Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 6472–6476, August 1991
Biochemistry

Interleukin 6 enhances a cellular activity that functionally substitutes for E1A protein in transactivation

(transcriptional activation/signal transduction/HepG2 cells/adenovirus)

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Communicated by Sidney Udenfriend, March 29, 1991

ABSTRACT **An interleukin 6 (IL-6)-regulated cellular activity in HepG2 cells is found to functionally substitute for the transcriptional transactivator product of the adenovirus transforming gene *E1A* in transactivating E1A-dependent and E1A-responsive viral early genes. Mutant viruses deficient in *E1A* expression replicate in HepG2 cells. Induction with IL-6 leads to significant enhancement of synthesis of viral early E1B and E2ae mRNAs by >30-fold and increases viral replication to the wild-type levels. The E1A-substituting activity activates E1A-responsive promoters in transient transfection, and this transcriptional activity is regulated by IL-6 induction. Formation of distinct protein–promoter complexes by binding of proteins in nuclear extracts prepared from HepG2 cells to the E1A-dependent *E2ae* promoter further supports the possibility that this activity may be a nuclear component in the IL-6 signal transduction pathway.**

Many viral transforming genes encode transcription regulators, among them the *E1A* gene product (E1A) of adenovirus (Ad) which regulates viral genes and a subset of cellular genes (for reviews, see refs. 1 and 2). Despite significant efforts, however, the mechanism of E1A action remains to be elucidated. This is due at least in part to the lack of sequence specificity in genes regulated by the E1A proteins and to the strong cell-type dependence of E1A action. Furthermore, although the efficiency of transactivation by E1A appeared to be correlated with the levels of E1A mRNAs (3), it also has been observed that replication of mutant viruses synthesizing very little E1A proteins was unabated in HeLa cells (4). We have found that transactivation of E1A-dependent viral genes is as efficient in some cultured human lymphoid cells when the E1A proteins are expressed at a level 1/50th of that of HeLa cells (5). Nevertheless, E1A is indispensable for the expression of Ad early genes in lymphoid cells (5) and in nearly all cell types so far studied.

It has been noted, however, that HeLa cells support a low level of replication of dl312, an *E1A*-deletion mutant virus, when the multiplicity of infection (moi) is high (6). Whereas this activity in HeLa cells may be attributed to complementation by human papilloma virus type 18 integrated in HeLa cells and expressing its *E7* gene, which can substitute for *E1A* in transfection (7, 8), a cellular activity (or activities) that functionally substitutes for E1A protein has been described in mouse embryonal carcinoma F9 cells (9, 10). The E1A-dependent *E2ae* promoter is active in F9 cells in transfection and correlates with the presence of E2F, a cellular factor that binds to the *E2* promoter (11), and the formation of distinct *E2* promoter–protein complexes (12, 13). However, the activity in F9 cells diminishes upon cellular differentiation (11, 13). A similar activity has been described in mouse oocytes and in preimplantation embryos (14). The nature of these activities and whether they are one and the same, has remained obscure.

Here we report a cellular activity in human hepatoblastoma HepG2 cells, which, in the absence of E1A, transactivates E1A-dependent viral genes in viral infection. This activity can be significantly increased by induction of HepG2 cells with the cytokine interleukin 6 (IL-6), resulting in full complementation in the replication of *E1A*-deletion mutant virus dl312. Regarding the two E1A-dependent promoters investigated, promoter elements of the *E2ae* and *E1B* genes, IL-6 regulates them in transfection, thereby confirming that the cellular activity functions at the transcriptional level. These results suggest that a component in the IL-6 transduction pathway may be the cellular activity that regulates the E1A-dependent promoters in the absence of E1A.

MATERIALS AND METHODS

Cells and Viruses. HepG2 cells, a human hepatoblastoma cell line (15), were cultured in Eagle's minimal essential medium (MEM-Eagle). HeLa cells were maintained as suspension cultures for viral infection (5). 293 cells, human embryonic kidney cells transformed with the left-hand 15% of Ad type 5 (Ad5) genome (16); HEL cells, a human embryonic lung fibroblastic cell line; HA22T/VGH (HA22T) cells, a human liver cell line (17); and HeLa cells for transfection were maintained as monolayer cultures in Dulbecco's modified minimum essential medium. All media were supplemented with L-glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 μg/ml), nonessential amino acids (GIBCO), and 10% (vol/vol) fetal calf serum (HyClone). Explants of human fetal liver were prepared by Joachim Bauer (18) and used for experiments on day 3 after explant, when major dedifferentiation had not yet occurred. Infection and propagation of Ads were as described (5). Inductions with IL-6 (provided by T. Kishimoto and T. Hirano of Osaka University), tumor necrosis factor α (TNF-α) (a gift from A. Cerami of Rockefeller University, New York), and dibutyryl cAMP (Bt_2cAMP) (Sigma) were performed 24 hr prior to infection and continued throughout viral infection. Southern and Northern analyses were performed as described (5).

Transfection. For IL-6 induction, HepG2 cells were transfected essentially as described by Won and Baumann (19). Briefly, 24 hr after plating (6×10^5 cells per 100-mm dish), HepG2 cells were transfected with 20 μg of total DNA, including carrier, by the calcium phosphate method (20). After dispersion by trypsinization on the following day, equal numbers of cells were plated onto two collagen (Boehringer Mannheim)-coated six-well dishes. One set of cells served as

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Abbreviations: Ad, adenovirus; Ad2 and Ad5, Ad types 2 and 5; Bt_2cAMP, dibutyryl cAMP; CAT, chloramphenicol acetyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-6, interleukin 6; moi, multiplicity of infection; pfu, plaque forming unit; TNF-α, tumor necrosis factor α; RSV, Rous sarcoma virus.

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a control, and the other was induced with IL-6 (500 units/ml) in serum-free medium beginning 4 hr after plating. Cell extracts were prepared 48 hr after the initial transfection as will be described elsewhere. Transfection of monolayer cells without cytokine induction was performed by the calcium phosphate method with desired amounts of experimental plasmid DNA in a total amount of DNA (including carrier DNA) empirically determined to be optimal for 6×10^5 cells of each cell line: 10, 20, 30, 30, and 30 μg of total DNA for 293, HepG2, HeLa, HA22T, and HEL cells, respectively. Plasmids containing the chloramphenicol acetyltransferase (CAT) reporter gene were as follows: the pE2ae-CAT plasmid contains the Ad5 *E2ae* promoter (−284 to +64 of the *E2ae* gene) (21); the pE1B-CAT plasmid contains the Ad5 *E1B* promoter (−365 to +1 of the *E1B* gene) (22); the positive control for transfection, pRSV-CAT, contains a 524-base-pair (bp) fragment of the 3′ long terminal repeat (LTR) of Rous sarcoma virus (RSV); and the negative control, pJYM-CAT, contains a TATA box from simian virus 40 but no enhancer. RSV-βgal contains the β-galactosidase gene driven by the RSV LTR enhancer/promoter. Assays for CAT and β-galactosidase activity were performed as will be described elsewhere. After normalizing transfection efficiency with the β-galactosidase activity, the CAT activity is expressed as the percentage of that of the positive control, pRSV-CAT. For example:

$$\% \text{ of pRSV-CAT activity} = \frac{(\text{pE2ae-CAT} - \text{pJYM-CAT})}{(\text{pRSV-CAT} - \text{pJYM-CAT})}$$

Electrophoretic Mobility-Shift Assay. The assay to be described elsewhere was performed with nuclear extracts prepared as described by Mattila *et al.* (23). Nuclear extracts (8 μg) were incubated with 1 ng of end-labeled *E1* (5′-CCATTTTCGCGGGAAACTGAATAAG-3′) or *E2* (5′-GATATGACGTAGTTTTCGCGCTTAAATT-TGAGAAAGGGCGCGAAACTAGTCCTT-3′; ref. 24) probe in 16 μl of binding buffer containing 20 mM Hepes (pH 7.9), 40 mM NaCl, 50 mM KCl, 0.5 mM $MgCl_2$, 1 mM EDTA, 4% Ficoll, and 2 μg of sheared salmon sperm DNA at room temperature for 20 min. For competition, an unlabeled probe at 100-fold molar excess, was preincubated with the extract at room temperature for 10 min in the same buffer prior to addition of the labeled probe. The DNA–protein complexes were separated from free DNA by electrophoresis on a 4% nondenaturing polyacrylamide gel containing 0.25× TBE (18 mM Tris base/18 mM borate, pH 8.1/2.5 mM EDTA) at 250 V at 4°C. The gels were transferred to dampened DEAE 81 membrane (DE-81, Whatman) and dried before exposure to Cronex x-ray films (DuPont).

RESULTS

A Cellular Activity Functionally Substituting for E1A Is Present in HepG2 Cells. The E1A proteins are required for transactivation of all early genes of adenovirus. Thus *E1A*-deletion mutants cannot express the E1A-dependent viral early genes and do not replicate in infected human cells with the exception of HeLa cells (see Introduction). However, the ability of E1A proteins to efficiently transactivate E1A-dependent viral early genes at very low protein concentrations in lymphoid cells (5) raises the possibility that in some differentiated human cells other than HeLa cells, cellular activities may be sufficient to complement the transactivation function of E1A. By using replication of an *E1A*-deletion mutant virus dl312 as an assay, it is apparent that HepG2 cells possess an activity that functionally substitutes for E1A and permits replication of dl312 (Fig. 1*A*). As quantified by the intensity of distinct viral genomic fragments after digestion

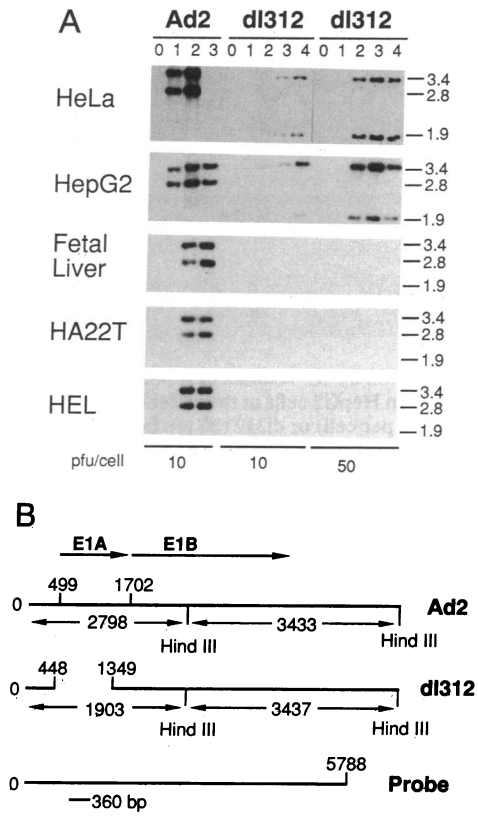

FIG. 1. Replication of the *E1A*-deletion mutant dl312 in HepG2 cells. (*A*) Total DNA was isolated from HepG2, HeLa, HEL, or HA22T cells, and human fetal liver explants were infected with Ad type 2 (Ad2) [10 plaque-forming units (pfu) per cell] or dl312 (10 or 50 pfu per cell) on days after infection indicated, except for HeLa cells in which Ad2 infection was completed at 2 days after infection. The DNA was digested with restriction enzyme *Hin*dIII, separated on a 1% agarose gel, and analyzed by Southern blotting (5) with a ^{32}P-labeled probe as shown in *B*. Each lane contained 1 μg of DNA ($\approx 6 \times 10^4$ cells). (*B*) The *Hin*dIII restriction fragments of Ad2 and Ad5 dl312 emcompass the E1A and E1B transcription units. dl312 (25) contains a 901-bp deletion in the *E1A* coding region, which renders the virus incapable of expressing E1A. Ad2 and Ad5 are phenotypically indistinguishable and nearly identical in this region of the genomes. The DNA probe is the insert of p3wT18, which contains the left-hand 5.8 kilobases (kb) of the Ad5 genome (5).

with restriction enzyme *Hin*dIII in Southern blot analysis, maximal replication of dl312, infecting at 10 pfu per cell, reached 5–10% of that of wild-type virus, albeit after a 2-day delay. When the moi was increased from 10 to 50 pfu per cell, replication of dl312 was proportionally increased and approached that of the wild-type virus at 10 pfu per cell. Since the activity in HepG2 cells appeared to resemble that in HeLa cells, which express integrated human papilloma virus 18, the possibility that HepG2 cells may also contain viral sequences known to complement E1A function was addressed. HepG2 cells have been shown not to harbor human hepatitis virus (15). Analyses with Southern blotting and the polymerase chain reaction, which detected human papilloma virus sequence in HeLa cells, failed to detect any human papilloma virus or Ad sequences in HepG2 cells (data not shown).

The E1A-substituting activity was not present, however, in either liver explants, which retain differentiated liver functions, or HA22T/VGH, another human liver cell line (17), because they did not support replication of dl312 (Fig. 1*A*). Replication of wild-type virus in these cells was identical to that in a human embryonic lung fibroblastic cell line, HEL, which served as a control. Taken together, these data suggest the existence of a cellular activity that functionally substi-

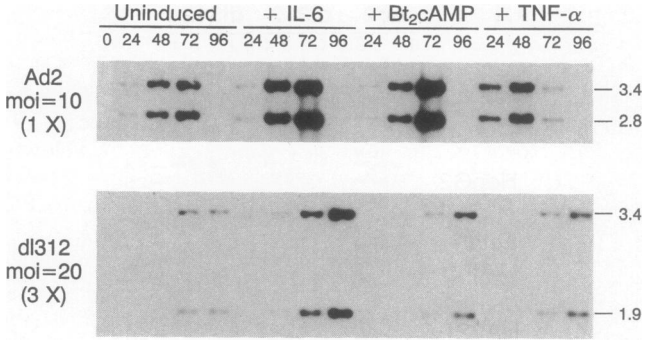

FIG. 2. Induction by IL-6 enhances replication of Ad2 and dl312. DNA isolated from HepG2 cells at the indicated hours after infection with Ad-2 (10 pfu per cell) or dl312 (20 pfu per cell) was analyzed as described in Fig. 1. Induction with IL-6 (100 units/ml), Bt_2cAMP (1 mM), or TNF-α (200 units/ml) were as described. "X" represents the relative length of exposure time of the autoradiographs.

tutes for E1A, is a property of HepG2 cells, and is not a liver-specific function.

E1A-Substituting Activity Is Regulated by IL-6. Among its pleiotropic effects, IL-6 induces cellular differentiation in a highly cell-type specific manner (for a review, see ref. 26). As in the case of induction of human B-cell differentiation by coordinated regulation of immunoglobulin genes at the level of transcription (27), IL-6 induces acute-phase genes in HepG2 cells transcriptionally (28), apparently in a promoter sequence-specific manner (19, 29, 30). Relative to uninduced HepG2 cells, IL-6 clearly accelerated the onset of replication of dl312 virus by 1 day and increased the maximal replication 15-fold to levels approaching that of the uninduced wild-type virus (Fig. 2). Similar results were obtained with other Ad mutants deficient in E1A expression (data not shown). Furthermore, enhancement of replication by IL-6 was not synergistic with E1A, since replication of wild-type virus was increased only 10-fold by IL-6 induction. Other cytokines were much less effective in enhancing replication of dl312. Bt_2cAMP and TNF-α increased dl312 replication by <3-fold (Fig. 2). These results showed that the requirement for E1A in viral replication can be compensated by an IL-6-regulated activity in HepG2 cells.

IL-6 Regulates the Synthesis of mRNAs Encoded by E1A-Dependent Viral Genes. Replication of *E1A*-deletion mutants suggested, but did not prove, that the E1A-substituting activity in HepG2 cells functions by activating E1A-dependent viral genes transcriptionally. Analyses of mRNAs transcribed from E1A-dependent *E2ae* and *E1B* genes in the course of viral infection showed that the E1A-substituting activity in HepG2 cells functioned at the level of mRNA synthesis (Fig. 3*A*). The mRNAs encoded by the *E1B* gene, which cooperates with E1A for transformation (31) and is dependent on E1A for its transcription, were detectable in HepG2 cells 72 hr after infection with dl312, as compared with 24 hr after infection with Ad2 (data obtained from a 20-fold longer exposure of Fig. 3*A*, not shown).

Induction of dl312-infected HepG2 cells with IL-6 resulted in a >30-fold increase of E1B mRNA levels, such that 24 hr after infection the levels equaled that of wild-type Ad2 infection without IL-6 induction (Fig. 3*B*, compare lane 24+ of dl312 with lane 24− of Ad2). Increases of E2ae mRNA levels by IL-6 induction in the absence of E1A, were even more dramatic—to levels at least 5- to 10-fold higher than that of the wild-type Ad2 infection in the presence of E1A without IL-6 induction (Fig. 3 *A* and *B*, compare lane 24+ of dl312 with lane 24− of Ad2). The mRNAs of a control cellular gene, *GAPDH* encoding glyceraldehyde-3-phosphate dehydrogenase, showed no appreciable difference after IL-6 induction. As expected, the mRNAs for the L2 family, transcribed from the major late promoter of Ad after viral DNA replication, were increased in proportion to the increases of E2ae and E1B mRNAs in IL-6 induction. As in the case of viral DNA replication, there appeared to be no synergy of E1A and IL-6 induction in viral early mRNA synthesis (Fig. 3*A*). Although IL-6 induced comparable increases of E1B mRNA levels in wild-type virus and in dl312-infected cells 24 hr after infection, the IL-6 effect diminished markedly as viral infection progressed (Fig. 3*A*, compare + and − lanes at 24 hr and 36 hr of Ad2 infection).

Together, these results provided strong evidence that, in their native chromosomal configuration, E1A-dependent viral early genes were activated for RNA synthesis by an endogenous activity present in HepG2 cells and that this activity was regulated in the IL-6 signal transduction pathway independent of E1A.

E1A-Substituting Activity Functions Transcriptionally. Increases in the accumulation of steady-state E2ae and E1B mRNAs by IL-6 induction was undoubtedly amplified by increases in the number of viral templates once viral replication began, even at the earliest times after infection consistent with yielding significant signals by Northern analysis. To address whether the endogenous E1A-substituting activity functions at the transcriptional level, promoters of *E2ae* and *E1B* were linked to the reporter gene encoding CAT and were assayed for their activity in cells with varying E1A-

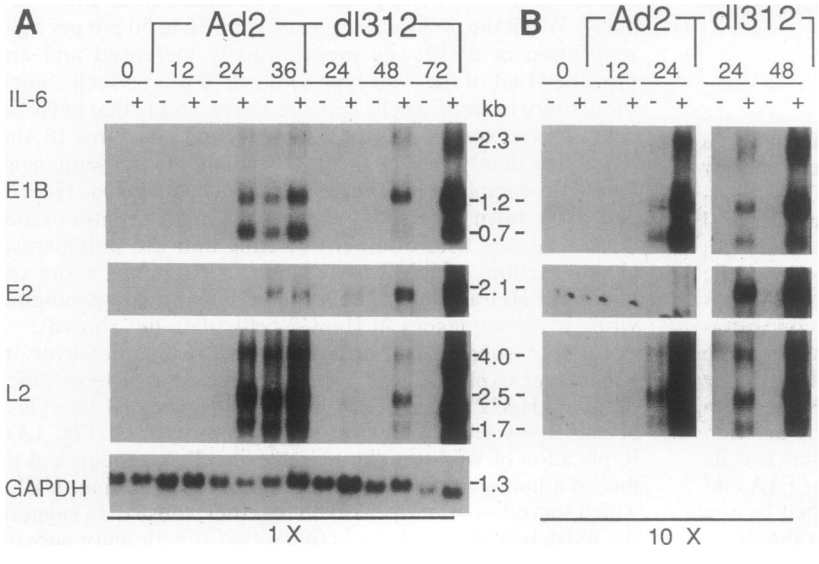

FIG. 3. IL-6 enhances accumulation of mRNAs encoded by E1A-dependent viral genes. Poly(A)$^+$ RNA from 1.0×10^7 HepG2 cells was isolated at the indicated hours after infection with Ad2 or dl312, with (+) or without (−) induction with IL-6 (500 units/ml) as indicated. Northern blot analysis with ^{32}P-labeled probe specific for GAPDH mRNA and AdE1B, E2, and L2 mRNAs was performed as described (5). The size of the RNAs in kb is indicated. "X" represents the relative length of exposure time of the autoradiographs.

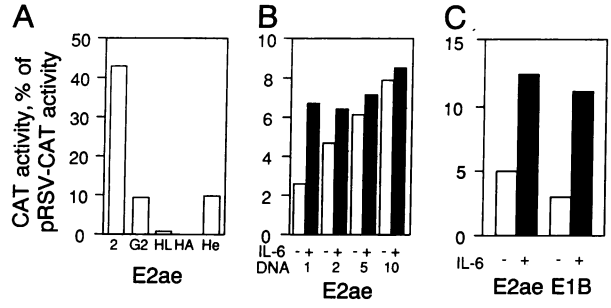

FIG. 4. Activation of *E2ae* and *E1B* promoters in HepG2 cells by IL-6 induction. (*A*) 293 (2), HepG2 (G2), HEL (HL), HA22T (HA), or HeLa (He) cells were transfected with pE2ae-CAT DNA (E2ae) (6 μg per 6 $\times$ 10^5 cells). (*B*) HepG2 (6 $\times$ 10^5) cells were transfected with increasing micrograms of pE2ae-CAT DNA as indicated, with (+) or without (−) induction by IL-6 (500 units/ml). (*C*) HepG2 (1 $\times$ 10^6) cells were transfected with 1 μg of pE2ae-CAT (E2ae) or pE1B-CAT (E1B) DNA with (+) or without (−) IL-6 induction. The cells were harvested 48 hr after transfection and were assayed for CAT activity as described.

substituting activities. Correlating with their abilities to support the replication of dl312, HepG2 and HeLa cells activated pE2ae-CAT expression to 15% of that of 293 cells—human embryonic kidney cells transformed with and constitutively expressing the *E1A* and *E1B* genes (Fig. 4*A*). HA22T and HEL cells, in which dl312 does not replicate, did not support significant *E2ae* promoter activity (Fig. 4*A*). Induction of HepG2 cells with IL-6 resulted in a reproducible 2- to 3-fold increase in the *E2ae* promoter activity, and this increase was inversely related to the amount of pE2ae-CAT transfected (Fig. 4 *B* and *C*). Likewise, the activity of the *E1B* promoter was increased 3- to 4-fold by IL-6 induction (Fig. 4*C*). Thus, the endogenous activity in HepG2 cells functionally substitutes for E1A by transcriptionally activating E1A-dependent early viral promoters.

Proteins in HepG2 Nuclear Extracts Form Distinct Complexes with E1A-Responsive Promoters. Transcriptional activation by the E1A-substituting activity in HepG2 cells suggested that nuclear factors in HepG2 cells may bind to E1A-responsive promoters and form DNA–protein complexes different in molar representation or composition from those formed by nuclear proteins in other cells. This was shown to be the case, at least in an *in vitro* binding assay (Fig. 5) in which binding of nuclear factors including E2F, thought to be important for the function of E1A-responsive *E2ae* promoter and the *E1A* promoter, was optimized (24). Regarding the *E2ae* promoter, as represented by the *E2* probe, which contains two E2F-binding sites, proteins present in HepG2 nuclear extracts formed complexes similar to those characterized in HeLa nuclear extracts (Fig. 5*A*, lane HeLa; ref. 24), except for a greater molar representation of a slowly migrating complex (Fig. 5*A*, G2 complexes in lanes HepG2 and HepG2/IL-6). These complexes were specific to the *E2*-probe and were not blocked by competition with a 100-molar excess of a *E1A* promoter probe (*E1*) or by a DNA probe containing the octamer sequence (lane Oct). Whether the G2 complexes formed in the presence of HepG2 and HeLa nuclear extracts are identical in composition remains to be examined. The G2 complex of HepG2 cells differed from the complexes formed in the presence of Ad-infected HeLa nuclear extracts in electrophoretic mobility (Fig. 5*A*, INF complexes in lanes HeLa/Ad; refs. 12, 13, and 32) and disappeared upon IL-6 induction, with the concomitant appearance of a more slowly migrating complex (Fig. 5*A*).

With respect to the promoter of the *E1A* gene, as represented by the *E1* probe, which contains one E2F site, formation of complex A was favored in the binding of proteins in HepG2 nuclear extracts (Fig. 5*B*, lanes IL-6), as compared with the predominant presence of complexes C and D, which resulted from binding of proteins in HeLa nuclear extracts (Fig. 5*B*, lane uninduced; ref. 24). Infection of HepG2 cells with Ad resulted in the diminution of complex A (Fig. 5*B*, lane HepG2 Ad) and the appearance of complexes distinct from those in Ad-infected HeLa cells (Fig. 5*B*, lane HeLa Ad; ref. 24). These results correlated transcriptional activation of E1A-responsive promoters by an endogenous activity in HepG2 cell with formation of specific protein–E1A-responsive promoter complexes by proteins in HepG2 nuclear extracts.

DISCUSSION

Two conclusions can be drawn from this study: (*i*) that an endogenous activity that can transcriptionally activate E1A-dependent viral promoters in the absence of E1A exists in HepG2 cells and (*ii*) that this activity is enhanced by IL-6. Transcriptional activation by this E1A-substituting activity leads to synthesis of viral early mRNAs and replication of viral DNA without the participation of *E1A* gene products. The >30-fold enhancement of accumulation of viral early mRNA and the 15-fold increases in viral DNA replication elicited by IL-6 apparently have their molecular basis in the activation of transcription of E1A-dependent viral early

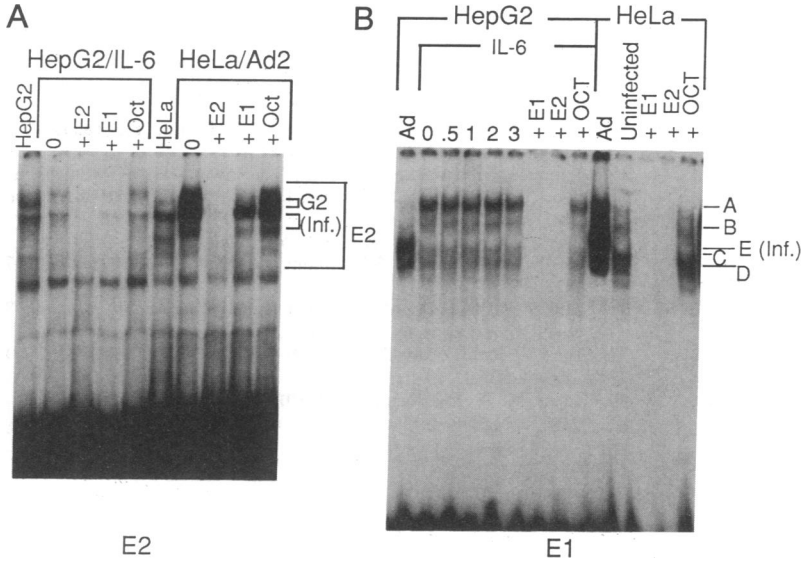

FIG. 5. Proteins in HepG2 and HeLa nuclear extracts bind to *E2ae* and *E1* promoters differently. (*A*) Nuclear extracts were prepared from HepG2 cells without or with IL-6 induction (500 units/ml for 2 days) and from HeLa cells without or with Ad-2 infection (18 hr after infection with 10 pfu per cell). In lanes 0, extracts were not preincubated with competitors. (*B*) Nuclear extracts were prepared from HepG2 and HeLa cells 24 and 18 hr, respectively, after Ad2 infection with 10 pfu per cell (lanes Ad), from uninfected HeLa cells (HeLa), and from HepG2 cells induced with IL-6 (500 units/ml) for the number of day(s) indicated. The *E2* probe (*A*) and *E1* probe (*B*) used for binding and competition and the octamer oligomer (Oct) used for competition were as described; "+" represents competition with the oligomer indicated. G2 and INF indicate HepG2 cell-specific and Ad infection-specific protein–DNA complexes, respectively. A–E, protein–DNA complexes.

genes. Thus, the E1A-dependence of viral early genes, which provides a severe constraint for expression of viral early genes and prevents the virus from replicating and entering the late cycle without E1A, can be abrogated by IL-6 signaling. This raises the question of the role of IL-6 in activating otherwise negligible expression of E1A-dependent and E1A-responsive genes physiologically. For example, in lymphoid cells, the most likely primary sites and carriers of Ad infection, the kinetics of Ad infection are markedly delayed relative to those in HeLa cells because of posttranscriptional regulation of *E1A* gene expression itself (5, 33). The *E1A* gene product has been shown to transactivate other viral and cellular genes. In those lymphoid cells with sufficient IL-6 receptors on their surface, can IL-6 enhance and accelerate infections by Ad and by other viruses?

Although the cDNAs encoding the two polypeptides comprising the high-affinity IL-6 receptors have been cloned (34, 35), the IL-6 signal transduction pathway is largely unknown. Signaling by IL-6 apparently leads to rapid and transient tyrosine phosphorylation of a 160-kDa cellular protein and activation of transcription of *tsl1* and *jun-B* genes in mouse B-cell lymphoma cells (36). Two results suggest that the E1A-substituting activity may be a nuclear component in the IL-6 signaling pathway: it functions transcriptionally and the patterns of protein–DNA complexes formed by HepG2 nuclear proteins with E1A-responsive promoters differ from those formed by HeLa nuclear proteins. The activity in HepG2 cells appears not to segregate with differentiated liver functions. However, it is enhanced by IL-6 induction, which also induces acute-phase genes in the liver and in cultured cells of liver origin. Is the E1A-substituting activity expressed in HepG2 cells due to cellular transformation, since HepG2 cells are derived from a hepatoblastoma? Can IL-6 induce this activity in cells not expressing the E1A-substituting activity? These questions and the question of whether the activity in HepG2 cells is related to the E1A-substituting activities expressed in early mouse development and embryonal carcinoma cells remain to be investigated.

Other cytokines such as cAMP and phorbol 12-myristate 13-acetate (PMA) have been shown to function in synergy with E1A (37–39) or independent of E1A (40) in a highly cell type-dependent manner. However, the participation of E1A is indispensable for the activity of E1A-dependent promoters during cAMP induction (37, 38). While PMA has been shown to replace the requirement of E1A in activating one of the E1A-responsive viral promoters, the *EIII* promoter, the PMA action is synergistic with E1A (39). In this regard, the magnitude of enhancement of mRNA synthesis from E1A-dependent viral early genes (Fig. 3), the full complementation of an *E1A*-deletion mutant in viral replication by IL-6, (Fig. 2), and the lack of synergy with E1A in IL-6 induction (Figs. 2 and 3) suggest that there is an IL-6-regulated activity present in HepG2 cells that has not yet been described. As a first step towards characterizing this E1A-substituting activity, we have found that a cDNA encoding an IL-6-regulated nuclear factor of the c/EBP family is sufficient to functionally substitute for the transactivating function of E1A when expressed in transfection (unpublished data).

We thank J. Bauer for providing liver explants; H. Baumann for the transfection protocol; G. Crabtree and K. Ullman for sharing the nuclear extract protocol prior to publication; T. Shenk, M. Marton, G. Acs, R. Banerjee, and members of Chen-Kiang laboratory for stimulating discussions; J. Bieker and J. Reddy for a critical reading of the manuscript; and L. Skinner for excellent preparation of the manuscript. J.M.S. acknowledges partial support from the Stanley Weil Fellowship. The work is supported by grants from National Institutes of Health (AI 24404), the American Cancer Society (IM 548), and a New Initiative in Immunology Award from the Irvington Medical Research Institutes to S.C.-K.

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