from A. Poley, contains an 8.4-kilobase (kb) Hind III–Eco RI fragment of HSV-1 (strain KOS) DNA including the entire coding sequence of the gD gene. Excess DNA beyond the distal end of the gD gene was removed by cleaving the plasmid with restriction endonucleases Sst I and Eco RI, removing the single-stranded ends with T4 DNA polymerase, and recircularizing the plasmid with T4 ligase. The new plasmid, containing a 2.5-kb HSV insert, was designated pMM27. Conveniently, ligation of the two blunt ends regenerated an Eco RI site. The Hind III–Eco RI segment of pMM27 was excised, the staggered ends were filled in with Klenow fragment of DNA polymerase, and then blunt end–ligated into the single Sma I site just downstream from the $P_{7.5}$ vaccinia virus promoter in the plasmid pGS20 (*16*). A resulting plasmid, containing the $P_{7.5}$ promoter and HSV coding segment in the correct orientation, was called pMM28. The HSV gene under control of the vaccinia virus promoter was then inserted into the TK locus of the vaccinia virus genome by homologous recombination as described (*15, 16*) and TK^- recombinants were selected and plaque purified. The predicted structure of the recombinant DNA was confirmed by restriction endonuclease digestion and hybridization to appropriate HSV DNA probes.

14. Construction of vgD52: The staggered ends of the 2.5-kb Hind III–Eco RI segment of pMM27 were filled in with the Klenow fragment of DNA polymerase, and the resulting DNA was blunt end–ligated into the single Hinc II site just downstream from the P_{28} vaccinia virus promoter in plasmid pLTP1 (*19*). A resulting plasmid having the P_{28} promoter and HSV coding segment in correct orientation was called pMM52. Homologous recombination was used to insert the gene into the TK locus of vaccinia virus.
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Identification of Human Glucocorticoid Receptor Complementary DNA Clones by Epitope Selection

Abstract. *Steroid hormones regulate cellular differentiation and physiologic functions predominantly through gene transcription. Regulation is achieved by the interaction of specific steroid receptor proteins and target genes. Expression cloning techniques were used to select human glucocorticoid receptor complementary DNA clones in order to define the mechanism by which the receptor exerts its transcriptional control. Immobilized fusion proteins from individual clones were used to select epitope-specific antibody which was subsequently eluted and identified by binding to protein blots of cellular extracts. Three cross-hybridizing clones containing inserts expressing antigenic determinants of the human glucocorticoid receptor were isolated.*

The regulation of eukaryotic gene expression in response to intercellular signals such as hormones represents a critical strategy for development and homeostatic regulation. Such regulation is modulated by compounds that bind intracellular receptors and those that interact with plasma membrane receptors. Steroid hormones regulate transcription as a consequence of binding specific intracellular receptors (*1*). The interaction between the steroid hormone and receptor initiates a transformation of the complex, after which it is capable of binding high-affinity receptor sites on chromatin and regulating transcription of a limited number of genes (*2*). The rates of transcription of mouse mammary tumor virus (MMTV), mouse metallothionein, and rat growth hormone genes are stimulated by one class of steroid hormones, the glucocorticoids, in cultured cell lines (*3*). Purified rat liver glucocorticoid receptor complexes bind a specific region of cloned MMTV DNA in vitro, suggesting that steroid receptors modulate transcription by binding specific regulatory sequences near promoters (*4*). Furthermore, deletion analysis of the human metallothionein II gene and of the MMTV promoter has defined regions that identify upstream control elements necessary for steroid response (*5*).

One model suggests the existence of distinct steroid-binding and DNA-binding domains in the receptor polypeptide as well as a major immunogenic region (*6*). Although sufficient protein for a direct structural analysis has not been available, polyclonal and monoclonal antibodies have been prepared against partially purified glucocorticoid receptor (*7, 8*). The receptor has been characterized as a 94-kilodalton (kD) polypeptide by biochemical and immunological criteria (*7–9*) as well as by covalent binding studies with labeled steroid analogs (*10*). In the absence of amino acid sequence information, we have attempted to isolate human glucocorticoid receptor complementary DNA (cDNA) clones with a polyclonal antiserum that is reactive with several proteins in addition to the receptor. An epitope selection technique has been used for the identification of these clones.

Rabbit polyclonal antiserum recognizing glucocorticoid receptor (GR 884) (*8*) was initially characterized for its ability to recognize denatured receptor epitopes by immunoblot analysis. Cytoplasmic extracts from human cells producing glucocorticoid receptor were fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) (*11*) and transferred to nitrocellulose filter paper (*12*). This filter was then incubated with GR 884 and specific binding was revealed by subsequent exposure of the filter to ^{125}I-labeled *Staphylococcus* protein A. This antibody identified a number of immunoreactive proteins (Fig. 1). If the antiserum was affinity purified with rat liver glucocorticoid receptor (*13*), only immunoreactivity against the 94- and 79-kD proteins was detected (Fig. 1, lane 3). This agrees with data from human and other mammalian species in which a 94-kD protein frequently copurified with a 79-kD putative cleavage product (*8, 9*).

Receptor from steroid-treated cells has a high affinity for chromatin and remains tightly associated with the nucleus, while receptor from untreated cells has a low affinity for chromatin and thus is found in the cytoplasm during the isolation procedure (*14*). There was a dramatic reduction in the levels of the 94- and 79-kD molecules in cytoplasmic extracts from cells after steroid treatment (Fig. 1, lanes 1 and 2). The relative levels of other nonreceptor proteins, however, were not affected. These data indicate that the antisera recognized an epitope of the glucocorticoid receptor.

To efficiently screen a large number of clones, a λgt11 complementary DNA (cDNA) library was prepared with size-fractionated polyadenylated [poly(A)$^+$] mRNA from the human IM-9 B-cell line (*8*). These cells contain 10^5 receptor molecules per cell, which is approximately tenfold more glucocorticoid receptors than overproducing tissues such as normal liver and lymphoid cells. The procedure used was based upon that of Young and Davis (*15*) and gave a library of approximately 2×10^5 independent members, 75 percent of which contained inserts. Approximately 7.5×10^5 recombinant phage were screened with GR 884

polyclonal antiserum. The initial screening gave 18 positive signals and the corresponding plaques were picked and subjected to secondary screening. Four of these plaques remained positive and were characterized by a clonal epitope selection technique.

Bacteriophages from each immunopositive isolate (2000 plaque-forming units) were separately used to infect *Escherichia coli* (Y1090) at 42°C for 5 hours on petri dishes and induced for the expression of fusion protein. The expressed bacterial proteins were adsorbed in situ to nitrocellulose filters and incubated for 1 hour with the polyclonal antiserum. Each of these isolates presumably expressed one or a small set of antigenic determinants that was recognized by the polyclonal antiserum. Affinity-purified antibodies immobilized on each filter were eluted and used to detect their cognate IM-9 proteins by Western blot analysis (*16, 17*). Three of the four clones selectively bound antibody specific for 94- and 79-kD proteins expressed in human lymphoid cells (Fig. 2A). Antibody purified from one clone (GR80) specifically recognized a protein doublet complex of approximately 38 kD. The fusion protein from the HGR2.9C lysogen was partially purified and covalently linked to an activated agarose resin to permit large-scale purification of epitope-specific antibody (*18*). Antibody retained on this affinity column was eluted and assayed for its ability to bind receptor. This antibody bound only the 94- and 79-kD proteins in extracts from human lymphoid cells (Fig. 2B).

If the transformed receptor becomes a DNA-binding protein with high affinity for chromatin, cytoplasmic receptor levels should decrease and nuclear levels should increase in response to steroid administration. In accord with this model, treatment of lymphoid cells with glucocorticoid resulted in a decrease of the 94- and 79-kD proteins in the cytoplasm (Fig. 2B, lanes 2 and 3). Combined with the appearance of receptor in nuclear extracts following a 2-hour steroid treatment (Fig. 2B, lanes 4 and 5), these results provide evidence that the fusion protein encodes a specific epitope representing the physiological glucocorticoid receptor.

The Eco RI insert from clone pHGR1.2A was labeled with ^{32}P by nick translation (*19*) and hybridized to the other immunopositive inserts. As shown in Fig. 3, the three immunopositive cDNA clones, pHGR1.2A, pHGR2.9C, and pHGR5.16A cross-hybridized, indicating that they all share a common nucleic acid sequence. In conjunction with the immunological characterization, these results suggest that this region encodes a common antigenic determinant. It has previously been reported that the glucocorticoid receptor contains a major immunogenic region and that many antibody preparations appeared to react with what may be a single antigenic site (*7*).

The procedure described for characterizing expression clones in the absence of a completely specific antiserum is both simple and rapid and should provide a convenient, widely applicable approach for identifying clones to rare messenger RNA's. This method has the advantage of rapidly characterizing unique epitopes expressed in fusion proteins with a procedure that avoids complex

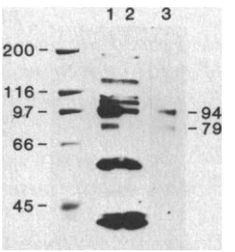

Fig. 1. Western blot characterization of glucocorticoid receptor polyclonal antiserum. Control and steroid-treated IM-9 cells were placed in steroid-free (charcoal-treated) media 24 hours prior to harvesting; maintenance of IM-9 cells and preparation of cytoplasmic extracts were as described (*8*). The synthetic glucocorticoid triamcinolone acetonide (TA) was added at $10^{-6}M$ 2 hours prior to harvest of steroid-treated cells. Cytoplasmic extracts (100 μg) were separated by electrophoresis through a 7.5 percent sodium dodecyl sulfate–polyacrylamide gel and transferred to nitrocellulose (*12*). Lanes 1 and 3 contain extracts from non–steroid-treated cells and lane 2 contains extracts from cells treated with 10^{-6} *M* TA. Lanes 1 and 2 were incubated with diluted crude glucocorticoid receptor antiserum (GR 884, 1:200 dilution) and lane 3 with antibody purified by affinity adsorption to immobilized, partially purified rat liver glucocorticoid receptor (*13*). Numbers to the left and right of the figure represent molecular size in kilodaltons.

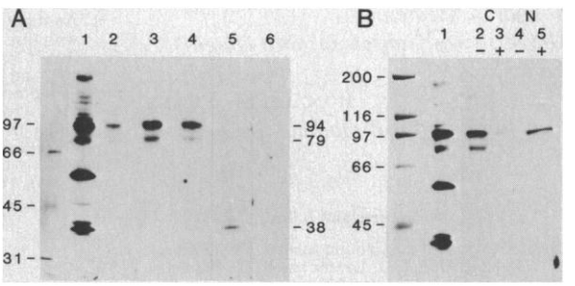

Fig. 2. (A) Identification of recombinant clones by epitope selection. Four immunopositive recombinants and λgt11 were used to select antibody. Following incubation with crude polyclonal antiserum for 1 hour, the filters were washed and antibody was eluted (*17*). Each selected antibody was then used to probe Western blots of IM-9 cytoplasmic extracts. Antibodies used are as follows: crude polyclonal antiserum (lane 1) and epitope-selected antibodies from clones HGR1.2A (lane 2), HGR2.9C (lane 3), HGR5.16A (lane 4), GR80 (lane 5), and λgt11 (lane 6). (B) Specificity of selected antibody. IM-9 cells were grown in suspension culture in the absence (−) or presence (+) of triamcinolone acetonide ($10^{-6}M$) for 2 hours prior to harvest. Protein (100 μg) from cytoplasmic and nuclear fractions were prepared (*20*) and subjected to Western blot analysis with epitope specific antibody which had been affinity purified against clone HGR2.9C (*18*). Lane 1, cytoplasm from control cells; lanes 2 and 3, cytoplasm (C) from control and steroid-treated cells, respectively; lanes 4 and 5, nuclear (N) fraction from control and steroid-treated cells, respectively. Lane 1 was incubated with crude (nonselected) polyclonal receptor antibody, lanes 2 to 5 were incubated with epitope-specific antibody that had been affinity purified against clone HGR2.9C. Numbers to the left and right of the panels represent size in kilodaltons.

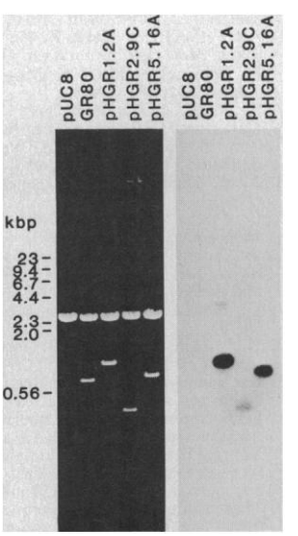

Fig. 3. Identification of cross-hybridizing DNA inserts from immunopositive clones. Recombinant bacteriophage DNA's were digested with Eco RI and the DNA inserts were isolated and subcloned into the Eco RI site of pUC8 DNA. Plasmid DNA's (0.5 μg) were digested with Eco RI, products were separated by electrophoresis on a 0.8 percent agarose gel and transferred to nitrocellulose (*19*). The 1100-base-pair Eco RI fragment from pHGR1.2A was nick-translated (*21*) and used as probe in a hybridization mixture of 50 percent formamide, 5× SSPE (1× SSPE contains 0.15 m*M* NaCl, 0.01 m*M* NaH_2PO_4, 1 m*M* EDTA, *p*H 7.4), 0.1 percent SDS, 10 percent dextran sulfate, and salmon sperm DNA (100 μg/ml). The blot was washed in 2× standard saline citrate, 0.1 percent sodium dodecyl sulfate at 68°C and autoradiographed. (Left) Ethidium bromide-stained gel. (Right) Southern blot of DNA's transferred from the gel in the left probed with nick-translated pHGR1.2A.

purification protocols. Isolation of full length cDNA for the glucocorticoid receptor with the immunopositive clone DNA's as probes will allow determination of its primary amino acid structure.

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Expression of the Chlamydial Genus-Specific Lipopolysaccharide Epitope in *Escherichia coli*

Abstract. *The obligate intracellular prokaryote* Chlamydia trachomatis *is the etiological agent of trachoma and is a primary causative pathogen of sexually transmitted genital tract disease; both diseases affect millions of people each year. The cloning of genes encoding the enzyme or enzymes producing the genus-specific lipopolysaccharide antigen of* Chlamydia *into* Escherichia coli *is reported here. The cloned chlamydial lipopolysaccharide antigen appears to be a hybrid lipopolysaccharide molecule composed of both* Chlamydia *and* Escherichia coli *components. The chlamydial lipopolysaccharide antigen is expressed on the surfaces of the viable* Escherichia coli *recombinants. These findings may have a significant impact on defining the role of this highly conserved antigen in the pathogenesis and diagnosis of chlamydial infections.*

Chlamydia are obligate intracellular prokaryotes that are structurally similar to gram-negative bacteria (*1*). There are two species, *Chlamydia trachomatis* and *C. psittaci*. *Chlamydia trachomatis* is a specific human pathogen, whereas *C. psittaci* is a pathogen of a variety of nonprimate species. Diseases caused by *C. trachomatis* present a major health problem throughout the world. In developing countries, *C. trachomatis* causes hyperendemic trachoma, which is the world's leading cause of preventable blindness. There are approximately 2 million people in the world today who are blind as a result of trachoma and an estimated 360 million more who are affected by this disease (*2*). In industrialized nations, *C. trachomatis* causes an array of sexually transmitted genital tract and associated infections whose incidence is increasing at epidemic rates (*3*). Conservative estimates indicate that in the United States alone there are at least 3 million *C. trachomatis* infections per year (*3*). In addition to their vertebrate hosts, chlamydial-like organisms have also been identified in tissues of spiders *(Coelotus luctuosus)* (*4*) and clams *(Mercenaria mercenaria)* (*5*);