pelleted and the pellet was washed twice with PBS containing 0.5% (v/v) Nonidet P-40 (Sigma) and twice with PBS. The pellet was used for SDS-PAGE. For affinity chromatography, 1 mg of RM3/1 antibody was coupled to cyanbromide activated sepharose (Pharmacia LKB) according to manufacturer's protocol. After incubation with a crude cell extract, RM3/1 antigen was eluted from the affinity column with 0.2 M glycine-HCl, pH 2.5. The eluate was immediately neutralized by addition of 1 M Tris (pH 8.5).

SDS-PAGE (Polyacrylamide Gel Electrophoresis)

Proteins were separated by SDS-PAGE under reducing conditions according to Laemmli (17) using a 8% running gel. For nonreducing conditions, mercaptoethanol or DTT (both Sigma) were omitted. Protein bands were either stained with 0.1% Coomassie Blue (R 250, Sigma) or with silver staining according to manufacturer's protocol (Sigma).

Deglycosylation

Detergent-rich phase was prepurified by gel filtration chromatography with a Sephadex G25 (Pharmacia LKB) column (70 cm length, inner diameter 3 cm). Proteins were eluted with 0.2 M ammonia acetate solution (pH 7.3). Protein containing fractions were concentrated by lyophilization and reconstituted in incubation buffer for glycosidases. Reaction volume for all incubations with glycosidases was 100 μl, 0.02 % sodium azide was included in all reaction mixtures. For deglycosylation with N-glycosidase (PNGase F, Boehringer Mannheim), 50 μl protein extract was denatured by heating for 2 min at 100°C with 40 mM phosphate buffer pH 7.5, 0.1 M EDTA, 5% mercaptoethanol and 0.5% SDS (all Sigma). Subsequently, 1.25% of octylthioglucosid and 5 U N-glycosidase was added to result in a final volume of 100 μl. For deglycosylation with O-glycosidase (Boehringer Mannheim), protein extract was incubated with 0.1 M sodium citrate/phosphate buffer pH 6.0, 100 μg/ml BSA and 5 mU O-glycosidase. For deglycosylation with sialidase (neuraminidase, Boehringer Mannheim), protein extract was incubated with 0.1 M sodium acetate buffer pH 5.0 and 0.2 U sialidase. Incubations were carried out at 37°C for 16 hrs. Reactions were stopped by addition of an equal volume of sample buffer and heating at 95 °C for 5 min. Shift in protein bands were detected by SDS-PAGE and Coomassie staining.

Metabolic Labelling with ^{3}H-Monosaccharides

Samples of 10^8 monocytes were incubated in 50 ml medium containing dexamethasone (10^{-7} M) and each 250 μCi

^{3}H-mannose, ^{3}H-glucosamin and ^{3}H-galactose overnight at 37°C. Cells were washed, lysed and immunoprecipitated. After SDS-PAGE radioactive proteins were detected by autoradiography.

RESULTS

Characterization of the RM3/1 Antigen

The monocyte surface epitope which is recognized by the RM3/1 antibody was assumed to have protein character. To demonstrate that RM3/1 expression is dependent on protein synthesis, actinomycin D—an inhibitor of RNA polymerase—and cycloheximide—an inhibitor of translocation at eucaryotic ribosomes—were incubated with monocytes and RM3/1 antigen density of 10^4 vital cells was quantified by FACscan analysis, corrected for isotype control (Figure 1). Both inhibitors of protein synthesis reduced RM3/1 antigen expression statistically significantly (paired t-test, $p \leq 0.05$).

After solubilization of equal numbers of glucocorticoid stimulated and nonstimulated monocytes and SDS-PAGE of the crude cell extract, a complex pattern of multiple proteins with molecular weights smaller than 120 kDa could be seen (Figure 2, lane 2: nonstimulated, lane 3: stimulated cells). Few proteins with molecular weights higher than 120 kDa could be identified on the gel, two prominent bands at approximately 150 (see arrow) and 170 kDa which are less expressed in the nonstimulated cells, can be found in the cell extract of stimulated monocytes. After prepurification of the crude cell extract via Sephadex G25 gel filtration chromatography (lane 4: nonstimulated, lane 5: glucocorticoid stimulated monocytes) the

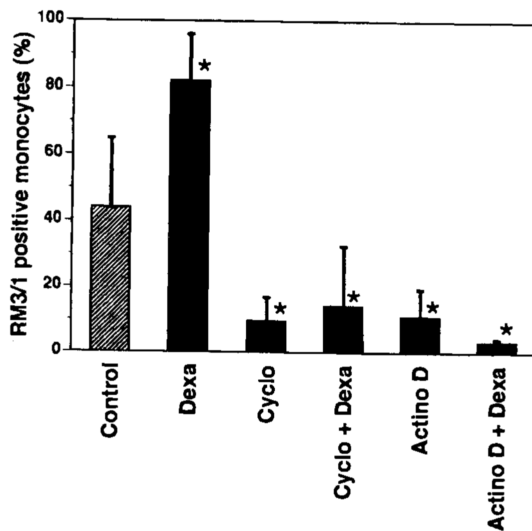

Fig. 1. Inhibition of RM3/1 expression by incubation of human monocytes with inhibitors of protein synthesis: cycloheximide (Cyclo, 2 μg/ml for 16 hrs) or actinomycine D (Actino D, 0.75 μg/ml for 16 hrs). Percentage of RM3/1 positive cells, corrected for isotype control, was determined by FACscan analysis, mean values of four independent experiments are shown. Cell viability was determined with propidium iodine, 10^4 vital cells were counted. Monocytes stimulated with dexamethasone (Dexa) alone or in combination with inhibitors of protein synthesis were statistically significant different (*) from control values ($p \leq 0.05$, paired t-test).

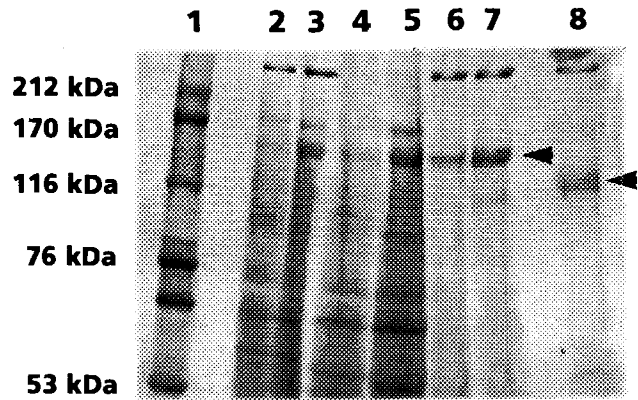

Fig. 2. Silver stained reducing 8% SDS-PAGE gel of crude and purified cell extracts. Lane 1 shows the high molecular weight standard, lane 2 and 3 crude cell extracts of nonstimulated and glucocorticoid stimulated monocytes, respectively, solubilized with octylthioglucosid, lane 4 and 5 cell extracts of nonstimulated and stimulated cells prepurified with gel filtration chromatography via Sephadex G25, lane 6 and 7 eluates from the RM3/1 affinity column incubated with nonstimulated and stimulated cell extracts, lane 8 shows the affinity column eluate of stimulated cells under nonreducing conditions. Arrows mark the migration distance of the RM3/1 antigen under reducing (150 kDa) and nonreducing (130 kDa) conditions. Monocytes were stimulated with 10^{-8} M fluticasone propionate.

pattern of bands changed only slightly, a high molecular weight protein at 220 kDa is absent after this preparation. Affinity chromatography of the crude cell extract (lane 6: nonstimulated, lane 7: stimulated cells) revealed that the band at 150 kDa (arrow) is bound by the RM3/1 antibody. The protein at 220 kDa is bound nonspecifically by the antibody which could be seen with control mouse IgG_1 affinity chromatography (not shown). The protein eluate from the affinity column was also chromatographed under nonreducing conditions (lane 8). A shift in the molecular weight was observed, in the absence of reducing agents the protein had an apparent molecular weight of 125 kDa.

To confirm that the prominent band around 150 kDa was affiliated to a single protein, a two-dimensional electrophoresis was performed. The 150 kDa band was found to be a single protein with an IP of 5.5 (data not shown).

Posttranslational modification of membrane proteins are a common, most often they are N-glycosylated. Thus, the RM3/1 antigen was analyzed for putative glycoportions (Figure 3). Therefore, the RM3/1 antigen was deglycosylated by incubation with N- and O-glycosidase (lanes 3 and 4) and sialidase (lane 5). After SDS-Page, a band shift of 25–30 kDa was detectable after incubation with N-glycosidase (arrow) compared to control (lane 2, arrow), whereas O-glycosidase did not cause any shifts. Incubation with sialidase caused a very slight shift so that the presence of some sialylated carbohydrates cannot be totally excluded. Incubation of the monocyte extract with combinations of O-glycosidase and sialidase (lane 6) or N- and O-glycosidase and sialidase (lane 7) resulted in no further shifts. Metabolic labelling with ^{3}H-mannose, ^{3}H-glucosamine and ^{3}H-galactose was performed to verify the presence of a glycan structure in the RM3/1 antigen. After incubation of the monocytes with a mixture of ^{3}H-mannose, ^{3}H-glucosamine and

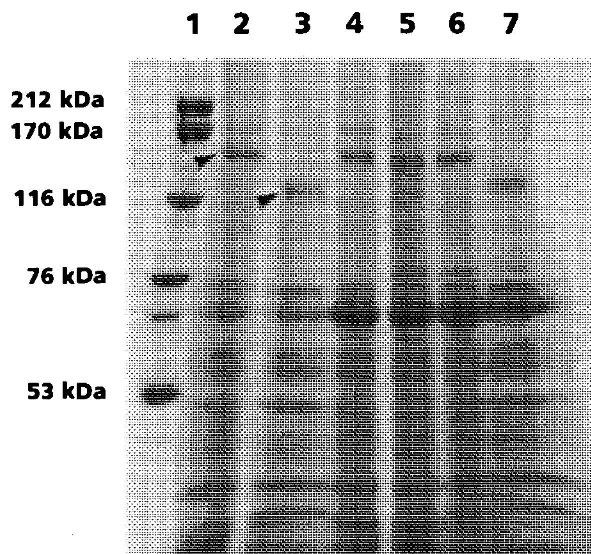

Fig. 3. Coomassie stained 8% SDS-PAGE gel of crude cell extracts of stimulated monocytes (10^{-8} M fluticasone propionate) treated with various glycosidases. Lane 1 shows the high molecular weight standard, lane 2 the crude, nontreated cell extract, lane 3 shows the cell extract after 16 hrs incubation with 5 U N-glycosidase, lane 4 after incubation with 5 mU O-glycosidase, lane 5 after treatment with 0.2 U sialidase, lane 6 after coincubation with 5 mU O-glycosidase and 0.2 U sialidase, lane 7 shows the cell extract after coincubation with all three glycosidases.

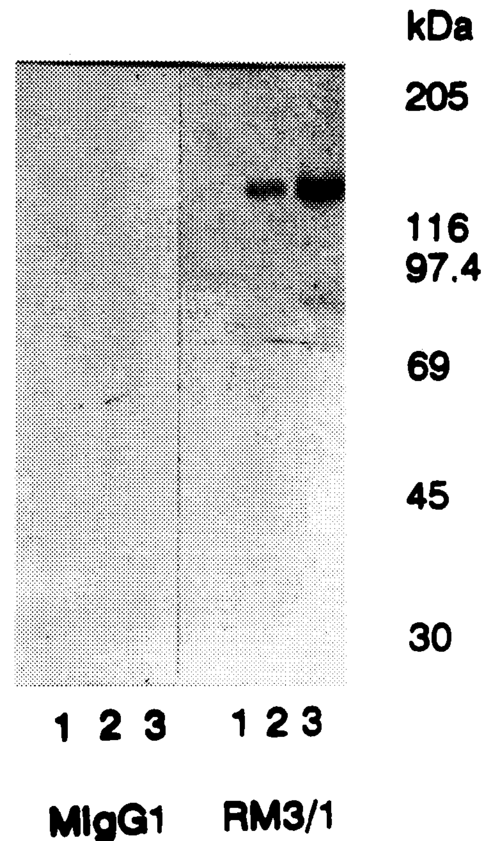

Fig. 4. Autoradiography of an 8% SDS-PAGE gel after immunoprecipitation of stimulated monocytes (10^{-7} M dexamethasone) labelled with a mixture of ^{3}H-mannose, ^{3}H-glucosamine and ^{3}H-galactose. Antibodies used for immunoprecipitation were RM3/1 and mouse IgG_1 as a negative control. Lane 1 shows immunoprecipitation of the culture supernatant, lane 2 immunoprecipitation of monocytes after nitrogen decompression, lane 3 immunoprecipitation of monocytes after Triton-X114 extraction.

^{3}H-galactose, the RM3/1 antigen was solubilized and immunoprecipitated. SDS-Page revealed bands after immunoprecipitation with the RM3/1 antibody, but not with control mouse IgG_1 antibody (Figure 4). No bands were detectable after immunoprecipitation with the RM3/1 antibody in the culture supernatant (lane 1). In the contrary, clear bands were seen after immunoprecipitation with the RM3/1 antibody of lysed monocytes after nitrogen decompression (lane 2) or Triton X-114 solubilization (lane 3).

Induction of the RM3/1 Antigen

The RM3/1 antigen expression was inducible by glucocorticoids. Time course of early surface expression on human monocytes was determined by indirect fluorescence analysis (Figure 6). Number of positive monocytes and antigen density increased significantly after 16 hrs. The antigen density and the number of positive cells always showed a high correlation with a coefficient of correlation of $r = 0.974$. Optimal dexamethasone concentration for stimulation was 10^{-7} M (data not shown).

To confirm that the RM3/1 induction is a glucocorticoid receptor-related effect, monocytes were coincubated with the glucocorticoid antagonist, RU486 and dexamethasone (Figure 5). Control monocytes exhibited a statistically significant increase in antigen density after stimulation with 10^{-7} M dexamethasone. When the glucocorticoid antagonist RU486 was added to the culture medium at a concentration of 10^{-6} M, a clear inhibition of the RM3/1 expression resulted, again statistically significant versus stimulated control. At equimolar concentrations of agonist and antagonist we observed a slight, but not significant rise in antigen density caused by dexamethasone in the presence of RU486. Finally, a 10-fold surplus of the agonist overcomes antagonistic effects and there is no difference to stimulated control cells. Significance of treatment effects was calculated with one-factorial ANOVA and subsequent Fisher PLSD test.

The influence of inflammatory activators—compared to the influence of the antiinflammatory glucocorticoids—on RM3/1 antigen expression was determined by incubation of PMA and LPS with monocytes. After PMA treatment the number of positive cells was statistically significantly reduced (paired t-test, $p \leq 0.05$) whereas LPS had no effect on RM3/1 expression compared to nontreated control cells (Figure 7).

Correlation of RM3/1 Antigen Induction and Relative Receptor Affinity of Glucocorticoids

For determination of antigen expression peripheral blood monocytes were incubated for one day with nine different glucocorticoids with known relative receptor affinity (RRA) (18–20). Various concentrations were used for each glucocorticoid, antigen expression was evaluated by FACscan analysis.

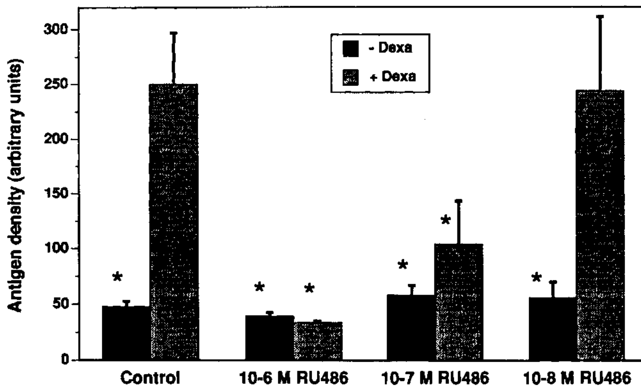

Fig. 5. Inhibition of RM3/1 expression by various concentrations of the glucocorticoid receptor antagonist RU486. Monocytes were stimulated for one day with 10^{-7} M dexamethasone in the presence or absence of RU486. Specific fluorescence intensity of 10^4 vital cells, corrected for isotype control, was determined by FACscan analysis. Means (n = 6) of three independent experiments are shown, statistically significant differences were evaluated by one-factorial ANOVA and subsequent Fisher PLSD test. Dexamethasone-stimulated control values were significantly different (*) from all non-dexamethasone stimulated values ($p \leq 0.001$). RU486 suppressed RM3/1 expression at concentrations of 10^{-6} M and 10^{-7} M, whereas 10^{-8} M failed to suppress antigen expression significantly.

After logit-log transformation (21) of percentage difference of the number of RM3/1 positive cells compared to nonstimulated control cells (Y values) according to:

$$\text{logit}(Y) = \log \frac{Y}{100 - Y}$$

the log concentration of glucocorticoid (x-axis) was plotted versus logit (Y). Intersection with the x-axis is found by linear regression and gives the concentration of glucocorticoid necessary to induce a 50% increase of RM3/1 antigen density (c_{50}). Relative antigen expression (RAE) induced by glucocorticoids (GC) was calculated with reference to dexamethasone. The relative antigen expression induced by dexamethasone was set 100, in parallel to receptor binding assays (20).

$$RAE_{GC} = \frac{C_{50}, \text{Dexa}}{C_{50}, \text{GC}} \cdot 100$$

Results of relative antigen expression are given in Table I. Three series of experiments were performed and, although RAE varies in terms of absolute values, rank order of glucocorticoids always remains the same. Correlation with published RRAs is very high with coefficients of correlation of r = 1.00, r = 0.995 and r = 0.989, respectively.

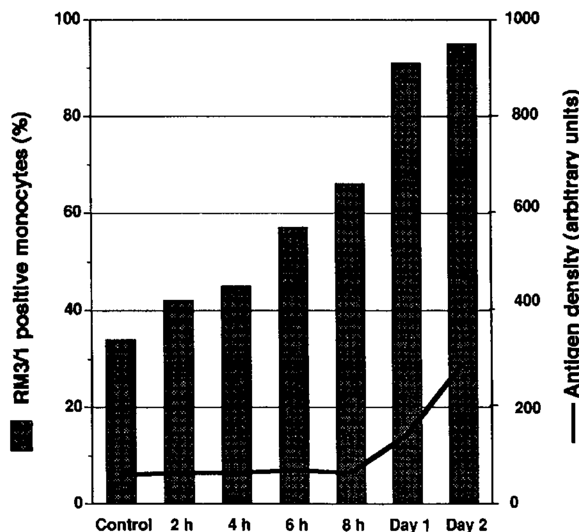

Fig. 6. Time course of increase of RM3/1 positive monocytes and antigen density on the cell's surface after 2, 4, 6, 8, 24, 48 hours stimulation with 10^{-7} M dexamethasone. Both parameters, corrected for isotype control, were determined by FACscan analysis. Cells' viability was determined with propidium iodine, dead cells were excluded from measurement.

DISCUSSION

The RM3/1 antigen is a membrane glycoprotein found on human monocytes and macrophages. It is constitutively present on 15–30% of circulating blood monocytes (11) and it is significantly induced by glucocorticoids. The RM3/1 expression is dependent on protein synthesis as we could demonstrate with inhibition experiments with actinomycin D and cycloheximide.

The RM3/1 protein could be solubilized with nonionic detergents which is typical for integral membrane proteins. SDS-PAGE revealed an apparent molecular weight of about 130 kDa (unreduced) and 150 kDa (reduced conditions). This migration behavior suggests the presence of several intramolecular disulfide bonds. The intrachain disulfide bonds might retain the protein in a more compact configuration which can migrate faster electrophoretically (22).

The RM3/1 antigen is extensively glycosylated posttranslationally, about 17% of the molecular weight are contributed by the oligosaccharide. The glycoportion consists predominantly of N-glycans, however, presence of sialic acids cannot be excluded. Glycoproteins which are N-glycosylated have a common core structure consisting of β-D-N-acetylgalactosamin-

Table I. Relative Antigen Expression (RAE) of Various Glucocorticoids in Relation to Their Relative Receptor Affinity (RRA) (derived from [20]) and Coefficients of Correlation Between RRA and RAE

Glucocorticoid	RRA [20]	RAE		
		Series 1	Series 2	Series 3
Dexamethasone	100	100	100	100
FP	1800	4799	2949	1786
Budesonide	935	2613	1770	—
TAAC	361	—	553	—
Flunisolid	180	272	445	426
Beclomethasone	76	40	67	49
Prednisolone	12	2	19	—
Triamcinolone	1	37	23	32
Correlation RRA/RAE		r = 1.00	r = 0.995	r = 0.989

Abbreviations: FP: fluticasone-17-propionate, TAAC: triamcinoloneacetonide

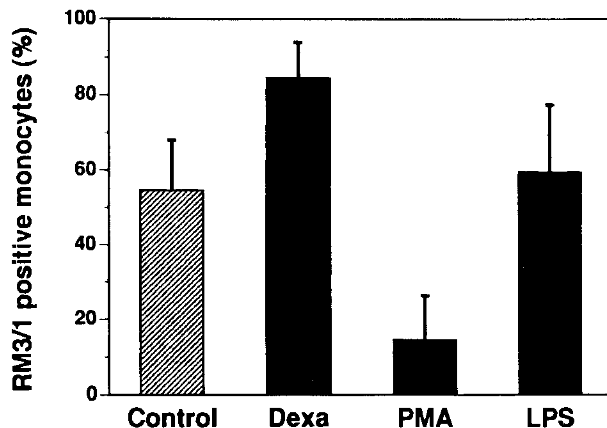

Fig. 7. Effect of PMA (0.3 ng/ml for 1 hr) and LPS (1 μg/ml for one day) on the number of RM3/1 positive monocytes, corrected for isotype control, compared to nontreated cells (control) and monocytes stimulated with 10^{-7} M dexamethasone (Dexa). Flourescence intensity was determined by FACscan analysis, 10^4 vital cells were counted. Means and standard deviation of four independent experiments are shown. Monocytes stimulated with dexamethasone alone or with PMA were statistically significant different (*) from control values ($p \leq 0.05$, paired t-test).

(1 → 4)-β-D-acetylgalactosamin-(1 → 4)-β-D-mannose-(1 → 6) and (1 → 3)-α-D-mannose (23). This core structure is further modified with additional mannose carbohydrates to result in high mannose-type oligosaccharides, with additional mannose, N-acetylglucosamine, galactose and sialic acid residues to result in hybrid-type oligosaccharides or with N-acetylglucosamine, galactose and sialic acid residues to result in complex-type oligosaccharides. Metabolic labelling revealed that at least the monosaccharides mannose, galactose and glucosamine are components of the glycoportion, thus supporting the presence of the N-glycan core structure in the RM3/1 antigen. Antigen density and number of RM3/1 positive cells manifested that early expression starts after 16 hrs. Previous time course studies showed that the peak expression of the RM3/1 antigen is after about 2–3 days in culture (11). The early expression kinetics are typical for glucocorticoid-induced effects at the molecular level. Glucocorticoid-induced up-regulation of IL-6 receptors on endothelial cells peaked after 15 hrs (24). Clear cut effects in up-regulation of GM-CSF receptor expression on monocytes induced by glucocorticoids was seen after 3 days (25). However, induction of RNA synthesis by glucocorticoids may occur fast, within 4 hrs (26).

It could be now demonstrated that the induction of the expression is a glucocorticoid receptor-mediated mechanism which could be inhibited by the glucocorticoid antagonist, RU486. The observation that 15–30% of human monocytes are RM3/1 positive without stimulation could thus be the result of endogenous stimulation by hydrocortisone. There is obviously a close relationship of RM3/1 expression and the antiinflammatory process (8,13,14). These previous observations are underlined by the finding that the appearance of the RM3/1 antigen is not related to the activation process of monocytes/ macrophages: PMA downregulated the RM3/1 antigen and bacterial LPS was lacking an effect on RM3/1 antigen expression.

Recently, a contribution of this antigen in the adhesion process of monocytes to activated endothelial cells was shown (27). The now described characteristics of this glycoprotein, carrying a substantial N-glycan portion, supports the notice that it is involved in the adhesion process since adhesion is often mediated by N-glycosylated proteins (28,29). The precise function of the RM3/1 membrane protein is still under investigation. However, the expression of this antigen was already found to be a very useful model system for testing synthetic glucocorticoids. Clinical glucocorticoid effects vary among different compounds, which can be mainly ascribed to the specific binding affinity of the corticosteroid to its receptor. Relative receptor affinity of glucocorticoids can be evaluated from *in vitro* binding tests with receptor containing cytosol (20,30,31). Experiments for determination of RRAs are usually performed with isolated and stabilized cytosolic receptors. Neither the glucocorticoid diffusion into the cell is part of the RRA calculation nor the time to initiate a DNA downstream effect. A conventional clinical approach for glucocorticoid screening is the skin blanching test (32). Several factors besides the glucocorticoid affinity to its receptor influence the outcome of this approach such as the type of vehicle used, the status of the skin or the absorption rate. Thus, this test system evaluates both pharmacokinetic and pharmacodynamic properties of a corticosteroid. This is valuable for assessment of glucocorticoids meant for dermal application, but may be misleading for mucous membranes in case of nasal or pulmonary application where a pronounced resorption phase is absent. For these types of application the RM3/1 model system is much more suitable because it mirrors pharmacodynamic glucocorticoid effects without preceeding pharmacokinetic events. Now, it could be shown that RM3/1 glycoprotein expression is dependent on glucocorticoid concentration and also highly correlated to the relative affinity (RRA) of the glucocorticoid to its receptor. Consequently, the very potent fluticasone propionate induced antigen expression at very low concentrations, whereas prednisolone influenced protein expression if present at high concentrations only. It has to be notified that only the poorly active beclomethasone, but not beclomethasone-17, 21-dipropionate (BDP, RRA 53) or its potent metabolite beclomethasone-17-monopropionate (17-BMP, RRA 1345), were tested because BDP and 17-BMP were not stable under incubation conditions and the addition of an esterase inhibitor was lethal for the monocytes. Although the results of single relative protein expression experiments varied up to 2-fold, the resulting ranking of the glucocorticoids was highly reproducible. This ranking, also shown by the RRA experiments, is also found for clinical efficacy of these corticosteroids (33).

Therefore, the RM3/1 expression may be a useful *in vitro* model to screen the potency of glucocorticoids, or to evaluate antagonistic effects. Using glucocorticoid-induced protein expression in monocytes as a natural model system, the glucocorticoid uptake as well as the effect of the glucocorticoid-receptor interaction—the production of the potentially antiinflammatory RM3/1 glycoprotein—can be measured. This system has even advantages over analogous methods which could be used, such as monitoring the reduced secretion of inflammatory mediators upon glucocorticoid addition. With the RM3/1 system it is not necessary to stimulate the monocytes first with inflammatory agents like LPS to measure a subsequent

glucocorticoid effect since the RM3/1 is directly induced by glucocorticoids. The RM3/1 antigen—as a membrane protein—can be measured easily by antibody labelling and subsequent FACscan analysis. Thus, this test system mimics parts of glucocorticoid action *in vivo* and is suitable for preclinical screening.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft DFG (grants to P.H. [Ho 1548/3-1] and C.S.).

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