A Mechanism of Resistance to Glucocorticoids in Multiple Myeloma: Transient Expression of a Truncated Glucocorticoid Receptor mRNA

By Pamela A. Moalli, Shafali Pillay, Dawn Weiner, Robin Leikin, and Steven T. Rosen

GLUCOCORTICOIDS are an important component of chemotherapy regimens used in the treatment of hematopoietic malignancies including multiple myeloma; however, the effectiveness of hormonal therapy is limited by the development of resistance. Previous studies in leukemias and lymphomas have demonstrated that glucocorticoids mediate their therapeutic effects via lymphocytolysis. However, myeloma cells in culture are typically resistant to glucocorticoid-induced cytosis, although some have been reported to be moderately growth-inhibited. Thus, despite the clinical response observed in myeloma patients treated with steroid hormones, the basis of this response is unclear. An understanding of the precise mechanisms by which glucocorticoids exert their therapeutic effects in multiple myeloma, as well as the progression of events leading to resistance, is critical for the successful treatment of patients. Here we address these issues by examining the effects of the glucocorticoid dexamethasone (DEX) on the human myeloma line, MM.1.17

Glucocorticoids mediate their direct effects on a cell through the glucocorticoid receptor (GR). In the absence of glucocorticoids, the GR is primarily cytoplasmic. On binding hormone, the receptor is activated, enabling the hormone-receptor complex to translocate to the nucleus, bind specific DNA regulatory sequences, and modulate the initiation of transcription from nearby promoters. In some cases, the GR acts as a positive regulator of transcription, while in others it represses expression. The primary amino acid sequence of the GR is composed of three major functional domains, within which several smaller domains have been identified. At the N-terminus is the hypervariable region (HVR), named as such because of its relative lack of homology to the other members of the steroid hormone receptor superfamily. The HVR is followed by the highly conserved DNA binding domain (DBD) and a hormone binding domain (HBD) at the C-terminus. In contrast to the DBD, the HBD is not absolutely required for receptor-mediated transcriptional activation. Mutagenesis studies have clearly demonstrated that a GR truncated of the HBD is converted to a constitutively active nuclear transcription factor.

As previously suggested, the mechanism of steroid hormone action and acquired resistance to glucocorticoids has been extensively studied in leukemias and lymphomas using both human and mouse cell lines as paradigms. Glucocorticoid-resistant variants in these malignancies, isolated by their lack of responsiveness to hormone-induced cytosis, have largely been found to express either reduced levels or altered forms of the GR. A smaller number have also been described in which apparently functional GR are present in unresponsive cells, suggesting that “postreceptor” mechanisms also contribute to resistance. From these studies, it has become clear that in leukemia and lymphoma cells, glucocorticoids act directly through the GR to induce cytosis. For this response to occur, the target cell must not only express a critical number of functional GR, but must also have specific postreceptor pathways intact.

In contrast, little is known regarding the mechanism of action of glucocorticoids in multiple myeloma. Indeed, several studies have reported on the presence of GR in myeloma cell lines and patient samples, and the growth inhibitory properties of hormone on myeloma cells in culture have been briefly described. However, to our knowledge, only one study has been conducted in which the molecular events involved in glucocorticoid action and resistance to glucocorticoids in multiple myeloma have been examined in detail. In the latter study, the investigators use a myeloma line sensitive to the lytic effects of dexamethasone (DEX) on a hormone-sensitive clone (MM.1S). MM.1S is lysed by DEX. DEX-induced cytolysis is effectively blocked by the glucocorticoid antagonist, RU 486, indicating the specificity of this response for the GR. In contrast to MM.1S, MM.1R is not lysed by hormone, has little hormone-binding activity, and expresses the 7.1-kb GR mRNA at low levels. Interestingly, we have found that two distinct phenotypes emerge from MM.1R with increasing periods of growth in culture. The first or “early” form, MM.1Re, expresses high levels of a variant GR mRNA of 5.5 kb that has a deletion in its 3’ end. With further growth in the presence or absence of selective media, the expression of this transcript is repressed, resulting in the second or “late” phenotype characteristic of MM.1RL. No discernible differences in the organization of the genomic GR sequence in DEX-sensitive and -resistant cells were detectable by Southern analysis, suggesting that no gross deletions, rearrangements, or allelic variations in the genomic sequence account for the resistant phenotypes of MM.1R.
glucocorticoids to show that this response is mediated directly through the GR. By demonstrating that two myeloma lines not lysed in the presence of glucocorticoid express functional GR, they provide evidence that resistance in the myeloma lines is mediated via postreceptor phenomena.

Here, we further investigate the direct action of glucocorticoids on myeloma cells using a human myeloma line, MM.1, which is sensitive to glucocorticoid-induced cytolysis. MM.1 was initiated from the peripheral blood of a patient in the leukemic phase of multiple myeloma whose MM.1, which is sensitive to glucocorticoid-induced cytolytic activity in the myeloma lines is mediated via postreceptor phenomena. The early phenotype characteristic of MM.1Re is a transient phenotype that is present immediately following selection of resistant cells from the parent population. At this time, MM.1Re expresses significantly decreased levels of the full-length 7.1-kb GR mRNA and high levels of a variant form of GR mRNA of approximately 5.5 kb. Analyses of the variant transcript and its protein product suggest that the 5.5-kb message codes for a protein that fails to bind hormone because of a truncation in the HBD. Eventually (usually following 4 to 6 months of continuous growth in culture), the expression of the truncated transcript ceases, resulting in the second or late resistant phenotype exemplified by MM.1RL. The latter follows the r- resistant phenotype previously described for leukemias and lymphomas.20,25-30

**Glucocorticoid binding assay.** Hormone binding was assessed in whole cell assays as described by Marchetti et al with some modifications. Briefly, 2 to 2.5 x 10^6 cells in mid-logarithmic growth were incubated in triplicate in the presence of increasing concentrations of ^3H-DEX (New England Nuclear, Boston, MA) and in the presence or absence of a 100-fold excess of cold hormone. Following a 1-hour incubation period, 1 mL of phosphate-buffered saline (PBS) was added to the cells and then applied to 2.4-cm GF-C Whatman glass filters (VWR Scientific, Irvine, CA) using a Model FH1200 vacuum transfer system (Hoeffer Scientific Instruments, Chicago, IL). Each tube was sequentially rinsed with four 1-mL washes of PBS to collect any adherent cells. The filters were dried and counted in a Beckman LS6800 Liquid Scintillation Counter (Irvine, CA) with an efficiency of approximately 50%.

Specific binding was calculated by subtracting the nonspecific bound ([^3H]-DEX + 100-fold excess cold) from the total ([^3H]-DEX alone). The best-fit line was determined by a computer-programmed linear regression analysis of the data points (Harvard Presentation Graphics, Mountain View, CA). The data were further analyzed by the Ligand computer program (P.J. Munson and D. Rodbard, National Institutes of Health, Bethesda, MD) to obtain the values for the slope and x-intercept. The maximum number of glucocorticoid binding sites and the dissociation constant (k_d) were calculated according to the method of Scatchard, assuming that each receptor binds one molecule of hormone.

**Probes for Northern, slot blot, and Southern analyses.** Specific fragments of the GR cDNA corresponding to the N-terminal HVR (1.3 kb), the DBD (2 kb), the HBD (0.75 kb), and the first 634 bp of the 3' UTR were used as probes. Oligonucleotide primers corresponding to the sequences flanking each region to be amplified were synthesized by Northwestern Biotechnology Facility (Evanston, IL). The position in the GR cDNA and the sequences of the primers are as follows: HVR: (+)47, 5'-GGCTTCAAGCTAAGTTGTTT-3'; (+)1361, 5'-TAATGACATCCTGGAACCTCAT-3'. DBD: (+)1360, 5'-CAAGAAGCA-CA A C A G G A C C C T C T-3'; (+)1650, 5'-TCTTGATGGCTGCTGAAAT-3'. The first 634 bp of the 3' UTR: (+)2635, 5'-TCCTTGAGGAAACCT-3'. The last 360 bp of the HBD includes the first 170 bp of the 3'-UTR: (+)2103, 5'-CAGGGATCTCTGAAAGATGTCCT-3'; (+)2635, 5'-GC-CAAACGTGGCCCTCTAACCACAT-3'. The first 634 bp of the 3' UTR: (+)2635, 5'-TCCTTGAGGAAACCT-3'.

**Materials and Methods**

**Cell culture and growth.** MM.1 was established from the peripheral blood of a 42-year-old woman with an IgA-λ multiple myeloma as previously described.17 MM.1 has been characterized in detail by cytochemical, immunohistochemical, flow cytometric, Northern, and Southern analysis and found to be an Epstein-Barr virus (EBV)-negative myeloma line that exclusively secretes the λ light chain.19 MM.1S, a sensitive clone of MM.1, was isolated using the polyclonal antibody (GIBCO, Grand Island, NY), supplemented with 10% heat inactivated fetal calf serum (GIBCO), glutamine (2 mmol/L), penicillin (100 U/mL), streptomycin (100 µg GIBCO) and fungizone (2.5 µg/mL; Irvine Scientific, Santa Ana, CA) in 5% carbon dioxide at 37°C.

For growth studies, cells were plated at a density of 1 x 10^5 cells/mL with three samples per time point in a 24-well plate (Costar, Cambridge, MA). DEX (Sigma, St Louis, MO) and/or RU 486 (Roussel Uclaf, Romainville, France) were added at the designated concentrations at the onset of each experiment, except to the control cells, which received an ethanol vehicle (< 1%). At each time point, cells were harvested by trypsinization and counted twice on a hemacytometer. The number of dead versus live cells was determined using the trypan blue exclusion assay.

**DNA analysis.** Genomic DNA was isolated by a standard sodium dodecyl sulfate (SDS) and proteinase K protocol. Thirty micrograms of DNA was digested to completion in separate experiments with a panel of enzymes (BamHI, EcoRI, HindIII,
**PstI, BglI, and XbaI** by incubating at 37°C for 3 hours. The samples were loaded in duplicate onto a 0.8% agarose gel, electrophoresed, and transferred onto a nylon filter (Hybond, Amersham, IL) using a 2016 VacuGene vacuum blotting unit (Pharmacia-LKB, Piscataway, NJ). The filter was cut in half, UV cross-linked, and the duplicate filters prehybridized in separate bags in a standard solution at 65°C for at least 3 hours. PCR amplified DNA fragments corresponding to specific domains of the GR cDNA were used to probe the blots in parallel for the presence/absence of specific DNA exon sequences. The probes were labeled with [³²P]dATP using a commercial kit (BRL, Gaithersburg, MD). Unincorporated nucleotide was separated from the labeled probe solution at 65°C for at least 3 hours. PCR amplified DNA containing progressively decreasing quantities of salt with the lowest stringency being a 1X SSC (1X SSC = 0.15 mol/L NaCl + 0.015 mol/L sodium citrate), 1% SDS solution at 65 to 68°C, and autoradiographed at 70°C.

**RNA analysis (Northern and slot blot analyses).** For Northern analysis, 20 μg of a poly A-enriched RNA (Fast Track mRNA isolation kit; Invitrogen, San Diego, CA) was electrophoresed on a 1% agarose/formaldehyde gel, and transferred to a nylon filter under a vacuum. For slot blot analysis, two decreasing concentrations (2.5 and 0.5 μg, respectively) of each sample were denatured and loaded onto a slot blot apparatus (Schleicher and Schuell) for transfer to a nylon filter (Hybond). For both assays, the RNA filters were dried to completion and UV cross-linked. Prehybridization was allowed to proceed for at least 3 hours at 45°C in a standard solution. Hybridization was conducted in freshly prepared buffer containing 1 × 10⁶ cpm/mL of labeled probe for at least 12 hours at 45°C. The filters were washed at 50°C, with a final stringency of 1X SSPE (20X SSPE = 0.15 mol/L NaCl, 2 mol/L sodium phosphate, pH 7.7, and 2 mol/L Na₂EDTA), 0.1% SDS, and autoradiographed by exposing to Kodak XAR film (Eastman Kodak Co, Rochester, NY) at -70°C. All filters were stripped and reprobed with actin to control for loading differences. For quantitative analyses, the filters were densitometrically scanned using an LKB-Ultroscan XL Densitometer (Gaithersburg, MD) and normalized to actin.

**RESULTS**

**Cell growth and sensitivity to DEX.** MM.1 grows well in culture without the addition of any exogenous growth factors other than those found in serum. When the glucocorticoid DEX is added to its media (10⁻¹ to 10⁻⁴ mol/L), MM.1 is killed in a concentration dependent manner (Fig 1). Addition of 10⁻⁴ mol/L DEX has the most profound effect on cell viability, with 85% of the initial cell population killed over a period of 1 week (Fig 1B). DEX-induced cytolysis is effectively blocked by the GR-binding glucocorticoid antagonist, RU 486, indicating that the lytic response is mediated through the GR (Fig 1B). The small population of cells resistant to glucocorticoid-induced cytolysis was subcultured by continuously exposing MM.1 to DEX and growing out the survivors. The resistant line, referred to as MM.1R, maintains a viability of greater than 90% when cultured in the presence of DEX (Fig 2B). Its growth rate appears unaffected by hormone, as it grows with a doubling time similar to that described for the sensitive parent line (Fig 2A). To test the stability of the lysis-resistant phenotype in our cell line, MM.1R was removed from media containing DEX for 6 months and then re-exposed to drug. We found that despite its prolonged growth in nonselective media, MM.1R had retained its resistance to hormone with a growth and viability that was similar to that of MM.1R grown in the presence of DEX (Fig 2). These findings demonstrate that the resistant phenotype is not a transient phenomenon and afforded us the option to perform our subsequent studies either in the presence or absence of hormone.

To compare MM.1R to a hormone-sensitive cell line, a glucocorticoid-sensitive clone of MM.1 was obtained by growing MM.1 in soft agar and testing sensitivity to DEX. To distinguish it from the parent line, MM.1, we refer to the sensitive clone used throughout this study as MM.1S.

**Analysis of GR mRNA.** GR mRNA from resistant and sensitive cell lines was examined on Northern blots. As probes, we used specific PCR amplified sequences of the GR cDNA corresponding to the HVR, the DBD, the HBD, the first 630 bp of the 3' UTR, and a fragment composed of the last 360 bp of the HBD and the first 170 bp of the 3' UTR (Fig 3A). By probing with these specific sequences, we were able to roughly assess which regions of the wild-type (WT) GR mRNA were present or absent from the receptor mRNA expressed by the resistant line.

In Northern blots probed with the HVR (Fig 3B), the full-length 7.1-kb WT GR transcript is present as a strong signal in the positive control HUT-78, which synthesizes the GR at high levels and MM.1S. mRNA isolated from the resistant line following a period of continuous growth in culture is represented in lanes 2 and 3. Lane 3 is representative of the early GR mRNA profile, which is present at the time MM.1R is selected from the parent line and typically persists for the first 4 to 6 months of growth in culture. We refer to the subtype of MM.1R with this pattern of GR mRNA expression as the "early" form (MM.1Re). The GR mRNA profile which follows that of MM.1Re is represented in lane 2. The subtype of MM.1R with the later pattern of GR mRNA expression is referred to as MM.1RL.

The lane containing mRNA from MM.1Re probed with the HVR demonstrates that the early events in resistance include a drastic decrease in the steady-state levels of the 7.1-kb GR message and the appearance of a variant form of the receptor mRNA of approximately 5.5 kb. With time, as exemplified by MM.1RL, the resistant cells express only very low levels of both the 7.1-kb and 5.5-kb transcripts (Fig 3B, lane 2). The level of expression of the 5.5-kb message expressed by MM.1Re was quantitated in a slot blot probe with the HVR. By this method, we have found that the 5.5-kb transcript is initially expressed in MM.1Re at levels comparable to the 7.1-kb transcript in MM.1S (Fig 3C), but steadily decreases with time.

Receptor mRNA from the resistant lines was further analyzed in Northern blots probed with fragments of the
receptor cDNA 3' to the HVR. The probes include the DBD, the HBD, the first 630 bp of the 3' UTR (UTR-1), and a fragment composed of the last 360 bp of the HBD and extending into the first 170 bp of the 3' UTR (HBD-2). A blot was also probed with the first 591 bp of the HVR to determine if the 5.5-kb transcript corresponded to the N-terminally truncated nt' transcript previously described in mouse lymphoma cells. For each of the probes used, with the exception of HBD-2 and UTR-1, the pattern of hybridization observed by Northern analysis was the same as that previously described for the HVR. That is, the probes hybridized well to the 7.1- and 5.5-kb transcripts expressed by MM.1S and MM.1Re, respectively, but did not bind to mRNA from MM.1RL. However, when the last 360 bp of the HBD and the first portion of the 3' UTR were used as probes, a different pattern of hybridization occurred. Although these probes hybridize well to the full-length 7.1-kb transcript, their binding to the 5.5-kb transcript is drastically reduced (Fig 3B). This suggests that at least part of the 1.6-kb deletion in this smaller form of the receptor mRNA includes the 3' end of the HBD and the 5' end of the 3' UTR. We were unable to probe with regions of the 3' UTR farther downstream of UTR-1 because of difficulties amplifying this region of the GR cDNA.

As in the previous Northern blots, probing with HBD-2 and UTR-1 resulted in very faint hybridization in the lane containing mRNA from MM.1RL (Fig 3B). Similarly, in slot blots, only a weak signal is seen for the GR transcript expressed by MM.1RL (data not shown). This confirms our previous hypothesis that transcription of the GR gene is repressed in this cell line. Importantly, the steady decrease in the expression of the 5.5-kb message occurs regularly, regardless of whether DEX is present in or absent from the growth media.

**Analysis of GR protein.** The results of the Northern suggest that resistance to hormone in MM.1R is due initially to the synthesis of a protein defective in hormone binding (MM.1Re) and eventually to a profound decrease in receptor number (MM.1RL). To test this hypothesis, the number of GRs in each cell type that bind hormone was determined in whole cell binding assays using [3H]-DEX. By this method, MM.1S showed specific and saturable
binding of [\textsuperscript{3}H]-DEX. A representative result of one such experiment analyzed by the method of Scatchard is shown in Fig 4A. A summary of several experiments is shown in Table 1. The data demonstrate that MM.1S expresses high levels (\sim 50,000) of a single class of receptors that bind hormone with a high affinity (kd of 2.98 \pm 62 nmol/L). In contrast, the binding activity in both MM.1R and MM.1RL is very low and erratic (Fig 4B). Thus, the decrease in the 7.1-kb GR mRNA in MM.1R following exposure to DEX is paralleled by a decrease in glucocorticoid-binding protein. In addition, the data suggest that if the 5.5-kb transcript expressed by the resistant line codes for a protein, the apparent deletion in the HBD must interfere with ligand binding.

Analysis of the GR gene. Since the two phenotypes of MM.1R could reflect a defect in the GR genomic sequence, the GR gene was examined in both the sensitive and resistant cell lines by Southern analysis. DNA was digested with a panel of enzymes to detect any allelic variation, deletions, or rearrangements. Southern blots containing duplicate samples were probed in parallel with two separate [\textsuperscript{32}P]dATP-labeled fragments generated by PCR from the GR cDNA. The HVR was chosen as the first probe, since it possesses the least amount of homology with the other members of the steroid receptor superfamily and, consequently, would hybridize with high specificity to the GR gene (<15%). The HBD was used as the second probe, because the hormone-binding assays and mRNA data suggest that this region of the receptor may be defective in MM.1R. Representatives of blots using these receptor fragments as probes are shown in Fig 5. For each of the enzymes used, the pattern of hybridization was the same in the resistant and sensitive cell lines as the positive control IM-9. (Note: the additional signal in the lane containing DNA from MM.1Re digested with PstI was determined to be background in a duplicate experiment.) The results indicate that the organization of the GR gene in the sensitive and resistant cell lines is indistinguishable.
DISCUSSION

Although it has been well documented that resistance to glucocorticoids in leukemias and lymphomas may reflect defects in either the functional and/or synthesis pathways of the GR or alternatively the postreceptor pathways, similar data are lacking for multiple myeloma. Here, we have explored the molecular basis of resistance in a human glucocorticoid-resistant myeloma line, MM.1R. MM.1R was derived from a patient with multiple myeloma that had become refractory to a treatment regimen that included intermittent corticosteroids. In culture, MM.1R grows uninhibited in the presence of high levels of DEX (10^{-7} to 10^{-5} mol/L) with a lysis-resistant phenotype that is stable for long periods in the absence of selective conditions. In contrast, the sensitive clone, MM.1S, derived from the same patient, is lysed in the presence of hormone.

We have found that, consistent with the data obtained from leukemia and lymphoma cells, a myeloma cell must express a certain level of WT GR mRNA and protein for it to be responsive to hormone-induced cytolysis. Thus, MM.1S, which expresses high levels of the GR (∼50,000 per cell), is killed when grown in the presence of glucocorticoids, but MM.1R, which expresses low to undetectable levels of a functional GR, is not lysed by hormone. Similarly, the full-length WT 7.1-kb GR mRNA is abundant in MM.1S, but is drastically decreased in both the early and late forms of MM.1R. Furthermore, the ability of the glucocorticoid antagonist, RU 486, to effectively block DEX-induced cell lysis demonstrates the specificity of the lytic response for the GR. Together, these data provide further evidence that in multiple myeloma, glucocorticoids can act directly through the GR to induce cell lysis.12

In place of the 7.1-kb transcript, MM.1Re preferentially synthesizes a 5.5-kb variant form of the GR mRNA. By probing Northern with different fragments of the GR cDNA, we have found that the 5′ end of the variant
transcript is intact, but an approximately 1.6-kb deletion occurs in the 3' end, including a small portion of the HBD. Our data indicate that the 5' end of the deletion occurs within the region of the first 400 bp of the HBD. Assuming the smaller message has retained the proper signals to be translated into protein, one can speculate from the results of the hormone-binding data that it is defective in hormone binding. The presence of a GR protein lacking a significant portion of the HBD is particularly intriguing in light of the previous reports that a GR truncated of the HBD is converted to a constitutively active molecule. Importantly, the studies have also shown that, despite their ability to act independently of hormone, the truncated mutants do not activate transcription as efficiently as WT. In this way, a hormone-independent GR would have enough activity to allow the normal GR-dependent homeostatic processes to proceed within a cell (e.g., gluconeogenesis), but insufficient activity to induce cytolysis.

Mutants truncated of a significant portion of the HBD have been previously implicated in the conversion to steroid hormone independence for other hormone-responsive malignancies such as breast cancer. In particular, a recent report on estrogen receptor negative/progesterone receptor positive (ER−/PgR+) breast tumors has demonstrated

| Table 1. GR Binding Characteristics in MM.1S |
|-----------------|-----------------|-----------------|
| Ro (fmol/10^-6 cells) | Receptors/Cell | kd              |
| 87.5 ± 4.4      | 52,695 ± 2,624  | 2.98 ± .62      |

Data are the mean ± SD of three experiments.
that these tumors arise as a result of the synthesis of a variant form of the ER mRNA that lacks exon 5 of the HBD. The alternatively spliced transcript codes for a protein that does not bind hormone, but is capable of activating transcription. For this reason, ER-responsive genes such as the PgR are transcribed even in the absence of estrogen, accounting for the ER⁻/PgR⁺ phenotype. On the other hand, alternatively spliced transcripts of the thyroid hormone receptor which code for proteins that neither bind ligand nor activate transcription, but inhibit receptor function because of their ability to bind DNA, have also been reported. The identification of similar ligand-independent receptors in glucocorticoid-responsive malignancies would have great implications for both the progression of the disease and the course of treatment. Further analysis of MM.1Re is required to determine whether the protein product of the 5.5-kb transcript is missing enough of the HBD to render it constitutively active.

Ultimately, the expression of both the 5.5-kb and the...
DEX RESISTANCE IN MULTIPLE MYELOMA

7.1-kb transcripts is repressed as MM.1R is maintained for longer periods in culture. The selective pressures resulting in the loss of the 5.5-kb message are not clear from the results of this study. However, our observation that 5.5-kb transcript expression consistently decreases independently of whether or not DEX is present in the media indicates that the event is not dependent on the presence of hormone. This is what one would predict of a GR variant lacking steroid-binding activity.

Although the receptor deficient (r−) phenotype of MM.1RL has been well studied in both mouse and human T-cell leukemias and lymphomas, the mechanism by which it arises is not yet clear.26-29 In this study, as in previous studies on r− variants, we have performed a restriction fragment length polymorphism analysis (RFLP) of the GR gene. Southern blots of DNA from sensitive and resistant cells digested with a series of enzymes were indistinguishable when probed with either the HVR or the HBD. Although the possibility remains that none of the enzymes cut within a small genomic region that is altered in the resistant line, we have interpreted the results of the DNA analysis as evidence that no gross rearrangements, allelic variation or large deletions account for the resistant phenotype of MM.1R.

Our data provide evidence that resistance to glucocorticoids in MM.1 is mediated by either a functional defect in or to decreased synthesis of the GR. However, it is noteworthy that Gomi et al29 report that the glucocorticoid resistance of the myeloma line (OPM-1) occurs via an alternative mechanism. Despite its resistance to DEX-induced cytolysis, OPM-1 was found to express a 95-Kd GR protein that behaved similarly to the GR expressed by the sensitive line (OPM-2) in functional assays. For this reason, the investigators conclude that “postreceptor” defects account for the resistant phenotype of OPM-1.29 In light of the aforementioned reports on myeloma cell lines that do not respond to hormone-induced cytolysis despite the presence of GR, it is likely that postreceptor mechanisms more commonly contribute to the resistant phenotype in multiple myeloma. However, additional patient samples must be examined to answer this question definitively, as well as to determine whether a particular mode of resistance correlates with a specific treatment regimen used in the patient. We are currently mapping the precise boundaries of the deletion in the 5.5-kb transcript. Eventually, we hope to design a probe specific for the transcript to screen multiple patient samples and perhaps to answer some of these questions.

ACKNOWLEDGMENT

This report is dedicated to Michael W. Rytel, MD (1934-1991) in memory of his leadership in medical education, contributions to scientific research, and service as a role model to aspiring medical scientists and clinicians. The authors would especially like to thank Helen Salwen for her technical expertise and assistance. We would also like to thank Laura Cundiff for her help in completing experiments and meeting deadlines; Dr John Cidlowski for his generous advice and assistance; Dr Susan Cohn, Dr Jeff Harmon, Dr Nancy Krett, Dr Sigmund Weitzman, and Dr Preethi Gunaratne for helpful discussions and editing; and Dr Stanley Hollenberg for providing us with the GR cDNA (pRSHGR).

REFERENCES

10. Thompson EB, Harmon JM: Glucocorticoid receptors and glucocorticoid resistance in human leukemia in vivo and in vitro, in


A mechanism of resistance to glucocorticoids in multiple myeloma: transient expression of a truncated glucocorticoid receptor mRNA

PA Moalli, S Pillay, D Weiner, R Leikin and ST Rosen