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Transcriptional and Posttranscriptional Regulation of Class I Major Histocompatibility Complex Genes following Transformation with Human Adenoviruses

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Transformation of rodent cells by human adenoviruses is a well-established model system for studying the expression, regulation, and function of class I antigens. In this report, we demonstrate that the highly oncogenic adenovirus type 12 operates at the transcriptional and posttranscriptional levels in regulating the activity of major histocompatibility complex class I genes and products in transformed cells. Adenovirus type 12 suppresses the cell surface expression of class I antigens in most cell lines. Nevertheless, in a number of cell lines suppression is the result of reduction in the amount of stable specific mRNA, while in another group of cell lines suppression involves interference with processing of a posttranscriptional product. The two mechanisms operate both for the endogenous *H-2* genes and for a miniature swine class I transgene that is expressed in the cells.

The major histocompatibility complex (MHC) class I genes play a key role in numerous immunological processes. Among these is the restricted recognition of foreign antigens by cytotoxic T lymphocytes (CTL) (35). It is therefore plausible that one of the mechanisms by which the immune system is able to control the growth of tumor cells depends on the presence of these antigens on the cell surface (17, 19).

The commonly accepted view is that lowered class I expression in adenovirus type 12 (Ad12)-transformed cells leads to escape from T-cell-mediated immune surveillance and is the cause of the increased oncogenicity of Ad12 (11, 16, 29, 31). Yewdell et al. (34) found a marked decrease in the susceptibility of influenza virus-infected Ad12-transformed cells to lysis by flu-specific CTL in comparison with Ad5-transformed cells. However, Mellow et al. (25) failed to find a similar decrease in susceptibility to allogeneic CTL among Ad12-transformed rat cell lines, and Haddada et al. (18) found no association between the level of class I antigens and tumorigenicity among adenovirus-transformed hamster cell lines.

The mechanism of regulation of class I genes following Ad12 transformation is also a subject to some controversy. Ad12 was reported to increase transiently the levels of class I transcripts following infection (28) but also to decrease the expression of class I antigens on transformed cells (29, 33). Down regulation of class I genes was reported to operate on the level of transcription (1, 15, 16, 21, 24) or maturation (32) of mRNA.

The reasons for the variable results found in different experimental systems are not clear. To further understand the regulatory mechanisms that operate to alter class I gene expression in Ad12-transformed cells, we infected and isolated a large panel of transformed cell lines that were derived from embryos of class I transgenic mice; these cell lines were recently described (12, 13). The transgene, which is a miniature swine class I gene (PD1) (14), was examined in parallel with the endogenous genes to determine whether the regulatory mechanisms under study are conserved among

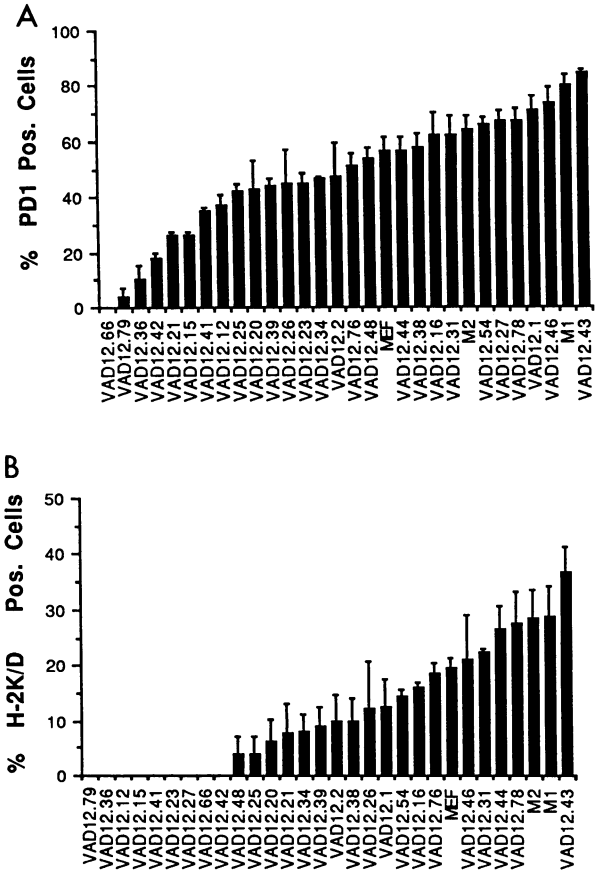

FIG. 1. Expression of class I MHC antigens by Ad12-transformed cell lines. Cell surface expression of PD1 (A) and H-2K^b/D^b (B) was analyzed by using the following antibodies: PT85A (10), 20-8-4S (recognizes a public determinant on H-2K^b and H-2D^b), and fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (Jackson ImmunoResearch Laboratories, Inc., ENCO, Jerusalem, Israel). Each cell line was tested four times. Results are mean relative number of positive cells ± standard error. M1 and M2, spontaneously transformed cell lines; MEF, mouse embryonal fibroblasts; VAD12, cell lines transformed by Ad12.

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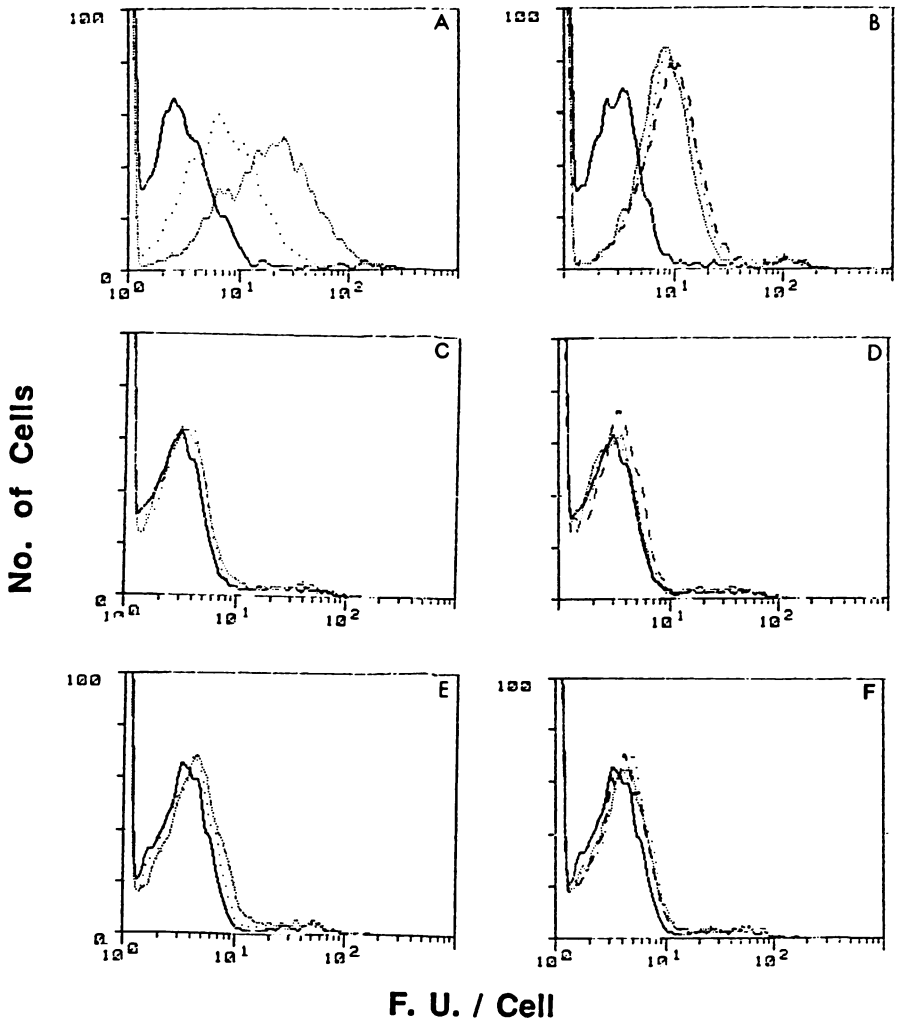

FIG. 2. General suppression of expression of class I MHC antigens and β2-microglobulin in Ad12-transformed cells. Cell surface expression of PD1, H-2K^b/D^b, and β2-microglobulin was analyzed as described in Materials and Methods. The following antibodies were used: (A, C, and E) PT85A (PD1; .....) and 20-8-4S (H-2K^b/D^b; . . . .); (B, D, and F) 28-14-8S (H-2D^b [2, 3, 26]; . . . .), B8-24-3 (H-2K^b [American Type Culture Collection]; .....), and mc-β2m-B, (β_2-microglobulin [7]; – – –). ——, background. Cell lines: (A and B) M1; (C and D) VAD12.36; (E and F) VAD12.44. F.U., fluorescein units.

different class I genes and among class I genes from different species and whether the positioning of the class I genes (which is the MHC for the endogenous genes but not for the transgene) has differential effects on their regulation. We demonstrate that in Ad12-transformed cells, regulation of the endogenous MHC class I genes and the transgene is highly complex and involves both transcriptional and post-transcriptional mechanisms.

Cell surface expression of H-2 and PD1 antigens following transformation with Ad12. Mouse embryonal fibroblasts from transgenic mice (PD1.C57BL/10) were infected with Ad12 (9, 23). The cells were kept in culture until colonies of transformed cells appeared, and individual colonies were picked and expanded (20). Twenty-seven cell lines transformed by the virus and two spontaneously transformed cell lines from the same pool of primary cells (30a) were tested for the expression of class I antigens. All of the Ad12-transformed cell lines but one (VAD12.66) have similar fully transformed phenotypes. The cells are not contact inhibited and form variable numbers of colonies in agar. The spontaneously transformed cells are contact inhibited. Figure 1 shows a large variability in the expression of class I antigens among the different cell lines. The variability is observed both for the endogenous antigens and for PD1. Nevertheless, 26 of the cell lines demonstrate weaker expression of the H-2K^b/D^b antigens than do the spontaneously transformed cell lines (M1 and M2), and 22 of the cell lines show weaker expression of these antigens than do primary embryonal fibroblasts. Twenty-two of the cell lines demonstrate weaker expression of PD1 than do the spontaneously transformed cell lines; in 18 cell lines, expression of these antigens is lower than in primary embryonal fibroblasts. Among the cell lines that are negative for cell surface expression of H-2 antigens, at least three (VAD12.27, VAD12.23, and VAD12.12) express significant levels of the PD1 antigen. These results indicate the existence of a common regulatory mechanism(s) for the two genes but also the existence of a regulatory mechanism that is gene or locus specific.

Coregulated expression of class I antigens and β2-microglobulin in transformed cell lines. To determine whether both

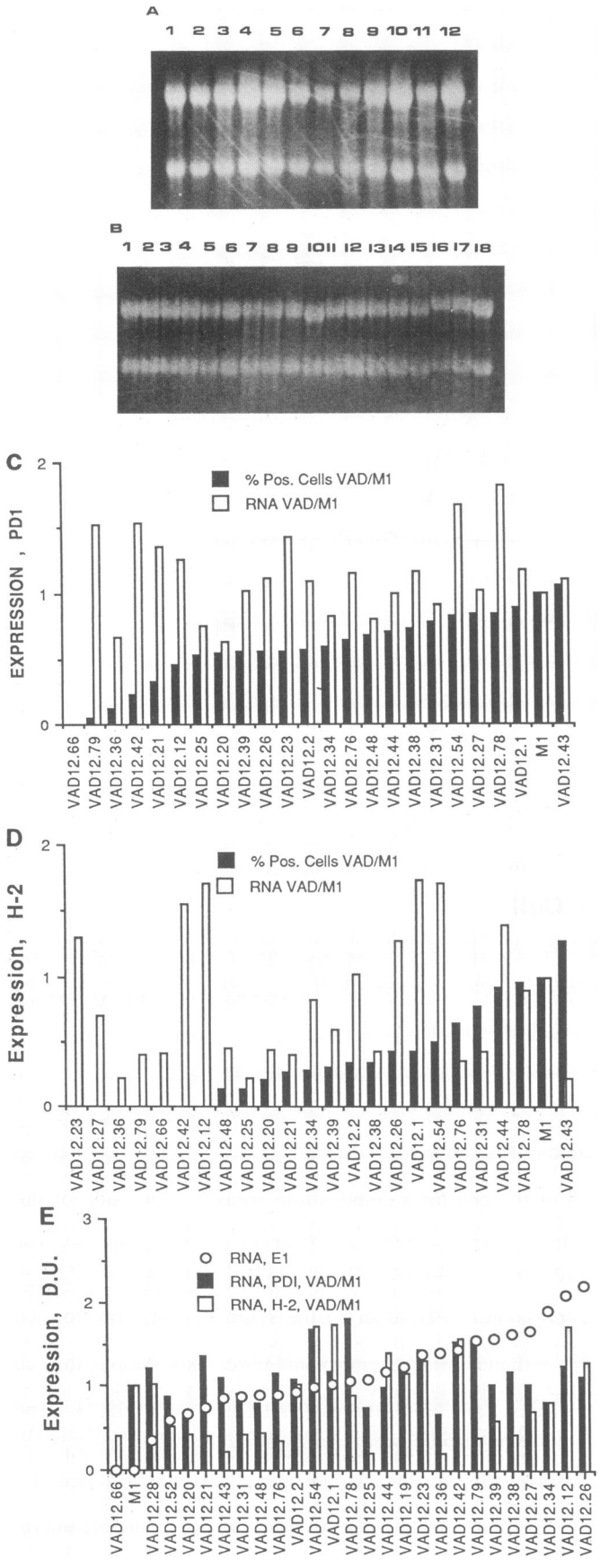

FIG. 3. Regulation of class I MHC genes in Ad12-transformed cell lines. Cytoplasmic RNA (15 μg) was loaded on formaldehyde-formamide gels and stained with ethidium bromide. (A) Lanes: 1, VAD12.39; 2, VAD12.26; 3, VAD12.44; 4, VAD12.42; 5, VAD12.12; 6, VAD12.52; 7, VAD12.28; 8, LPD1; 9, Ltk$^-$; 10, VAD12.78; 11, VAD12.25; 12, M1. (B) Lanes: 1, VAD12.1; 2, VAD12.23; 3, VAD12.27; 4, M2; 5, VAD12.2; 6, VAD12.36; 7, VAD12.48; 8, VAD12.34; 9, VAD12.54; 10, VAD12.19; 11, VAD12.20; 12, VAD12.38; 13, VAD12.31; 14, VAD12.79; 15, VAD12.43; 16, VAD12.21; 17, VAD12.66; 18, VAD12.76. (C to E) Expression of class I MHC genes in Ad12-transformed cell lines (Fig. 1) after densitometric scanning of the hybridization signals in the Northern (RNA) blot (see text), normalized to expression of the spontaneously transformed cell line, M1. Results are sorted according to the cell surface expression of either PD1 (C) or H-2K^b/D^b (D). (E) Results of mRNA expression sorted according to expression of E1A. D.U., densitometric units (results of densitometric scanning of the specific hybridizing signals from the relevant X-ray films).

H-2K and H-2D antigens are suppressed in the transformed cell lines, specific monoclonal antibodies against the two antigens and a monoclonal antibody against C57BL β2-microglobulin were used to analyze cell surface expression. Figure 2 demonstrates that the expression of all three antigens is suppressed in Ad12-transformed cell lines.

Transcriptional and posttranscriptional regulation of MHC class I genes in Ad12-transformed cells. Cytoplasmic RNA was prepared from transformed cell lines (5) (Fig. 3A and B) and hybridized with probes specific for H-2 (22), PD1 (30), E1 (an *Eco*RI fragment of pAd12 *Eco*RI-C), and actin (8) (results not shown). All of the cell lines but one (VAD12.66) express class I and E1 mRNA (Fig. 3). Additional hybridization with a β2-microglobulin probe showed that hybridization occurred with this probe as well (results not shown). The findings that (i) all of the cell lines express class I RNA and (ii) even in lines that express weakly, only a twofold decrease was seen in the amount of stable specific RNA raise the possibility that at least two types of regulatory mechanisms can occur in this system. To distinguish between the two groups of cell lines, we analyzed the results by normalizing both cell surface expression and hybridization signals following densitometric scanning to the expression of one of the spontaneous cell lines, M1. The results in Fig. 3 lead to the following conclusions. First, in four cell lines that express high levels of PD1 RNA (VAD12.79, VAD12.42, VAD12.21, and VAD12.12), expression of cell surface levels of PD1 antigen is very low (Fig. 3C). Second, in seven cell lines that express H-2-specific RNA, no expression of cell surface H-2^b antigens occurred (Fig. 3D). Third, all of the cell lines but one (VAD12.66) expressed similar levels of E1 RNA (Fig. 3E). Thus, the variability in expression of class I RNA and the suppression in cell surface expression of class I antigens cannot be directly attributed to variabilities in the levels of E1 expression. Expression of E4 sequences was not evident in any of the cell lines. Finally, both types of regulatory mechanisms appear to operate both for endogenous *H-2* genes and for the transgene, PD1.

To analyze the regulation of class I MHC genes following viral oncogenesis, we tested the effects of Ad12 transformation on the expression of these genes. We used primary cells from class I transgenic mice to investigate whether the position of the class I gene and its origin play a role in Ad12-mediated regulation. We screened a large panel of transformed cell lines for expression of the endogenous antigens and the transgene product. Our results establish

that most cell lines express lower levels of class I antigens than do primary embryonal fibroblasts and spontanously transformed cell lines. Thus, following transformation, the selected cells are those that express low levels of class I antigens.

The data presented in this report clearly show that both transcriptional and posttranscriptional mechanisms operate to regulate class I gene expression in Ad12-transformed cells. Preliminary data in our laboratory demonstrate that in the group of cell lines that express low levels of cell surface expression of class I antigens but high levels of class I mRNA, the class I MHC molecules are highly unstable. A mechanism that involves the binding of class I heavy chains in the endoplasmic reticulum by the adenovirus product E3/19K is known to exist following infection of susceptible cells with adenoviruses of the nononcogenic subgroups B, C, and D but not for viruses of the oncogenic group A, to which Ad12 belongs (4, 6, 27). It is possible that a similar mechanism exists in Ad12-transformed cells which express class I mRNA. Such cells escape the viral suppressive mechanisms that operate on the transcriptional level but are subjected to a second suppressive mechanism that is mediated by the viral antigens. Further experiments are under way to determine the nature of the common and specific regulatory elements and to identify the binding factors involved in both the transcriptional and posttranscriptional control of class I MHC genes in Ad12-transformed cells.

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