Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7630–7634, August 1992
Biochemistry

Retinoic acid receptors initiate induction of the cytomegalovirus enhancer in embryonal cells

(hormone response element/congenital defects/pathogenesis/human cytomegalovirus/vitamin A)

PETER GHAZAL*†‡, CORDELL DEMATTEI*†, EMI GIULIETTI*, STEVEN A. KLIEWER§, KAZUHIKO UMESONO§¶, AND RONALD M. EVANS§¶

*Department of Immunology and †Department of Neuropharmacology, Division of Virology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037; §The Salk Institute for Biological Studies, La Jolla, CA 92186-5800; and ¶Howard Hughes Medical Institute, La Jolla, CA 92186-5800

Contributed by Ronald M. Evans, April 24, 1992

ABSTRACT **Reactivation of latent virus is believed to result from a signal transduction event that induces immediate-early (IE) gene transcription. Evidence is presented that the major IE promoter (MIEP) of human cytomegalovirus (hCMV) is activated by physiological levels of retinoic acid (RA) in human embryonal carcinoma cells. Mutagenesis experiments localized in the MIEP enhancer, a retinoic acid-responsive element composed of a direct repeat separated by five nucleotides. Protein–DNA binding experiments revealed that this element functions as a specific target site for the direct interaction of nuclear receptor proteins for RA. These findings implicate the biologically active derivative of vitamin A (RA) as a potential modulator of hCMV pathogenesis in infants and immunocompromised adults.**

The identification of signals that trigger the activation of a latent virus is central to elucidating biological mechanisms of pathogenesis manifest in persistent viral infections, characteristic of the herpesvirus group. Human cytomegalovirus (hCMV), a potent teratogenic agent, establishes an incurable latent infection and is the leading cause of congenital infections in humans (1–3). Symptomatic infection of neonates with hCMV predominantly affects the immature nervous system (1–6). Common congenital abnormalities noted in the brain include microcephaly, hydrocephaly, cerebral necrosis, and microgyria (1–11). Interestingly, vitamin A and its active derivative retinoic acid (RA) can induce a similar pattern of congenital abnormalities, including microcephalic and hydrocephalic malformations (12–16). The activity of hCMV in RA-responsive sites evokes the question of whether retinoids at normal physiological levels might directly signal the activation of hCMV during embryogenesis. The cellular responses to RA are primarily mediated by the nuclear RA receptor (RAR) family of proteins (17–20). These proteins belong to a superfamily of intracellular receptors that, upon ligand activation, function as multimeric transcription factors, controlling gene expression through interactions with discrete sequences in the promoter regions of specific target genes (21, 22).

In the presence of RA, the human embryonal carcinoma cell line NT-2/D1 differentiates *in vitro*, predominantly into cell types of neuronal lineage (23–25). hCMV can replicate in RA-induced differentiated cells but not in uninduced NT-2/D1 cells, suggesting a link between RA action and CMV replication in this *in vitro* model for developmental expression (24, 26). The failure of hCMV to replicate in the uninduced cells, which resemble cells in very early embryonic stages, is due to extremely low levels of transcription initiation by the major immediate-early (IE) gene promoter (MIEP) (27–29). Thus, the effect of RA on the progress of a hCMV infection in NT-2/D1 cells is determined by the level of transcription by the MIEP. There are two possible pathways, derepression and induction pathways, by which the action of RA could influence the MIEP. Evidence for a derepression mechanism has been previously presented (28, 30, 31) in which a candidate repressor protein present in undifferentiated cells has decreased DNA binding activity 5 days after RA induction (30). The derepression pathway necessitates events downstream from the primary action of RA. In contrast, the induction pathway would require either a direct primary or secondary stimulatory response by RA. Whether or not RA might act as an extracellular stimulus that directly activates the MIEP remains unclear. Therefore, we have explored whether the control of transcription by the MIEP in the induced NT-2/D1 cells could involve a primary signaling event by RA that is mediated by the direct participation of ligand-activated RARs.

MATERIALS AND METHODS

Plasmids and Mutagenesis. The parent MIEP reporter construct, pMIEP(−1145/+112)CAT in which the gene for chloramphenicol acetyltransferase (CAT) is the reporter gene, has been described (32). All deletion mutants were made by using the native restriction endonuclease sites. The numbers in parentheses for each clone name indicate the deletion (5′ and 3′, respectively) endpoint relative to the cap site. The clustered point mutations present in the reporter constructs pMIEP(RA1)CAT, pMIEP(RA2)CAT, and pMIEP(RA3)CAT were engineered by PCR-directed mutagenesis. The plasmids pRARE(ab), pRARE(cd), pRARE(ef), and pRARE(gh), encoding the RA-responsive element (RARE), were constructed by insertion of the specific oligonucleotide (shown in Fig. 3*B* and Fig. 4*A*) at the *Hin*dIII–*Bam*HI sites upstream of the HSV herpes simplex virus (HSV) thymidine kinase gene (*tk*) promoter, *tk*-CAT (pRSCAT$_4$; ref. 33). The identity of all of the recombinant clones was confirmed by sequencing. Expression plasmids for the three subtypes of RAR used in the cotransfection assay have been described and are pRShRAR$_\alpha$ (17) and pRShRAR$_\beta$ and pRShRAR$_\gamma$ (34).

Transfections and CAT Assays. The cell lines NT-2/D1, U373-MG, and CV-1 were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) fetal bovine serum. DNA-mediated transfections were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RA, retinoic acid; IE, immediate early; MIEP, major IE promoter; CMV, cytomegalovirus; hCMV, human CMV; RAR, RA receptor; hRAR, human RAR; RARE, RA-responsive element; CAT, chloramphenicol acetyltransferase; *tk*, viral thymidine kinase gene; EMSA, electrophoretic mobility-shift assay; HSV-1, herpes simplex virus type 1.

‡To whom reprint requests should be addressed.

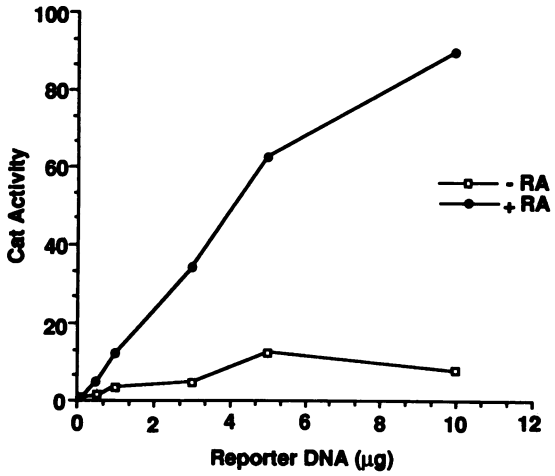

FIG. 1. RA induction of the hCMV MIEP. NT-2/D1 cells were transfected with various amounts (from 0.1 μg to 10 μg) of a MIEP reporter construct, pMIEP(−750/+54)CAT, and incubated with 10 μM RA (●) or with ethanol (□). RA induction of the MIEP in the transient assay required a minimum of 2–6 hr of exposure to ligand (data not shown). Quantitation is expressed as the percentage conversion of chloramphenicol to the acetylated forms (CAT activity).

essentially carried out as described (32, 35). After removal of the DNA–calcium phosphate precipitate, the cells were kept in DMEM with 10% charcoal–resin-treated fetal bovine serum in the presence of ligand for 30–36 hr. Control transfections received solvent only. Plasmid ratios used in the specific experiments are indicated in the figure legends. CAT assays were performed as described (32) except that cell extracts were normalized to β-galactosidase activity before performing CAT assays. The CAT activity was quantified by liquid scintillation counting.

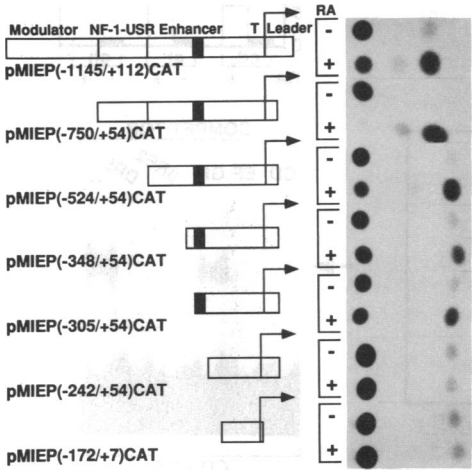

FIG. 2. Identification of a RARE in the MIEP of hCMV. RA responsiveness of the various mutants was monitored by assaying for CAT activity from NT-2/D1 cells treated with (+) or without (−) 10 μM RA. Identical results were also obtained with gene-transfer experiments in permissive U373-MG glial cells. Note that the CAT activities shown for the various promoter constructs represent independent experiments; therefore, their relative levels of expression are not directly comparable in this figure. A schematic of the MIEP mutant is shown to the left of the CAT assays with the name of the mutant indicated below. The numbers correspond to the 5′ and 3′ deletion endpoints of the MIEP. The various transcription control domains are designated by the modulator, NFI-USR (nuclear factor I-unique sequence region) enhancer, T (TATA box) and leader regions. The location of the RA-responsive region is marked by the black box.

Nuclear Extract Preparation and DNA-Binding Assays. Nuclear extracts from uninduced and RA-induced NT-2/D1 cells were prepared as described for HeLa cell nuclear extract (32, 48). The electrophoretic mobility-shift assay (EMSA) was performed as described (32). The specific probes used in the EMSA are shown in Fig. 4*A*.

Preparation of Recombinant Human RARα (hRARα) from *Escherichia coli*. The hRARα protein was expressed in BL21(DE3)plysS cells by using the PET-8C expression vector as described (36). The expressed hRARα protein was

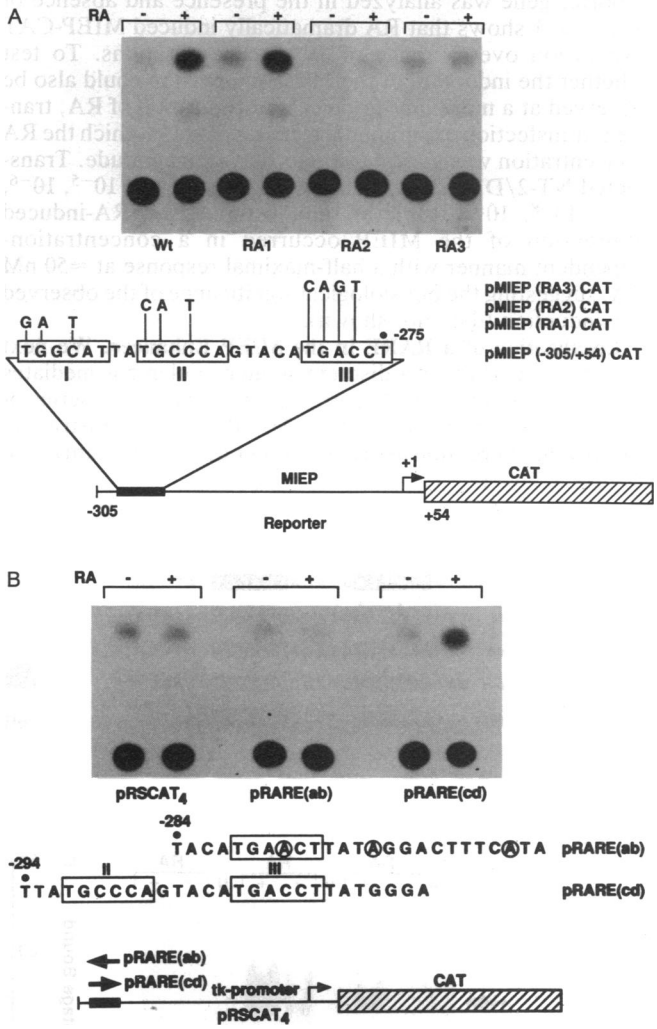

FIG. 3. Mutational analysis of the MIEP-RARE. (*A*) Clustered point mutations were introduced into the parental wild-type construct pMIEP(−305/+54)CAT. NT-2/D1 cells were transfected in the presence (lanes +) and absence (ethanol only) (lanes −) of RA with either wild-type (Wt) or the following mutant constructs: RA1, pMIEP(RA1)CAT; RA2, pMIEP(RA2)CAT; and RA3, pMIEP-(RA3)CAT. A schematic of the reporter constructs is shown below the CAT assays with the point mutations in motif I, II, and III indicated above the wild-type sequence. The numbers refer to the nucleotide position relative to the initiation site of transcription. Identical results were also obtained with gene-transfer experiments in permissive U373-MG cells. (*B*) The MIEP-RARE localized to positions −294 to −269 was transferred to the heterologous HSV-1 *tk* promoter ($pRSCAT_4$) to create the reporter plasmid pRARE(cd). In addition, a control oligonucleotide encompassing nucleotides −284 to −258 with point mutations (indicated by circled bases) was also cloned upstream of the *tk* promoter to generate the reporter plasmid pRARE(ab). The plasmids, $pRSCAT_4$, pRARE(ab), and pRARE(cd) were cotransfected with the β-galactosidase internal control expression vector into NT-2/D1 cells and then treated with 10 μM RA (lanes +) or with ethanol (lanes −). Identical results were obtained with transient expression experiments in U373-MG cells.

chromatographically enriched from the clarified crude bacterial lysate by using a heparin-agarose column as described (36).

RESULTS AND DISCUSSION

Regulation of the MIEP by RA. To pursue the possibility that the MIEP is transcriptionally activated through a direct effect of RA, transient expression by NT-2/D1 cells of a recombinant plasmid containing the MIEP linked to the CAT reporter gene was analyzed in the presence and absence of RA. Fig. 1 shows that RA dramatically induced MIEP-CAT expression over a range of DNA concentrations. To test whether the induction of the MIEP expression could also be observed at a more physiological concentration of RA, transient transfection experiments were repeated in which the RA concentration was varied over 6 orders of magnitude. Transfected NT-2/D1 cells were incubated with RA at 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} M, and without RA. RA-induced expression of the MIEP occurred in a concentration-dependent manner with a half-maximal response at ≈50 nM RA, suggesting the physiological significance of the observed RA induction (data not shown).

Localization of a RARE in the MIEP Enhancer. We next investigated whether a discrete sequence element mediates responsiveness to RA. Accordingly, we analyzed a series of 5′ and 3′ deletion mutants of the MIEP reporter construct in a transient expression assay. Promoter deletion mutants with 5′ endpoints at nucleotide positions −1145, −750, −524, −348, and −305 and with 3′ endpoints at nucleotide positions +112, +54, and +7 responded to RA with a 4- to 10-fold induction of CAT expression (Fig. 2 and data not shown). Deletion of the MIEP sequences to nucleotide positions −242, −172, −132, and −117 were nonresponsive to RA induction. These results suggest that sequences between nucleotide positions −305 and −242 mediate a stimulatory effect of RA on MIEP transcription. Inspection of this 63-base-pair (bp) RA-responsive domain revealed sequences closely resembling RAREs, consisting of a TGACC-related repeat that recently has been identified in natural and artificial promoters (33, 35, 37–42). These sequences are TGGCA (motif I), TGCCC (motif II), and TGACC (motif III) at nucleotide positions −299, −291, and −280, respectively. To determine the precise location of the MIEP RARE and the role of motifs I–III, a series of clustered point mutations were introduced in the three specific TGACC-related motifs. When these constructs were transiently expressed in NT-2/D1 cells in the presence and absence of RA, mutation of motif I did not alter the ability of the MIEP to be induced by RA (Fig. 3*A*). In contrast, mutations in the TGACC-related motifs II and III abrogated the RA-inducibility of the MIEP, although in some experiments pMIEP(RA2)CAT showed a marginal response to RA. We interpret these results to indicate that motifs II and III constitute an optimal "core" RARE. Indeed, when transferred to the *tk* promoter of HSV-1 that is nonresponsive to RA, motifs II and III but not

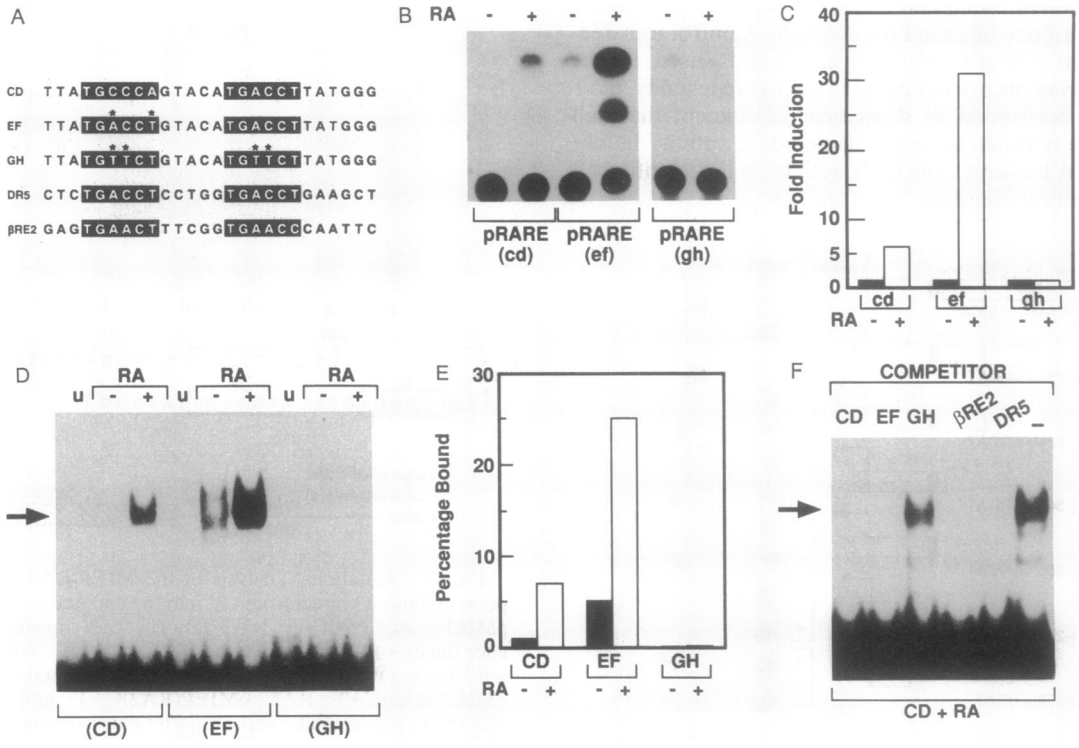

FIG. 4. RA-induced RAR binding to the MIEP-RARE. (*A*) Primary sequence structure of the CD, EF, GH, DR5 (35), and βRE2 (33) probes. Asterisks indicate nucleotide point mutations relative to the wild-type CD template. Reverse print marks the hexad repeat. (*B*) The CD, EF, and GH probes were ligated into the pRSCAT$_4$ vector to generate reporter plasmids pRARE(cd), pRARE(ef), and pRARE(gh). These plasmids were cotransfected with pRSV$_\beta$gal internal control vector into NT-2/D1 cells and then treated with (lanes +) or without (lanes −) 10 μM RA. (*C*) The response of pRARE(cd), pRARE(ef), and pRARE(gh) to RA (bars +) is plotted as the relative fold-induction observed in these experiments. (*D*) EMSA of CD, EF, and GH probes with 5 μg of nuclear proteins from uninduced (lanes −) and RA-induced (lanes +) (96-hr exposure to ligand) NT-2/D1 cells. The arrow marks specific nucleoprotein complex, and "u" refers to unbound probe. (*E*) Comparison of the relative affinity of CD, EF, and GH probes for uninduced (−) and RA-induced (+) NT-2/D1 nuclear receptor proteins. No specific complex was obtained with the GH probe corresponding to an *in vivo* down-mutant, whereas a stronger complex was seen with the EF probe corresponding to an *in vivo* up-mutant. The quantity of complex formed with each of the probes was determined by scintillation counting of excised, free, and bound bands. The quantity of complex formed is expressed as a percentage of the total probe, and the value for each template is shown on the ordinate. The same results were obtained in at least three independent experiments. The lower affinity of receptor complexes formed for CD, when compared with EF, probably reflects intrinsic binding properties of RAR (see Fig. 5). (*F*) Competition of CD complex formation with a 35-molar excess of unlabeled CD, EF, GH, βRE2, and DR5 probes. The arrow indicates specific nucleoprotein complex.

a control sequence containing a mutant motif III were able to confer RA inducibility (Fig. 3*B*). Based on these data we refer to the sequence motifs II and III as the MIEP-RARE, representing one of few naturally occurring RAREs identified.

Previous studies with artificial RAREs suggest that a direct repeat of the hexad sequence TGACCT spaced by 3, 4, and 5 nucleotides corresponds to response elements for the vitamin D_3 receptor (VDR), thyroid hormone T_3 receptor (TR), and RAR, respectively (35). The MIEP-RARE agrees well with the spacing rule of 5, with motif III identical with the canonical hexad sequence, while motif II diverges by two nucleotides. Hence, the MIEP-RARE constitutes an imperfect direct repeat with a spacing of 5.

Functional Binding of the RARs to the MIEP-RARE. To explore the interaction of sequence-specific factors with the MIEP-RARE, the EMSA was used with [^{32}P]DNA probes. The probes consisted of a wild-type and two mutant forms of the MIEP-RARE. The EF mutant converts the MIEP-RARE to the canonical RARE structure, while GH mutations exhibit a dysfunctional MIEP-RARE (Fig. 4 *A–C*). Nuclear proteins prepared from uninduced and RA-induced NT-2/D1 cells were examined for binding to the various probes in the EMSA. Fig. 4 *D* and *E* shows the dramatic increase in the appearance of nucleoprotein complex bound to the CD and EF probes after RA treatment. Mutation of motifs II and III present in the GH probe inhibited nucleoprotein complex formation, suggesting those factors that interact with CD and EF probes recognize the hexad repeat. The sequence requirement for the formation of the induced nucleoprotein complex bound to the CD probe was investigated further by performing competition experiments in the EMSA system. The induced CD complex was inhibited from forming by an excess of the unlabeled CD and EF probes but not by the GH probe (Fig. 4*F*). The inhibition of CD complex formation by EF and the reciprocal inhibition of EF complex formation (data not shown) suggest that these probes recognize identical factors. Since the EF probe represents a synthetic canonical RAR binding site, it is possible that RARs are associated with the RA-induced CD and EF nucleoprotein complexes. To test this suggestion, competition assays were performed with a naturally occurring RARE defined from the RAR promoter, βRE2 (33), and a strong artificial RAR binding site, DR5 (35). The βRE2 and DR5 probes competed effectively for the formation of the nucleoprotein complexes associated with both CD and EF (Fig. 4*F* and data not shown). We infer from these observations that the RARs participate in the formation of the RA-induced complex from NT-2/D1 cells.

To demonstrate a direct interaction between the MIEP-RARE and RAR, bacterially expressed RARα was used in the EMSA. As predicted, when increasing amounts of RAR were incubated with labeled CD and EF probes, a major nucleoprotein complex could be formed. The formation of this complex could be specifically inhibited by unlabeled probe and the βRE2 probe but not by a nonspecific oligonucleotide (data not shown). For comparison, labeled DR5 probe was also included in these studies. The affinity of binding of EF and DR5 with the RAR was greater than the binding affinity observed between the CD probe and RAR (Fig. 5). This observation agreed well with the affinity of binding detected for CD and EF probes with the RA-induced nuclear proteins in NT-2/D1 cells (Fig. 4 *D* and *E*). Further, the *in vivo* RA-responses for CD and EF correlate well with *in vitro* DNA-binding affinities of the RAR proteins (Figs. 4 and 5). Finally, overexpression of RAR effector plasmids in cells was able to activate dramatically reporter constructs in a MIEP-RARE-dependent fashion (Fig. 6). Together, these results strongly support the conclusion that RAR directly binds the MIEP-RARE and thereby enhances transcription.

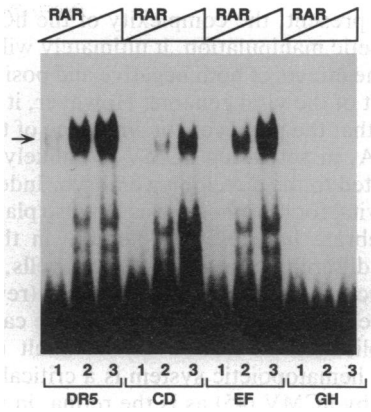

FIG. 5. Direct binding of bacterially expressed RAR to the DR5, CD, EF, and GH probes. Increasing amounts of hRARα bind to radiolabeled probes for DR5, CD, and EF. Conditions for binding have been described (36) and included 120, 300, and 600 ng of partially purified RARα for lanes 1–3, respectively. Specific RAR–DNA complex is indicated by the arrow.

The RAR-mediated activation of the MIEP supports the proposal that a link between vitamin A and viral activation in fetal development could play a key role in hCMV teratogenesis.

CONCLUSIONS

Our observation that hCMV contains a primary target promoter for RA transcriptional activation and previous studies on the secondary influence of RA on negative elements in the MIEP (28, 30) suggest a sequential multistep series of events initiated by RA that can lead to a state of permissiveness of an embryonal cell. Our results do not imply that hCMV replication is exclusively dependent on the presence of RA but simply that RA can signal, as an extracellular stimulus, viral IE gene expression by direct interaction with the RARs.

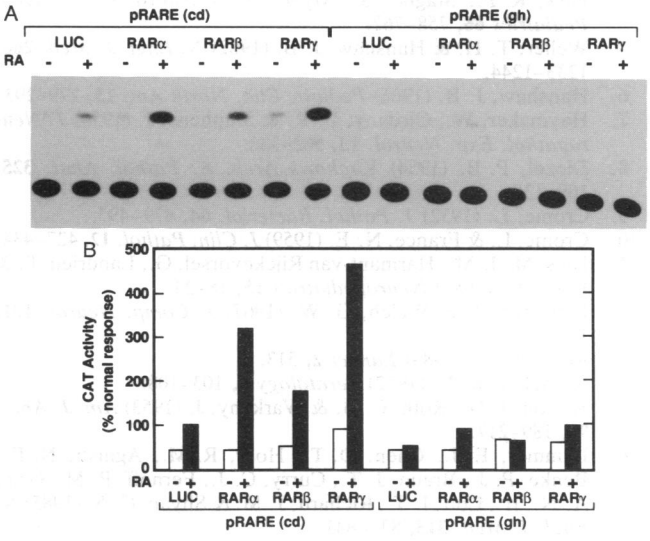

FIG. 6. Trans-activation of MIEP-RARE by RARs. CV1 cells were cotransfected in duplicate in 3-cm diameter dishes, with 250 ng of either pRARE(cd) or the control reporter vector pRARE(gh) and 50 ng of expression plasmids, pRSV-LUC (control) (LUC), pRS-hRARα (RARα), pRS-hRARβ (RARβ), or pRS-hRARγ (RARγ) (36). CAT assays were standardized by using equivalent units of β-galactosidase by cotransfecting 125 ng of pRSVβgal. A total of 4 μg of DNA per transfection was made up by using pGEM-4, and cells were exposed for 40 hr to either ethanol (−) or 1 μM RA (+). One set of the duplicate CAT assays is shown.

Although, at present, the complexity of the hCMV genome prohibits genetic manipulation, it ultimately will be essential to examine the effects of both negative and positive elements in the context of the viral genome. However, it is interesting to speculate that the pathogenetic influence of the vitamin A congener, RA, in activating hCMV is unlikely to be exclusively restricted to the developing embryo. Indeed, retinoids are not only vital for morphogenesis but also play an essential role in vertebrate homeostasis—notably in the control of growth and differentiation of epithelial cells, cells of the hematopoietic system, and cells of the retina (reviewed in ref. 43). In parallel, epithelial cells represent the cardinal tissue-type for replication of hCMV in the adult (1–3, 44). In addition, the hematopoietic system is a critical target tissue for infection by hCMV (45) as is the retina, in which hCMV retinitis often leads to blindness (46, 47). Thus, correlation in the temporal and spatial identity during expression of hCMV and within the physiological action of RA is exhibited in both neonate and adult. The discovery of a link between a molecularly characterized RA-signaling event and viral gene activation provides an exquisite example of how viral pathogenesis, in general, may be finely integrated with a principle homeostatic control mechanism of the host.

We thank M. B. Oldstone, D. Mangelsdorf, and S. Hoffman for helpful comments on the manuscript, and J. Nelson for his support and encouragement throughout this project. Our thanks go to G. Whitman for expert secretarial assistance. This work was supported in part by a National Institutes of Health grant (to P.G.) and funds provided by the State of California and allocated on the recommendation of the University-wide Task Force on AIDS (to P.G.). K.U. is a research associate, and R.M.E. is an investigator of the Howard Hughes Medical Institute at the Salk Institute for Biological Studies. This is publication 7127-IMM from the Departments of Immunology and Neuropharmacology of The Scripps Research Institute.

1. Weller, T. H. (1991) *Transplant. Proc.* **23**, 5–7.
2. Ho, M. (1991) in *Biology and Infection*, ed. Ho, M. (Plenum, New York), pp. 205–224.
3. Yow, M. D. (1989) *J. Infect. Dis.* **159**, 163–172.
4. Pass, R. F., Stagno, S., Myers, J. & Alford, C. A. (1980) *Pediatrics* **66**, 758–762.
5. Weller, T. H. & Hanshaw, J. B. (1962) *N. Engl. J. Med.* **266**, 1233–1244.
6. Hanshaw, J. B. (1966) *Pediatr. Clin. North Am.* **13**, 279–293.
7. Haymaker, W., Girdany, B.-R. & Stephens, J. (1954) *J. Neuropathol. Exp. Neurol.* **13**, 562–586.
8. Diezel, P. B. (1954) *Virchows Arch. A: Pathol. Anat.* **325**, 109–130.
9. Crome, L. (1952) *J. Pathol. Bacteriol.* **64**, 479–495.
10. Crome, L. & France, N. E. (1959) *J. Clin. Pathol.* **12**, 427–434.
11. Dias, M. J. M., Harmant-van Rijckevorsel, G., Landrieu, P. & Lyon, G. (1984) *Neuropediatrics* **15**, 18–24.
12. Langman, J. & Welch, G. W. (1967) *J. Comp. Neurol.* **131**, 15–26.
13. Rosa, F. W. (1983) *Lancet* **2**, 513.
14. Shenefelt, R. E. (1972) *Teratology* **5**, 103–108.
15. Wilson, J. G., Roth, C. B. & Warkany, J. (1953) *Am. J. Anat.* **92**, 189–217.
16. Lammer, E. J., Chen, D. T., Hoar, R. M., Agnisti, N. D., Benke, P. J., Braun, J. T., Curry, C. J., Fernoff, P. M., Grin, A. N., Jr., Lott, I. T., Richard, J. M. & Shyan, C. S. (1985) *N. Engl. J. Med.* **313**, 837–841.
17. Giguere, V., Ong, E. S., Segui, P. & Evans, R. M. (1987) *Nature (London)* **330**, 624–629.
18. Mangelsdorf, D. J. & Evans, R. M. (1992) *Transcriptional Control and Regulation*, eds. Yamamoto, K. R. & McKnight, S. L. (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
19. Mangelsdorf, D. J., Ong, E. S., Dyck, J. A. & Evans, R. M. (1990) *Nature (London)* **345**, 224–229.
20. Petkovich, M., Brand, N. J., Krust, A. & Chambon, P. (1987) *Nature (London)* **330**, 444–450.
21. Green, S. & Chambon, P. (1988) *Trends Genet.* **4**, 309–314.
22. Evans, R. M. (1988) *Science* **240**, 889–895.
23. Andrews, P. W. (1988) *Dev. Biol.* **103**, 285–293.
24. Andrews, P. W., Gonczol, E., Plotkin, S., Dignazio, M. & Oosterhuis, J. W. (1986) *Differentiation* **31**, 119–126.
25. Andrews, P. W., Damjanov, I., Simon, D., Banting, G. S., Carlin, C., Dracopoli, N. C. & Fogh, J. (1984) *Lab. Invest.* **50**, 147–162.
26. Gonczol, E., Andrews, P. W. & Plotkin, S. (1984) *Science* **224**, 159–161.
27. Nelson, J. A. & Groudine, M. (1986) *Mol. Cell. Biol.* **6**, 452–461.
28. Nelson, J. A., Reynolds-Kohler, G. & Smith, B. (1987) *Mol. Cell. Biol.* **7**, 4125–4129.
29. Lafemina, R. & Hayward, G. S. (1986) *J. Virol.* **58**, 434–440.
30. Shelbourn, S. L., Kothari, S. K., Sissons, J. G. P. & Sinclair, J. H. (1989) *Nucleic Acids Res.* **17**, 9165–9171.
31. Nelson, J. A., Gnann, J. W. & Ghazal, P. (1990) *Curr. Top. Microbiol. Immunol.* **154**, 75–103.
32. Ghazal, P. & Nelson, J. A. (1991) *J. Virol.* **65**, 2299–2307.
33. Sucov, H. M., Murakami, K. K. & Evans, R. M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5392–5396.
34. Ishikawa, T., Umesono, K., Mangelsdorf, D. J., Aburatani, H., Stanger, B. Z., Shibasaki, Y., Imawari, M., Evans, R. M. & Takaku, F. (1990) *Mol. Endocrinol.* **4**, 837–844.
35. Umesono, K., Murakami, K. K., Thompson, C. C. & Evans, R. M. (1991) *Cell* **65**, 1255–1266.
36. Mangelsdorf, D. J., Umesono, K., Kliewer, S. A., Borgmeyer, U., Ong, E. S. & Evans, R. M. (1991) *Cell* **66**, 555–561.
37. Smith, W. C., Nakshatri, H., Leroy, P., Rees, J. & Chambon, P. (1991) *EMBO J.* **10**, 2223–2230.
38. Munoz-Canoves, P., Vik, D. P. & Tack, B. F. (1990) *J. Biol. Chem.* **265**, 20065–20068.
39. Vasios, G., Mader, S., Gold, J. D., Leid, M., Lutz, Y., Gaub, M. P., Chambon, P. & Gudas, L. (1991) *EMBO J.* **10**, 1149–1158.
40. Lucus, P. C., O'Brian, R. M., Mitchell, J. A., Davis, C. M., Imai, E., Forman, B. M., Samuels, H. H. & Granner, D. K. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2184–2188.
41. Duester, G., Shean, M. L., McBride, M. S. & Stewart, M. J. (1991) *Mol. Cell. Biol.* **11**, 1638–1646.
42. de The, H., Vivianoco-Ruiz, M. D. M., Tiollais, P., Stunnenberg, H. & Dejean, A. (1990) *Nature (London)* **343**, 177–180.
43. Sporn, M. B., Roberts, A. B. & Goodman, D. S., eds. (1984) *The Retinoids 1 and 2* (Academic, New York).
44. Myerson, D., Hackman, R. C., Nelson, J. A., Ward, D. C. & McDougall, J. K. (1984) *Hum. Pathol.* **15**, 430–439.
45. Nelson, J. A., Ibanez, C., Schrier, R., Wiley, C. & Ghazal, P. (1991) in *Viruses that Affect the Immune System*, eds. Fan, H. Y., Chen, I. S. Y., Rosenberg, N. & Sugden, W. (Am. Soc. Microbiol., Washington), pp. 231–246.
46. Freeman, W. R., Lerner, C. W., Mines, J. A., Lash, R. S., Nadel, A. S., Starr, M. B. & Tapper, M. L. (1984) *Am. J. Pathol.* **97**, 133–142.
47. Palestine, A. G., Rodrigues, M. M. & Macher, A. M. (1984) *Ophthalmology* **91**, 1092–1099.
48. Ghazal, P., Lubon, H., Fleckenstein, B. & Hennighausen, L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3658–3662.