entire Eco RI site with four extra base pairs at the 5′ end for stability and recutting efficiency. The PCR fragment was cloned in-frame into the Eco RI site of the pGEX-1λT vector (Pharmacia) The rest of the DNA fragments encoding the peptides listed in Table 1 were derived from synthetic oligonucleotides. The oligonucleotides, which were cloned into the Bam HI and Eco RI sites of the pGEX2T vector, contained coding sequences and the sequence 5′-GATCC-3′ at the 5′ end of the sense strand oligos and the sequence 5′-AATT-3′ at the 5′ end of the antisense strand oligos. The 5′ end of the DNA fragments derived from these oligos were in frame with the GST of pGEX2T and the 3′ ends were out of frame, resulting in amino acid residues NSS at the COOH-terminus of the GST-peptide fusion proteins. The oligonucleotides that were cloned into the Eco RI site of the pGEX1λT vector contained coding sequences and the sequence 5′-AATTC-3′ at the 5′ end of both sense and antisense strand oligos. The 5′ end of these oligos were in frame with the GST of pGEX1λT vector The synthetic oligos were phosphorylated with T4 polynucleotide kinase under conditions recommended by the manufacturer (New England Biolabs). The sense and antisense oligos were phosphorylated and annealed by boiling for 2 min and then cooling slowly to room temperature The DNA fragments were cloned into pGEX vectors The recombinant DNA was transformed into either *Escherichia coli* DH5α or *E. coli* NB42 as described (*26*)

6. Filter binding assay. Transformants were grown into log phase and induced by 0.1 mM isopropyl-β-D-thiogalactopyranosid galactoside (IPTG) for 1 hour at 37°C Cells were lysed on ice in Triton lysis buffer (phosphate-buffered saline, 100 mM EDTA, 1% Triton X-100, 1% aprotinin, and 1 mM phenylmethylsulfonyl fluoride) by sonication. The cell extract was boiled in Laemmli sample buffer, and proteins were separated by SDS-PAGE (12 5% gel) and transferred to nitrocellulose in 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) (pH 11), 20% (v/v) methanol Filters were blocked in 50 mM tris (pH 8.0), 150 mM NaCl, 0.1% Tween 20 (TBST buffer) containing 0.2% gelatin or 2% nonfat dry milk. Biotinylated probes were added in the same buffer at 0.2 to 1 μg/ml, incubated at 4°C for 2 hours, and washed extensively in TBST. Filters were incubated with streptavidin-conjugated alkaline phosphatase (Boehringer Mannheim Biochemicals) at a dilution of 1:5000 in TBST-gelatin buffer at 4°C for 1 hour, washed, and developed with Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega Biotec), as described by the manufacturer.
7. The Abl SH3 probe contains amino acids 84 through 138 of murine type IV c-Abl (*27*) fused in-frame at the Bam HI site of pGEX-2T. Biotinylation was done as described (*28*).
8. For immunoblotting with affinity-purified polyclonal antibody to GST (0.5 μg/ml), filters were treated as described at room temperature (*6*). The bound antibody was detected with goat antibody to rabbit immunoglobulin conjugated to alkaline phosphatase (Promega Biotec).
9. Sequencing of the constructs to confirm their identity was done with Sequenase according to the manufacturer's directions (U.S. Biochemicals).
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11. The Src and N-Src SH3 probes were prepared as described (*4*). The GST-GRB2 and GST-Nck fusion proteins were from J Schlessinger Biotinylation of these proteins was done as described (*28*). GST and GST–3BP2-40 were purified as described (*28*).
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Mineralocorticoid and Glucocorticoid Receptor Activities Distinguished by Nonreceptor Factors at a Composite Response Element

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Mineralocorticoid and glucocorticoid hormones elicit distinct physiologic responses, yet the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) bind to and activate transcription similarly from a consensus simple hormone response element (HRE). The activities of GR and MR at plfG, a 25–base pair composite response element to which both the steroid receptors and transcription factor AP1 can bind, are analyzed here. Under conditions in which GR represses AP1-stimulated transcription from plfG, MR was inactive. With the use of MR-GR chimeras, a segment of the NH_2-terminal region of GR (amino acids 105 to 440) was shown to be required for this repression. Thus, the distinct physiologic effects mediated by MR and GR may be determined by differential interactions of nonreceptor factors with specific receptor domains at composite response elements.

Mineralocorticoids and glucocorticoids, two classes of adrenal steroid hormones, differ greatly in their physiologic effects. They can elicit opposing effects on ion transport within a single tissue (*1*), a single cell type (*2*), or within an individual cell (*3*). However, molecular biological studies of mineralocorticoid and glucocorticoid action suggest a paradox: The varied effects of these hormones are mediated by receptor proteins that are closely related (*4*) and that bind to and enhance transcription from a common consensus DNA sequence, designated originally as a glucocorticoid response element (GRE) (*5*). Receptors for two other classes of steroid hormones, progestins and androgens, also recognize and function from this element (*6*). Because receptor binding alone is sufficient to confer hormone-mediated transcriptional enhancement from promoters linked to the GRE, this consensus sequence has been denoted a simple hormone response element (HRE) (*7, 8*). Thus, the specificity and complexity of hormone effects must be conferred by other means such as differential actions of receptors that are contingent upon communication between receptor and nonreceptor factors (*9–11*).

Interactions of steroid receptors with other transcription factors may explain responses from at least three types of regulatory regions (*9–16*). (i) When binding sites for various regulatory factors are placed in proximity to simple steroid response elements, a mutual increase in the stimulatory activity of both factors is commonly observed (*16*), suggesting that they may interact with each other or with a common target. (ii) Transcriptional activation mediated by sequence-specific binding of AP1 transcription factors can be repressed by the glucocorticoid recep-

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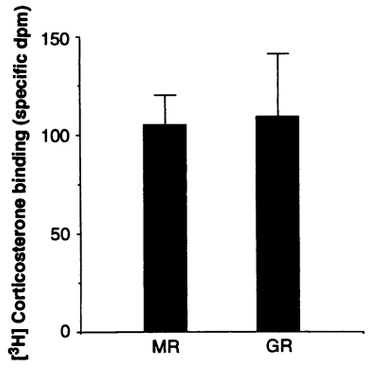

Fig. 1. MR and GR are expressed at comparable levels in COS-7 cells. COS-7 cells, growing on 10-cm dishes in Dulbecco's modified Eagle's medium (DMEM H16) with 5% charcoal-treated fetal calf serum [100 ml of serum was mixed with 2 g of acid-washed charcoal for 90 min at 4°C as previously described (*34*)], were transfected with 8 μg of mineralocorticoid (6RMR) or glucocorticoid (6RGR) receptor expression vectors (*35*) by the calcium phosphate precipitation method. After 24 hours, cells were harvested, extracts prepared, and the receptors labeled with [^{3}H]hormones (*35*). Shown is the average of four experiments (±SEM).

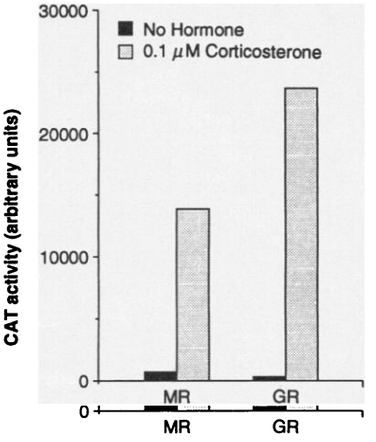

Fig. 2. MR and GR both confer hormone-dependent activation from a simple GRE. Subconfluent cultures of CV-1 cells were cotransfected as in Fig. 1 with either 0.5 μg of expression vector for the rat mineralocorticoid receptor (VA/rMR) or rat glucocorticoid receptor (VARO), 2 μg of a simple GRE-containing reporter gene (GTCO), and pUC carrier DNA to a total of 5 μg. Cells were treated as in Fig. 1. Where indicated medium contained 0.1 μM corticosterone (Sigma). Twenty-four hours after addition of hormone, cells were harvested and whole cell extracts were prepared by freeze-thaw in dry ice–ethanol (four cycles of −70°C, +37°C) followed by centrifugation for 15 min at 150,000*g*. Nonchromatographic CAT assays (*36*) were performed with the use of heat-treated extracts (68°C for 5 min) and normalized to protein. Extracts (20 μg of protein) were incubated with unlabeled chloramphenicol (Sigma; 1.6 mM final concentration) and [^{14}C]acetyl-CoA (New England Nuclear; 125,000 to 150,000 cpm per assay) in a total volume of 50 μl for 1 hour at 37°C. Reactions were extracted with ethyl acetate (Aldrich) and counted. Shown is a representative experiment; average fold-activations in 11 experiments for MR and nine experiments for GR were: MR, 24 ± 2.1; GR, 34 ± 3.9 (mean ± SEM). MR and GR basals differed by <10% on average. Corticosterone (or aldosterone or dexamethasone) stimulated reporter activity <1.5-fold in the absence of cotransfected receptor expression vector.

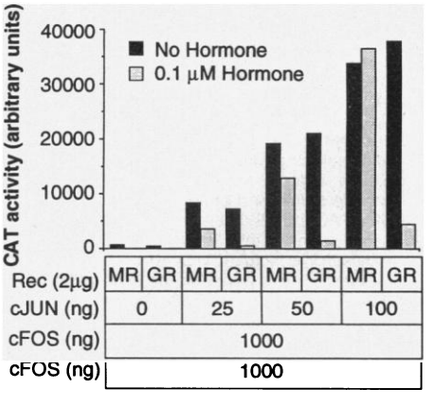

Fig. 3. Effect of varying cFos:cJun ratios on regulation from plfG3 by MR and GR. F9 embryonal carcinoma cells were transfected as in Figs. 1 and 2 with 2 μg of 6RGR or 6RMR receptor expression vector, 3 μg of plf-G3 reporter, 1 μg of cFos, and varying amounts of cJun expression vectors as shown; 125 ng of 6RZ was included as an internal transfection control. After incubation with the calcium phosphate precipitate for 16 hours, cells were glycerol-shocked (15% glycerol in DMEM H16 for 1 min), then washed twice with phosphate-buffered saline before addition of hormone-containing medium. Cells were incubated with hormone for 24 hours; extracts were prepared and CAT activities measured as described (Fig. 2). Shown is a representative experiment; a minimum of three experiments was performed at each cJun concentration, with less than twofold variation in the amount of cJun required for half-maximal reduction of MR repression. Average repression by MR at 0.25 μg of cJun was 0% (±6.8%) (Fig. 4B). Rec, receptor.

tor and other nuclear receptor family members (*12, 13, 15, 17, 18*). (iii) Composite GREs have been identified that contain binding sites both for the receptor and for nonreceptor factors that are essential for receptor activity (*9, 10*). At a 25-bp composite GRE, plfG (*9, 19*), the interaction of GR with AP1 is essential for hormonal regulation, and the subunit composition of the AP1, homodimers of cJun or heterodimers of cJun and cFos, specifies enhancement or repression, respectively, by GR (*9*). Thus, at the composite element, receptor activities are determined, not merely accentuated, by nonreceptor factors.

We first compared GR and MR activity at the composite element plfG to ascertain whether conditions could be defined in which the two receptors displayed distinct behaviors. Expression vectors for the MR or GR receptors were transfected into cultured cell lines (simian CV-1 and COS7, human HeLa, and murine F9) that express little or no endogenous functional MR or GR (*20*). To assess directly the amount of MR and GR expression, we quantitated hormone binding at saturating levels of [^{3}H]corticosterone, which is an agonist for both receptors. Hormone binding activity provides a measure of steady state amounts of receptor (the important parameter for our experiments) without concern for differential plasmid levels, transcription rates, or mRNA or protein stabilities. The hormone binding activities in extracts from cells transfected with MR and GR were indistinguishable under these conditions (Fig. 1). Thus, differences observed between the effects of the two receptors likely reflect intrinsic functional differences, not merely differential expression.

We confirmed that like the human MR, the rat MR could enhance transcription from a simple HRE (*21*). In cotransfections of simian CV-1 cells with rat MR or GR expression vectors together with reporter plasmid GTCO [containing a synthetic GRE derived from the mouse mammary tumor virus long terminal repeat (MMTV LTR) fused to the thymidine kinase (TK) promoter and chloramphenicol acetyltransferase (CAT) sequences (*22*)], each receptor strongly stimulated CAT expression upon addition of 100 nM corticosterone (Fig. 2). GR displayed slightly greater activity on average. Similar results were obtained with reporter constructs containing various simple HRE-promoter combinations (the intact MMTV LTR, the GRE from the tyrosine aminotransferase gene, or GRE fusions to the β-globin promoter), and in cotransfections of GTCO into other cell types (COS7, F9, HeLa, or AtT20). Thus, the similar behavior of MR and GR on simple HREs appears to be independent of context effects caused by specific reporter constructs or cell types.

The functional ratio of two AP1 family subunits, cJun and cFos, is a determinant of GR function at plfG (*9*). GR enhances transcription from plfG when AP1 is composed of cJun homodimers, it represses transcription when AP1 is composed of cJun-cFos heterodimers, and it lacks activity in the absence of AP1 or at intermediate ratios of cJun and cFos (*9*). We compared the activities of MR and GR on plfG3 CAT, a reporter construct containing three tandem plfG composite elements fused to a minimal promoter and CAT sequences (*9*). We used F9 cells, which lack endogenous AP1 activ-

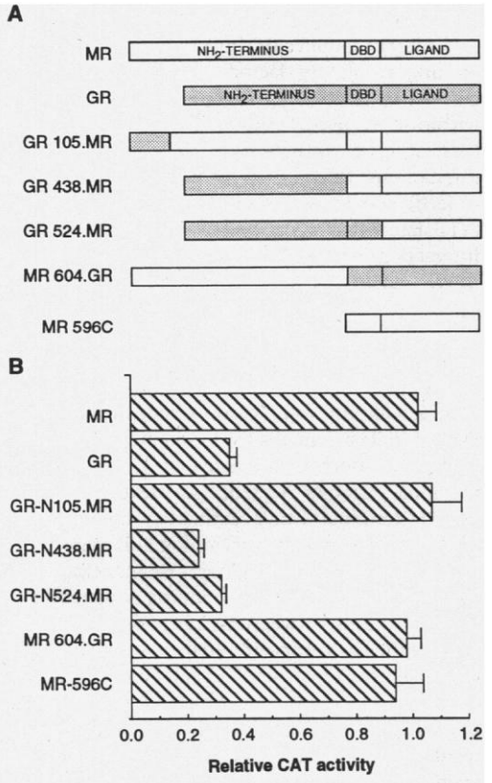

Fig. 4. Mapping a domain of GR that selectively confers repression of cJun-cFos action at plfG. (**A**) MR-GR chimeras. Upstream GR sequences were fused at homologous sites to downstream MR sequences. All chimeras except GR105.MR and MR105.GR were constructed by polymerase chain reaction (PCR) to introduce restriction sites at homologous points in the two receptors without changing the amino acid sequence, as described (*36*). Oligonucleotides to be used as PCR primers contained sequences complementary to the target site and a 5′ tail corresponding to the desired restriction site. A return primer was directed at a region such that amplification yielded a convenient fragment for cloning. Chimeras were made with the use of the PCR fragment to bridge between GR and MR. The exceptions, GR105.MR and MR105.GR, were made with an Nco I site at amino acid 105 of both cDNAs. All regions of the chimeras made with PCR were sequenced (UCSF Biomolecular Resource Center Sequencing Facility) and found to be free of mutations. Chimeras are named according to the amino acid residue at which the upstream portion ends; for example, GR 438.MR has GR amino acids 1 to 438 fused (at a homologous site) to the MR zinc finger and signaling domains. (**B**) Chimeric receptors identify domains of GR necessary for repression. F9 cells were transfected as above with 2 μg of either expression vectors for MR, GR, or GR.MR chimeras as shown together with 2 to 3 μg of plfG3 CAT, 0.25 μg of cJun expression vector, an excess of cFos expression vector, and 0.125 μg of 6RZ. Hormone treatment, preparation of extracts, and determination of CAT activities were as described (Fig. 2). Data shown are CAT activities in the presence of 1 μM corticosterone and are expressed relative to CAT activity in the absence of hormone, which is assigned a value of 1.0. Wild-type and chimeric receptors were all tested on a simple GRE-containing reporter (GTCO, Fig. 2 and text) and found to have comparable activity (<twofold variation).

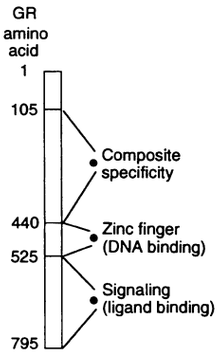

Fig. 5. A novel functional domain of GR. Diagram depicts the composite specificity domain and other key domains of the receptor. Composite specificity refers to the function of this domain in distinguishing GR behavior from MR at plfG. This domain maps to a region of the receptor that also includes a potent enhancement domain; whether there is functional overlap between the composite specificity domain and this enhancement domain is not yet known.

ity (*23*), to assess MR action on plfG3 CAT under conditions in which GR represses transcription (that is, a high ratio of cotransfected cFos:cJun expression vectors). Both MR and GR repressed transcription from plfG3 CAT at high ratios (>20) of cotransfected cFos: cJun (Fig. 3). However, as relative cJun expression increased (cFos: cJun ratio of 10 or 4; Figs. 3 and 4B), MR failed to repress, whereas GR-mediated repression was maintained. Enhancement of transcription by MR from a simple HRE was unaffected by transfection of cFos and cJun into F9 cells.

Two aspects of these results merit consideration: (i) Transfected cFos alone stimulated CAT expression by 1.5- to 2-fold above the basal expression from the plfG3 CAT reporter, although cFos alone does not dimerize and has no AP1 activity (*24*). This suggests that F9 cells express a Jun family member (*23*, *25*) with low intrinsic activity that is stimulated by cFos, probably by way of heterodimer formation. (ii) MR progressively loses regulatory activity with increasing amounts of cotransfected cJun. This implies that MR binds to plfG and selectively interacts with and represses the putative heterodimers of the Jun-like factor and cFos, but not the bona fide cJun-cFos heterodimers. Thus, MR and GR activity can be clearly distinguished at plfG. To examine this difference we chose conditions (cFos: cJun ratio of 10:1) for further experiments in which GR represses strongly and MR is inactive.

The differential behavior of MR and GR at plfG could reflect distinct DNA binding specificities, AP1 interactions, or interactions with other factors. If GR-specific behavior were determined solely by DNA binding specificity (uninfluenced by interaction with other factors), the zinc finger region of GR, but not that of MR, might determine repression at plfG. However, if differential interactions between one of the receptors and another factor (AP1 or other) were essential for distinct behaviors of MR and GR, the zinc finger region of the molecules might be interchangeable whereas another region might confer repression. To examine these possibilities, we constructed a series of GR-MR chimeras (Fig. 4A) and introduced them into F9 cells under conditions in which GR, but not MR, represses plfG3 CAT.

Chimeras containing GR information upstream of the DNA binding domain (between amino acids 105 and 438) were competent for repression at plfG (compare GR 105.MR with GR 438.MR) (Fig. 4B). The GR NH_2-terminal region conferred repression activity upon chimeras bearing either the MR or GR DNA binding domains. In contrast, point mutations in the MR DNA binding domain that make this domain virtually identical to that of GR (*26*) were not sufficient to convert MR into a repressor (*20*). Furthermore, NH_2-terminal deletion mutants of MR also failed to repress under these conditions, indicating that the MR NH_2-terminus does not inhibit a cryptic MR repressing function residing elsewhere on the molecule (Fig. 4B). Finally, fusions of the MR NH_2-terminus to the GR DNA and ligand-binding domains (MR 604.GR) did not repress, demonstrating that GR does not contain additional repression domains outside of the NH_2-terminal region. We conclude from these results that the NH_2-terminal domain functionally distinguishes GR from MR with respect to repression of cJun-cFos activity at plfG.

At least four mechanisms may explain the specificity of hormone action: (i) A given target cell might express only one of the closely related receptors; (ii) a given target cell might metabolically inactivate one of the hormonal ligands; (iii) sequences distinct from the common DNA element might exist and be differentially bound by the receptors; or (iv) the receptors might interact differentially with other factors. In support of (i), Strahle *et al.* (*27*) demonstrated that an endogenous glucocorticoid-responsive gene as well as a transfected reporter could be rendered responsive to progestin by cotransfected PR in hepatoma cells. Thus, certain glucocorticoid-regulated liver genes may be unresponsive to progestins solely because liver cells do not express the

appropriate receptor. Selective ligand metabolism (ii) has been invoked to explain the mineralocorticoid specificity of ion transport regulation in the kidney collecting tubule, which expresses both GR and MR. In tubule cells, glucocorticoids but not mineralocorticoids are converted to inactive metabolites by 11-β-hydroxysteroid dehydrogenase (11-HSD) (*28, 29*). Model (iii) has not yet received experimental support. Although possible, this scheme seems unlikely to provide complete discrimination in view of the near-identity of MR and GR zinc finger regions (*26*), as well as their functional interchangeability at both simple and composite GREs. Moreover, each of these first three models contains regulatory constraints: The first two preclude regulation by the excluded hormone or receptor at any locus in the cell, whereas the third seems to complicate differential regulatory effects by the overlapping hormones on a given gene. In contrast, model (iv) is free of those constraints.

Whereas MR and GR activate transcription similarly at simple GREs, their behavior at a composite response element can be distinct. MR is incapable of repressing cJun-cFos–stimulated transcription from a plfG-linked gene. A simple interpretation is that in the presence of AP1, a segment of GR between amino acids 105 and 438 interacts with a cellular factor, perhaps AP1 itself, and this interaction results in repression (or more precisely, anti-enhancement) of AP1-stimulated activity from the composite element. We call the region of the receptor that specifies distinct receptor behavior at a composite response element, a composite specificity domain (Fig. 5).

The mechanism of composite specificity domain function and the nature or limits of its specificity are unknown. Because GR can associate with cJun (*9, 13, 15*), it is conceivable that the composite specificity domain directly contacts AP1. This interaction might stabilize the ternary complex and thereby favor GR over MR occupancy at plfG (a putative MR-AP1 interaction has not been tested), and it may abolish the activation potential of AP1. We speculate that MR also contains a composite specificity domain, perhaps in the same region as that for GR, but that its activity will be manifested only in a different context of factors and composite elements. The composite specificity domain maps outside of the zinc finger region (Fig. 5), implying that although this region is essential for composite regulation (9), it does not distinguish the two receptors. The composite specificity domain resides within a single large exon that encodes the entire portion of GR upstream of the zinc finger region (*30*). This segment of the nuclear receptor family is highly divergent in size and sequence; even the most closely related receptors, such as MR and GR, display significant differences in this region (*31*).

Different families or subfamilies of DNA binding regulatory factors may be distinguished by their DNA binding specificities; within a given subfamily, individual members may be distinguished by differential interactions with other regulatory factors. Indeed, the activities of the AP1-CREB-ATF family members are distinguished by interactions with members of their own family as well as interactions with members of other transcription factor families, such as the nuclear receptor family. A notable parallel is found between the MR and GR and the octamer binding factors Oct-1 and Oct-2, which also rely on regions outside of their DNA binding domains for functional specificity (*32*). Both Oct-1 and Oct-2 bind to the same octamer sequence; however, the COOH-terminal Oct-2 activation domain (but neither Oct-1 activation domain) stimulates octamer-linked β-globin transcription when fused to either DNA binding domain.

Interactions between members of different families at composite elements expand the regulatory repertoire for each family and provide a mechanism by which closely related family members can confer distinct regulatory effects. Composite elements have been identified in other glucocorticoid responsive genes such as the alpha-fetoprotein gene and the gene encoding phosphoenol pyruvate carboxykinase (PEPCK) (*10*). In addition, interactions between AP1 family members and other members of the steroid receptor superfamily have been described (*9, 10, 12–15, 33*). We predict that the identification and activity of other composite specificity domains will depend on both the cellular context and the nature of the composite element. Our present findings establish that differential factor interactions can distinguish receptor activities that are not discernible in the context of simple elements, thus providing a mechanism for hormone-specific modulation of distinct gene networks.

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26. Within the core of the DNA binding region of the receptors (amino acids 440 to 500 of GR and 604 to 664 of MR), the two receptors differ at four residues, MR G608 = GR S444, MR V619 = GR L455, MR L661 = GR Y497, MR Q662 = GR R498. None of these residues is involved in making either specific or nonspecific DNA contacts, as shown by x-ray crystallography [B. F Luisi, W. X Xu, Z Otwinowski, L. P Freedman, K. R Yamamoto, P B Sigler, *Nature* **352**, 497 (1991)]. One of the differences is a conservative substitution (MR V619 = GR L455). Mutations in MR, L661Y and Q662R, which convert residues 661 and 662 of MR to their GR counterparts (residues 497 and 498) did not restore repression activity Abbreviations for the amino acid residues are G, Gly, S, Ser; V, Val, L, Leu, Y, Tyr, Q, Gln; and R, Arg
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29. The physiologically significant glucocorticoids, corticosterone (in the rat) and cortisol (in primates), are potent agonists for MR in cell culture and bind MR in vitro with an affinity equal to that of the principal mineralocorticoid, aldosterone Glucocorticoids do not elicit mineralocorticoid effects in the intact mammalian kidney because they are inactivated in the renal collecting tubule by 11-HSD (*28*). This nicotinamide adenine dinucleotide phosphate (NADP)–dependent enzyme catalyzes the oxidation of glucocorticoids to their inactive 11-oxo forms. Mineralocorticoids, although they contain an 11-hydroxyl group, are nevertheless protected from the actions of 11-HSD; the presence of a reactive aldehyde at C 18 results in cyclization of mineralocorticoids to the hemiketal form leaving the 11-hydroxyl inaccessible to the enzyme 11-HSD activity has not been identified in hippocampus, a tissue rich in MR, and it appears that glucocorticoids occupy and activate MR in hippocampal neurons
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35. The 6R plasmids are SP65-based vectors which have the Rous sarcoma virus (RSV) promoter fused to receptor cDNA sequences followed by the SV40 polyadenylation signal. To monitor transfection efficiency, 0.375-μg 6RZ (β-galactosidase in a 6R background) was included in each transfection. After 16 hours at 37°C, the cells were washed and transferred to fresh medium for an additional 24 hours. Cells were harvested in phosphate-buffered saline, resuspended in receptor buffer at 4°C with protease inhibitors (1 μg/ml each of pepstatin A, aprotinin, and leupeptin) and lysed by sonication (5 s at setting 4 with the use of a microprobe of a Branson Sonifier 350). Sonicate was transferred to a microcentrifuge tube and spun for 10 min at 14,000 rpm, 5-μl aliquots were removed for protein and β-galactosidase assay. Remaining supernatant was split into three tubes. Two were made 0.2 μM [^{3}H]corticosterone (New England Nuclear) The third tube was incubated with 0.2 μM [^{3}H]corticosterone plus 330-fold excess cold corticosterone (Sigma) to determine nonspecific binding. After 16 hours at 0°C, the reaction was stopped by addition of acid-washed charcoal and counted on GF/C filters (Whatman). Nonspecific counts were subtracted from the average of the two specifically labeled groups, and counts were then expressed as specific disintegrations per minute per microgram of protein. Transfection efficiency was comparable (<50% variation) as determined by β-galactosidase assay
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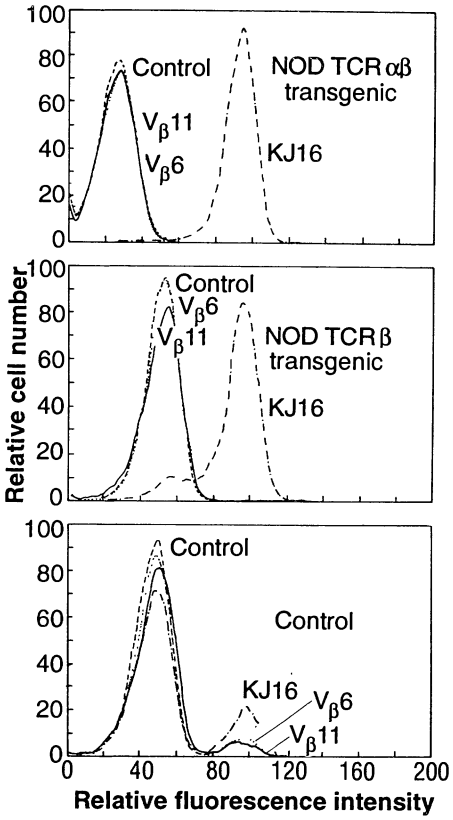

Fig. 1. Phenotype of peripheral T lymphocytes of transgenic αβ (upper), β (middle), and control (nontransgenic littermate) (lower) NOD mice by FACS analysis. Cell suspensions from pooled mesenteric, axillary, and inguinal lymph nodes were prepared by gentle teasing through a wire screen and T cells enriched by passage over an anti–immunoglobulin M immunoaffinity column (Biotex). T cells were stained as described (*3*) and analyzed on an Ortho Diagnostics Inc. Cytofluorograf IIs analyzer, gating for size by forward light scatter. The following MAb and antisera were used for staining: KJ16 [(*6*), reactive to $V_β$8.1 and $V_β$8.2], RR-4.7 [anti-$V_β$6; (*8*)], RR3.15 [anti-$V_β$11; (*9*)]; FITC-conjugated goat antibody to rat immunoglobulins (KPL) was used as a secondary antibody. Equivalent numbers of cells were counted for the control and the transgenic mice. Flow cytometry on other transgenic β and αβ NOD mice confirmed the data shown.

Progression to Diabetes in Nonobese Diabetic (NOD) Mice with Transgenic T Cell Receptors

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The T cell receptor (TCR) requirements in the pathogenesis of insulin-dependent diabetes were examined with transgenic NOD mice bearing nondisease-related TCR α and β chains. In both TCRβ and TCRαβ transgenic NOD mice the β chain transgene was expressed by >98% of peripheral T cells. The α chain transgene was also highly expressed. Insulitis developed in both sets of transgenic animals with most of the lymphocytes in the lesion expressing the transgenic β chain and with depletion of the endogenous TCR $V_β$ genes. Nonetheless, NOD animals transgenic for TCRβ and TCRαβ developed diabetes similar to controls. Thus, skewing the TCR repertoire did not diminish autoimmune susceptibility in NOD mice.

The NOD mouse develops spontaneous insulin-dependent diabetes mellitus (IDDM), characterized by the invasion of lymphocytes into pancreatic islets (insulitis) and the selective destruction of the insulin-producing pancreatic beta cells, similar to human type I diabetes. IDDM in both NOD mice and humans is probably T cell–mediated (*1*), but the autoantigens that trigger the autoimmune response are unknown. One approach to examining the role of T cells in IDDM and the antigens that drive them has been the derivation of islet-specific T cell clones. Characterization of the TCR gene usage from these clones has shown that T cells with receptors of diverse sequences can transfer insulitis and diabetes (*2*). Nonetheless, the issue of preferential TCR gene usage in islet targeting remains unsettled.

To investigate the importance of the TCR repertoire in IDDM we created transgenic NOD mice. These mice predominantly expressed single TCRβ or TCRαβ sequences that were not related to diabetes; their endogenous repertoire was suppressed by allelic exclusion. We reasoned that if the pathogenic T cells in diabetes were focused on a very limited set of peptide epitopes, autoantigen recognition would be dependent on a limited number of specific TCR and depleting these sequences would pro-

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