Abrogation of Cell Surface Expression of Human Class I Transplantation Antigens by an Adenovirus Protein in *Xenopus laevis* Oocytes

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ABSTRACT Class I transplantation antigens form complexes with a virus protein encoded in the early region E3 of the adenovirus-2 genome. The interaction between this viral glycoprotein, E19, and nascent human class I antigens has been examined by microinjecting purified mRNA into *Xenopus laevis* oocytes. Both E19 and the two class I antigen subunits, the heavy chain and β_2-microglobulin (β_2M), were efficiently translated. The heavy chains did not become terminally glycosylated, as monitored by endoglycosidase H digestion, and were not expressed on the oocyte surface unless they were associated with β_2M. The E19 protein did not become terminally glycosylated, and we failed to detect this viral protein on the surface of the oocytes. Co-translation of heavy chain and E19 mRNA demonstrated that the two proteins associate intracellularly. However, neither protein appeared to be transported to the *trans*-Golgi compartment. Similar observations were made in adenovirus-infected HeLa cells. Heavy chains bound to β_2M became terminally glycosylated in oocytes in the presence of low concentrations of E19. At high concentrations of the viral protein, no carbohydrate modifications and no cell surface expression of class I antigens were apparent. Thus, β_2M and E19 have opposite effects on the intracellular transport of the heavy chains. These data suggest that adenovirus-2 may impede the cell surface expression of class I antigens to escape immune surveillance.

Major histocompatibility antigens regulate various cell–cell interactions of importance to the immune system (1). This is exemplified by the recognition of viral antigens by cytotoxic T-lymphocytes, which only occurs provided that the foreign molecules are in juxtaposition to major histocompatibility complex class I antigens (2). Whether this requirement signifies that the two types of molecules actually are in physical contact is as yet unresolved (3, 4).

It has been shown that class I antigens interact with an adenovirus-2 protein (3–8). This viral protein is one of the few that associate with class I antigens to form complexes stable enough to allow for their isolation (5, 7). The adeno-protein is encoded in the early region E3 of the viral genome (9–11). Due to its origin and the molecular weight of the polypeptide portion, which is ~19,000 (12), this protein is called E19. However, E19 contains two Asn-linked carbohydrate moieties (13) so the glycosylated protein has an apparent molecular weight of ~25,000. In vivo it exists predominantly as a dimer.

Class I antigen–E19 complexes are held together by noncovalent interactions only, but the precise nature of the association is not understood. However, preliminary studies indicate that it is the glycosylated, membrane-spanning heavy chain of the class I antigens that binds E19 (7).

Complex formation between class I antigens and E19 may be initiated in the endoplasmic reticulum, since all polypeptides of the complex are manufactured by membrane-bound ribosomes (14). Such an association may affect the intracellular transport of individual constituents of the complex. This is particularly likely as regards the class I antigens since the interaction between β_2-microglobulin (β_2M)[1] and heavy chains regulates the intracellular transport of these molecules (15, 16). We have examined the biogenesis of the class I antigen–E19 complexes by microinjecting relevant mRNA into *Xenopus laevis* oocytes, which previously have been shown to faithfully reproduce the intracellular transport of class I antigens in mammalian cells (17). In this paper, we demonstrate that in oocytes the E19 protein abrogates the intracellular transport of class I antigens.

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[1] *Abbreviations used in this paper:* β_2M, β_2-microglobulin; DME, Dulbecco's modified Eagle's medium; Endo H, endoglycosidase H.

MATERIALS AND METHODS

Antisera: A rabbit antiserum against human class I antigens was rendered specific for the heavy chain by extensive absorptions on a column containing β_2M (18). A previously described rat monoclonal antibody against β_2M was used (7). A mouse monoclonal antibody against E19 (a kind gift of Mats Andersson and Carmen Fernandez, manuscript in preparation) was also used.

Isolation of mRNA and Cell-free Translation: Human class I antigen heavy chain mRNA and β_2M mRNA were separately isolated from Raji cells. E19 mRNA was obtained from HeLa cells 6–7 h postinfection with 10,000 adeno-2 virions per cell. A detailed description has been published (14). Briefly, microsomes were isolated from lysed cells, and the microsomal RNA was extracted by phenol/chloroform/isoamylalcohol (49:49:2, vol/vol/vol). Poly A–containing mRNA was isolated by two cycles of oligo dT column chromatography and then size fractionated by sucrose-density gradient centrifugation. The distribution in the sucrose gradient fractions of mRNA coding for the class I antigen subunits and the E19 protein, respectively, was monitored by subjecting aliquots of each fraction of the various gradients to cell-free translation. A previously described rabbit reticulocyte lysate translation system containing dog pancreas microsomes was used (19).

Fractions containing mRNA coding for only one of the polypeptides were used individually or in various combinations for the microinjection experiments.

Purification of mRNA by Hybridization Selection: The EcoRI-D fragment of the adenovirus-2 genome, encompassing the sequence coding for the E19 protein (12), was cloned into pBR322. Plasmid DNA was isolated and immobilized onto nitrocellulose filters as described (20). To one-half of the filter, 2.5 μg of mRNA was hybridized according to the protocol of Ricciardi et al. (21). mRNA, eluted from the filter after extensive washings, was used for microinjection into *Xenopus* oocytes.

The purity of the hybridization-selected E19 mRNA fraction was assessed by microinjecting aliquots into oocytes, which then were labeled with [^{35}S]-methionine (Amersham Corp., Amersham, Buckinghamshire, England) for 24 h. The total glycoprotein fraction of the labeled oocytes was analyzed by SDS PAGE. Noninjected, labeled oocytes served as the control. By this procedure, it was established that the purified E19 mRNA fraction gave rise to a single extra component with an apparent molecular weight of 25,000, as compared with noninjected oocytes (not shown).

Microinjection into Oocytes: Large *Xenopus laevis* females were injected with 1,000 IU of human chorionic gonadotropin (Sigma Chemical Co., St. Louis, MO) 1–2 wk before they were killed and the oocytes isolated. In a typical experiment, 30–40 oocytes were microinjected with 50 nl each of a mRNA fraction or combinations of mRNA fractions containing 200–500 μg mRNA/ml of distilled water. If not otherwise stated, the oocytes were then incubated in 150 μl of Bart's medium (22) containing 0.2 mCi [^{35}S]methionine at 19°C for 20–24 h. After the labeling period, the oocytes were homogenized in 0.5 ml of ice-cold 20 mM Tris-Cl buffer, pH 8.0, 1% Triton X-100, 0.15 M NaCl, and 1% Trasylol (Bayer AG, Leverkusen, FRG) in a small Dounce homogenizer, and the suspension was centrifuged for 15 min at 10,000 g. Glycoproteins in the supernatants were usually enriched by chromatography on Lens Culinaris hemagglutinin–Sepharose 4B columns (23). Bound glycoproteins were desorbed by the addition of 10% α-methylmannoside to the eluant, which was identical to the lysis buffer, and subjected to indirect immunoprecipitation.

Oocyte Surface Expression of Translation Products: To assess the expression on the oocyte surface of translation products from microinjected mRNA, a modification of a previously described method was used (24). Injected oocytes, incubated with [^{35}S]methionine for 20–24 h, were washed with Bart's medium and defolliculated by incubation for 50 min at 22–24°C with 2 mg/ml of collagenase (Worthington Biochemical Corp., Freehold, NJ) in 0.1 M phosphate buffer, pH 7.6. After extensive washing in Bart's medium and a final wash with buffer B (50 mM phosphate buffer pH 7.5, 80 mM NaCl, 0.5% bovine serum albumin), aliquots of the oocyte fraction were separately incubated in the cold for 60 min with dilutions, ranging from 1:10 to 1:20, of the various antisera made up in buffer B. The oocytes were washed five times with buffer B and then homogenized as described above. Immediately after homogenization, the material was centrifuged for 15 min at 10,000 g, and 50 μl of a 20% suspension of formalin-fixed *Staphylococcus aureus* Cowan I strain bacteria was added to the supernatant. After 30 min, the bacteria were extensively washed according to a standard protocol (16) and the adsorbed material was subjected to electrophoresis. After the first immunoprecipitation, the resulting supernatant was subjected to indirect immunoprecipitation. Material recovered in this second immunoprecipitation was presumed to be located inside the cell.

Cell Culture Growth, Adenovirus-2 Infection, and Radioactive Labeling: Monolayer cultures of HeLa cells were grown in Dulbecco's modified Eagle's medium (DME, Gibco Laboratories, Grand Island, NY) containing 2.5% normal calf serum, 2.5% fetal calf serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin in flat-bottom tissue culture flasks (Costar, Cambridge, MA). Infections of HeLa cells by human adenovirus-2 were carried out with ~1 × 10^4 virions per cell using 2 × 10^6 cells growing in a 25-cm^2 flat-bottom tissue culture flask. The virus particles were added to subconfluent cell monolayers in serum-free medium. After 1 h, normal serum-containing medium was added, and the cells were incubated further for 3 h before they were labeled with [^{35}S]methionine.

Cells were washed once with phosphate-buffered saline (pH 7.4) before addition of methionine-free DME supplemented with 4 mM glutamine, 5% dialyzed fetal calf serum, and 0.25 mCi [^{35}S]methionine. Pulse–chase experiments were performed by labeling the cells for 10 min, and then cells were either solubilized at 0°C or washed and further incubated in the normal culture medium containing 20 mM of unlabeled methionine. The incubation was terminated by lysing the cells with 0.02 M Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl, 1% Triton X-100, and 1% Trasylol. After 30 min at 0°C, cell debris was removed by centrifugation at 10,000 g for 15 min at 4°C. The supernatants were enriched for glycoproteins on Lens Culinaris hemagglutinin–Sepharose 4B columns (23), and the eluted material was subjected to indirect immunoprecipitation.

Immunoprecipitations and Electrophoretic Analyses: Indirect immunoprecipitations were done according to a standard protocol (16). Immunoprecipitated proteins were separated by gradient slab SDS PAGE, essentially as described by Blobel and Dobberstein (25). Ovalbumin (molecular weight 46,000), carbonicanhydrase (molecular weight 30,000), and lysozyme (molecular weight 14,300) were always run in parallel as marker proteins. Gels were fixed in 10% trichloroacetic acid for 15 min at 4°C, treated with Enhancer (New England Nuclear, Boston, MA) for 60 min, and finally soaked in water containing 1% glycerol for 15–30 min. After drying, fluorography of the gels was performed as described (26). Kodak XAR film was used throughout. For quantitation of protein bands, fluorographs were scanned using a Beckman Densitometer (Beckman Instruments, Inc., Palo Alto, CA).

In accordance with previous studies (16, 17), terminally glycosylated heavy chains have been assigned an apparent molecular weight of 45,000, whereas core glycosylated and endoglycosidase H (Endo H)–digested heavy chains display apparent molecular weights of 43,000 and 41,000, respectively. The E19 protein exhibits an apparent molecular weight of 25,000 when fully glycosylated, but is called E19 to adhere to the nomenclature used earlier (4–8).

Enzyme Digestion: Asn-linked core-sugar moieties were removed from immunoprecipitated proteins using Endo H (Seikagaku Kogyoco, Tokyo, Japan) according to the protocols previously described (16).

RESULTS

Translation of E19 mRNA in the Absence and Presence of mRNA of Class I Antigen Subunits

To examine the translation and processing of the adenovirus E19 protein, *Xenopus* oocytes were microinjected with mRNA encoding that protein. The oocytes were labeled with [^{35}S]methionine for 1 h and after various periods of chase, accomplished in the presence of 20 mM methionine, samples were withdrawn and glycoproteins were isolated and subjected to indirect immunoprecipitation using a monoclonal antibody against the E19 protein. The immunoprecipitates were then analyzed by SDS PAGE. After the labeling period, the E19 protein gave rise to a single electrophoretic band (Fig. 1, lane *A*). After a chase period of 3 h, the major E19 band displayed a slightly increased electrophoretic mobility, and an additional, lower molecular weight band emerged (Fig. 1, lane *B*). After chase periods of 8 and 24 h (Fig. 1, lanes *C* and *D*), respectively, the same two bands were apparent as after 3 h of chase. The E19 protein apparently became degraded considerably during the chase period. Thus, only a minor fraction of the radioactivity present in the E19 band at the end of the labeling period remained immunoprecipitable throughout the entire chase period (compare lane *D* with lane *A* of Fig. 1).
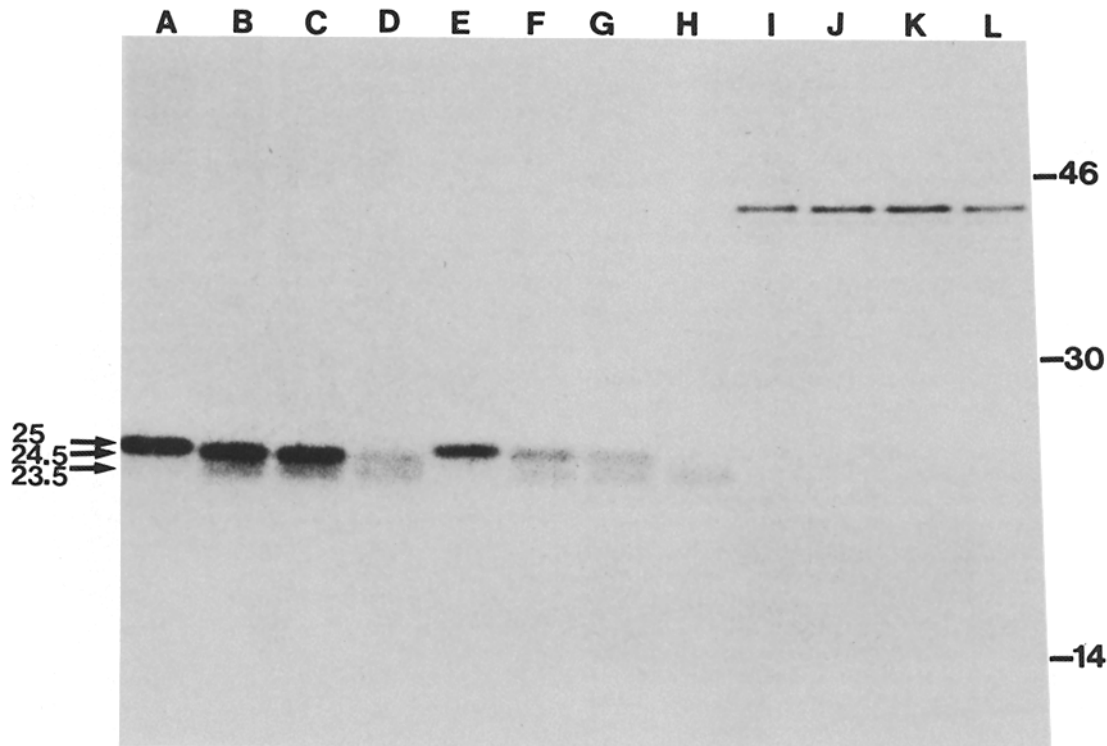
FIGURE 1 Biosynthesis of the E19 protein in oocytes in the absence and presence of class I transplantation antigens. Oocytes were microinjected with a fraction enriched for E19 mRNA alone (lanes A–D) and combined with fractions enriched for heavy chain and β₂M mRNA (lanes E–L). The oocytes were labeled with [³⁵S]methionine for 1 h, washed, and chased in the presence of 20 mM methionine for various periods of time. Samples were withdrawn, glycoproteins were isolated and precipitated with an anti-E19 monoclonal antibody (lanes A–H) or with an anti-heavy chain serum (lanes I–L). Samples withdrawn at the end of the labeling period are shown in lanes A, E, and I; after 3 h of chase in lanes B, F, and J; after 8 h of chase in lanes C, G, and K; and after 24 h of chase in lanes D, H, and L. The figures denote apparent molecular weights in kilodaltons.

To examine whether mRNA encoding class I antigen subunits would affect the translation and processing of the E19 protein, mRNA encoding the E19, class I antigen heavy chains, and β₂M were mixed before injection into the oocytes. The electrophoretic analyses are summarized in Fig. 1 (lanes E–L). It can be seen that in the presence of class I antigens, the E19 protein bands behaved qualitatively identical to the translation products obtained in the absence of class I antigens (compare lanes A–D with lanes E–H of Fig. 1). However, the shift toward E19 species with enhanced electrophoretic mobilities was more pronounced in the presence than in the absence of class I antigens. Furthermore, the degradation of the E19 protein seemed to increase by the presence of the class I antigens, which themselves, as revealed by the immunoprecipitated heavy chains, were only slowly eliminated during the chase period (Fig. 1, lanes I–L). In any event, these data demonstrate that the E19 protein is efficiently translated by Xenopus oocytes also in the presence of class I antigens. The two new species of E19 that appear during the chase period are fully consistent with the appearance of the E19 protein in mammalian cells after adenovirus infection. Since the E19 protein contains two Asn-linked carbohydrate moieties (13), the novel forms of the protein may represent molecules whose carbohydrate moieties are in the process of being trimmed. In the presence of class I antigens, the conversion of E19 to the apparently lower molecular weight forms is more complete than in the absence of transplantation antigens.

Effect of E19 on the Terminal Glycosylation of Class I Antigen Heavy Chains

In the previous experiments, neither E19 nor class I antigen heavy chains displayed increased apparent molecular weights during the chase period. Thus, no evidence for terminal glycosylation of either protein was obtained. To directly address this issue, we examined whether E19 and class I antigens, separately and together, reach the trans-Golgi compartment. This was accomplished by monitoring the susceptibility of the carbohydrate moieties of the two proteins to digestion by Endo H. This enzyme digests core sugar moieties but not terminally glycosylated oligosaccharides (27).

Oocytes were injected with class I antigen heavy chain mRNA alone or together with β₂M mRNA. The microinjected oocytes were labeled for 26 h, and glycoproteins were isolated and subjected to indirect immunoprecipitation with use of an anti–heavy chain serum. Part of the immunoprecipitates were treated with Endo H before SDS PAGE analysis. Fig. 2 (lanes A and B) shows that class I antigen heavy chain mRNA was translated into a single electrophoretic heavy chain species whose carbohydrate moiety was fully susceptible to Endo H digestion. In contrast, heavy chain mRNA translated in the presence of β₂M produced two closely spaced protein bands, of which the major one with the highest molecular weight was resistant to the enzymatic treatment (Fig. 2, lanes C and D). A similar experiment, in which E19 mRNA was substituted for the mRNA of the class I subunits,
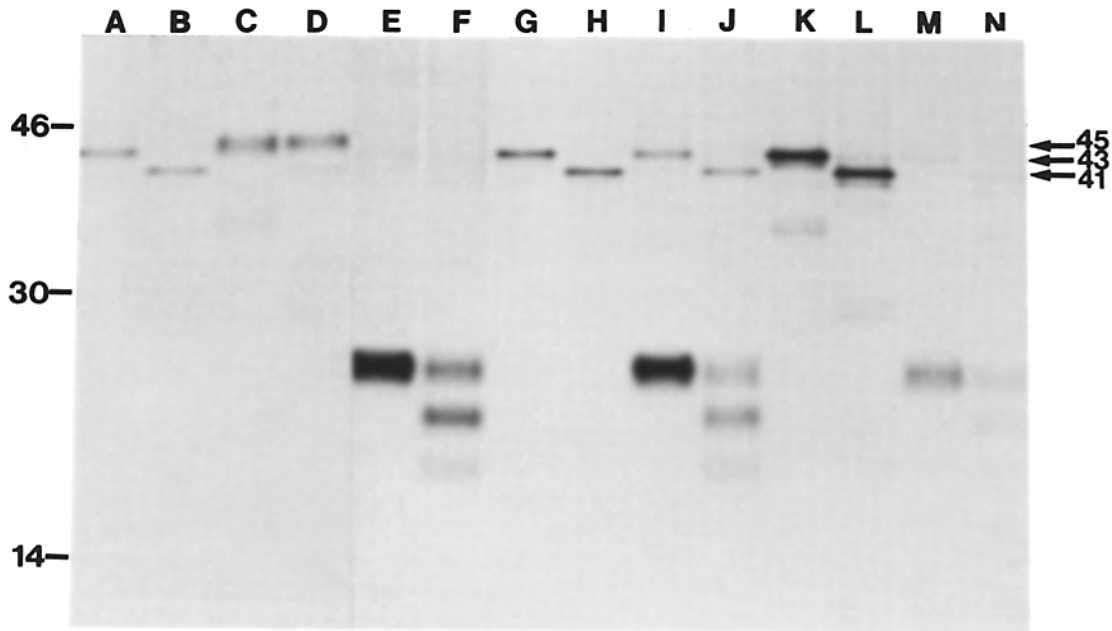
FIGURE 2   Influence of the E19 protein on the terminal glycosylation of class I antigens. Oocytes were microinjected with heavy chain mRNA alone (lanes A and B) and together with β_2M mRNA (lanes C and D). E19 mRNA was also used alone (lanes E and F) and together with heavy chain mRNA in the absence (lanes G-J) and presence of β_2M mRNA (lanes K-N). After 26 h of [^{35}S]-methionine labeling, glycoprotein fractions were isolated and immunoprecipitated with an anti-heavy chain serum (lanes A-D and G, H, K, and L) and with an anti-E19 monoclonal antibody (lanes E, F, I, J, M, and N), respectively. Part of each immunoprecipitate was treated with Endo H (lanes B, D, F, H, J, L, and N), while the remainder served as the control (lanes A, C, E, G, I, K, and M). mRNA present in the fraction enriched for β_2M mRNA gave rise to the contaminants visualized in lanes C, D, K, and L (see reference 17). Figures denote apparent molecular weights in kilodaltons.

revealed that the viral protein immunoprecipitated with an E19 monoclonal antiserum was partly susceptible to Endo H–induced carbohydrate elimination. Thus, Fig. 2 (lanes E and F) demonstrates that after enzymatic treatment, E19 resolved into three equally spaced electrophoretic bands. Although the pattern of the E19 bands suggests incomplete digestion, a fivefold increase of the Endo H concentration produced results identical to those shown.

These data are consistent with the notion that class I antigen heavy chains are transported to the *trans*–Golgi compartment only if they are associated with β_2M (15–17). However, since the majority of the E19 molecules were sensitive to the enzyme Endo H, these observations do not warrant any definite conclusions about the putative transport of E19 beyond the *cis*–Golgi complex.

The effect of the E19 protein on the terminal glycosylation of the class I antigens was examined by injecting various combinations of mRNA into oocytes, which were then processed as described above. Co-translation of heavy chains and E19 did not promote terminal glycosylation of the individual chains. This can be inferred from the observation that both heavy chains and E19 were as susceptible to Endo H digestion as when translated individually (in Fig. 2, compare lanes A and B with G and H, and lanes E and F with I and J). Despite the fact that the particular antiserum used against the heavy chains did not co-precipitate noticeable amounts of the E19 protein, the two types of molecules do indeed associate. This was established by the monoclonal antibody against E19, which co-precipitated at least a fraction of the heavy chains (see Fig. 2, lanes I and J).

Also, when mRNA encoding β_2M was mixed with the E19 and heavy chain mRNA and injected into oocytes, the ter-

minal glycosylation of the class I antigen heavy chains was inhibited. Fig. 2 (lanes K and L) demonstrates that virtually all immunoprecipitated heavy chains were sensitive to Endo H digestion. Likewise, Endo H treatment of molecules immunoprecipitated by the monoclonal antibody against E19 revealed a very similar if not identical electrophoretic pattern to that obtained when E19 was translated alone (in Fig. 2, compare lanes E and F with M and N). The monoclonal antibody co-precipitated small amounts of heavy chains, whereas the anti–heavy chain serum did not bring down measurable amounts of the E19 protein. Despite these weak to insignificant co-precipitations, it seems reasonable to conclude that the class I antigens and the E19 protein interacted.

The experiments described above were repeated, but rather than using a fraction enriched for E19 mRNA, we used hybridization-selected E19 mRNA. Translation of this mRNA in oocytes gave rise to only one detectable product apart from the endogeneous proteins. The hybridization-selected mRNA alone and co-translated with mRNA encoding the class I antigen subunits generated results identical to those depicted in Fig. 2. The translated viral protein also displayed susceptibility towards Endo H digestions identical to that shown in the figure. These data unequivocally demonstrate that it is the E19 protein and no other virus products that impedes the terminal glycosylation of the class I antigen heavy chains.

Class I Antigen Heavy Chains Do Not Become Terminally Glycosylated in Adenovirus-2-infected HeLa Cells

To verify that the E19 protein affects the terminal glycosyl-

ation of class I antigen heavy chains also in mammalian cells, HeLa cells were infected with adenovirus-2. Thus, mock- and virally infected cells were labeled with [^{35}S]methionine for 10 min and then chased with an excess of unlabeled methionine for 2 h. Samples were withdrawn at the beginning and end of the chase period, glycoproteins were isolated, and class I antigens were indirectly immunoprecipitated. Before SDS PAGE analysis, part of each sample was treated with Endo H. Fig. 3 summarizes the results. It can be seen that at the beginning of the chase period, the majority of the class I antigen heavy chains were susceptible to the enzymatic treatment regardless of whether the chains were derived from mock (Fig. 3, lanes A and B) or virally (Fig. 3, lanes E and F) infected cells. After 2 h of chase, the heavy chains of the mock-infected cells were completely resistant to the Endo H digestion (Fig. 3, lane D) while at the same time in the virally infected cells, all heavy chains appeared to remain in the high mannose form as evidenced by their sensitivity to the enzyme (Fig. 3, lane H). The E19 protein, which co-precipitated with the class I antigens, was virtually completely susceptible to the Endo H digestion at both times investigated. These data demonstrate that class I antigen heavy chains in an adenovirus-infected mammalian cell display abrogated terminal glycosylation in a way apparently identical to that encountered in the oocytes (see above).
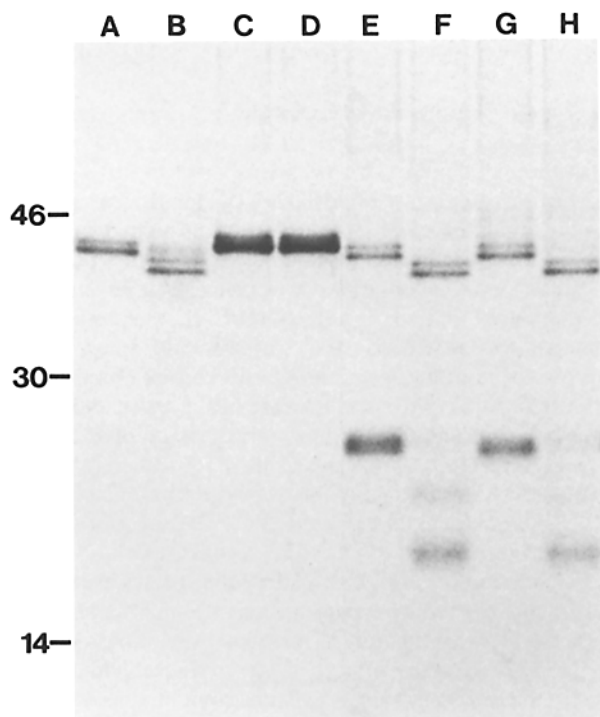

FIGURE 3   Effect of the E19 protein on the terminal glycosylation of class I antigen heavy chains in adenovirus-2-infected HeLa cells. HeLa cells were mock-infected or infected with adenovirus-2 for 4 h. Mock-infected (lanes A-D) and infected HeLa cells (lanes E-H) were labeled with [^{35}S]methionine for 10 min and either immediately chilled to 0°C (lanes A, B, E, and F) or chased for 2 h with an excess of unlabeled methionine (lanes C, D, G, and H) before being lysed. Glycoprotein fractions were immunoprecipitated with an anticlass I heavy chain serum. Before SDS PAGE, half of each sample was digested with Endo H (lanes B, D, F, and H). Figures denote apparent molecular weights in kilodaltons.

Cell Surface Expression of Class I Antigens in the Presence and Absence of the E19 Protein

The experiments described above do not rule out that the E19 protein becomes transported beyond the cis-Golgi compartment despite lack of evidence of terminal glycosylation of its carbohydrate moieties. Likewise, the experiments do not formally rule out that the E19 protein impairs the terminal glycosylation of the class I antigen heavy chains without affecting their intracellular transport. To examine these possibilities, we explored the cell surface expression of the two proteins. Consequently, oocytes were separately injected with mRNA encoding heavy chains, heavy chains and β_2M, heavy chains and β_2M and E19, heavy chains and E19, and E19 alone, respectively. After labeling with [^{35}S]methionine for 24 h and removal of visually damaged oocytes, the cells were defolliculated and the cell surface expression of heavy chains and E19 was examined as described in Materials and Methods. To avoid unnecessary dilution of the samples, no lectin-column chromatography step preceded the immunoprecipitations. Fig. 4 summarizes the results. While heavy chains were efficiently translated in the absence of β_2M (Fig. 4, lane H), they could, as expected, not be discovered on the oocyte cell surface (Fig. 4, lane A). This result ascertained that the adopted methodology discriminated between intracellular and cell surface–expressed heavy chains.

After translation in the presence of β_2M, two distinct heavy chain species could be discerned on the cell surface (Fig. 4, lane B). In addition to the major core-glycosylated component, a slightly higher molecular weight form of the heavy chains was evident in the intracellular fraction (Fig. 4, lane I). The small amounts of heavy chains in the cell surface fraction may have represented contaminating intracellular molecules, although it is known that the oocyte system may give poor cell-surface expression also of other proteins (see reference 24). To rule out the possibility of contamination, the experiment was repeated. However, before SDS PAGE analysis, the immunoprecipitated heavy chains were subjected to Endo H digestion. Whereas all heavy chains in the cell surface fraction were unaffected by the enzymatic treatment, ~40% of the heavy chains in the intracellular fraction displayed enhanced electrophoretic mobility upon Endo H digestion. Thus, these data make it unlikely that the heavy chains in the cell surface fraction were contaminants derived from the intracellular fraction.

The E19 protein was also efficiently translated in the oocytes (Fig. 4, lane J) but if present on the oocyte surface, it was below the level of detection (Fig. 4, lane C). A consequence of these observations was that translation of mixtures of heavy chain and E19 mRNA did not promote measurable cell surface expression of either polypeptide (Fig. 4, lanes D and E). When the two class I antigen subunits were translated in the presence of E19, neither heavy chains (Fig. 4, lane F) nor E19 (Fig. 4, lane G) could be detected at the cell surface. The heavy chains present in the intracellular fraction did not display the electrophoretically more slowly migrating band that corresponds to terminally glycosylated molecules (compare lane I with lane M of Fig. 4).

From these data, it may be suggested that in contrast to class I antigens, the E19 protein is not transported to the occyte cell surface. Moreover, the E19 protein seems to abolish the cell surface expression of the class I antigens.
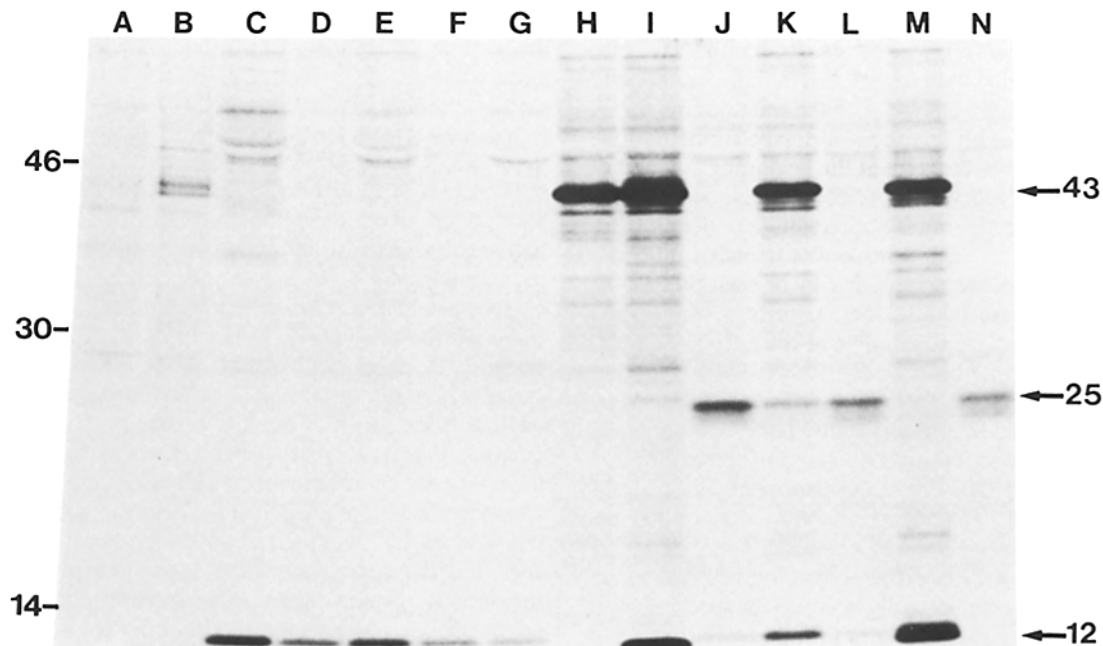
Figure 4 Abrogation of cell surface expression of class I antigens by the E19 protein. Oocytes were microinjected with heavy chain mRNA alone (lanes A and H) and together with β_2M mRNA (lanes B and I). Separately, oocytes were injected with E19 mRNA alone (lanes C and J) and together with heavy chain mRNA in the absence (lanes D, E, K, and L) and presence (lanes F, G, M, and N) of β_2M mRNA. After incubation with [^{35}S]methionine for 24 h, the oocytes were processed as described in Materials and Methods. In lanes A, B, D, F, H, I, K, and M the anti-heavy chain serum was used and in lanes C, E, G, J, L, and N an anti-E19 monoclonal antibody was used. Lanes A-G depict proteins located on the oocyte surface and lanes H-N, intracellular proteins. Contaminating proteins are present due to the fact that no enrichment of glycoproteins on the Lens Culinaris hemagglutinin columns had been performed. In lanes C-G and J-N, a contaminating protein with an apparent molecular weight slightly greater than that of β_2M is evident. This component, which was precipitated by nonimmune serum, was derived from the E19 mRNA fraction since it was absent when hybridization-selected E19 mRNA was used. Figures denote apparent molecular weights in kilodaltons.

Quantitative Aspects of the Interaction between E19 and Class I Antigens

To gain further insight into the quantitative effects of β_2M and E19 on the intracellular processing of the heavy chain, oocytes were injected with constant amounts of mRNA encoding heavy chains and increasing amounts of β_2M mRNA in the absence and presence of two different concentrations of E19 mRNA. Glycoproteins were isolated from the microinjected and [^{35}S]methionine-labeled oocytes. Heavy chains were precipitated with an anti-heavy chain serum, and part of each immunoprecipitate was treated with Endo H before analysis by SDS PAGE. The relative amounts of Endo H-resistant heavy chains, expressed as the percentage of total heavy chains precipitated, were estimated by densitometry. Fig. 5 shows that on increasing the amounts of β_2M mRNA injected, progressively more heavy chains became resistant to the enzymatic treatment. In the presence of low amounts of E19 mRNA, β_2M also promoted the generation of terminally glycosylated heavy chains, but the effect was considerably less pronounced than in the absence of the virus protein. At higher concentrations of the viral mRNA, β_2M could not confer Endo H resistance to the heavy chains (Fig. 5).

In a similar set of experiments, constant amounts of mRNA encoding class I antigen heavy chains and β_2M were mixed with various amounts of E19 mRNA before injection into oocytes. Glycoproteins, labeled with [^{35}S]methionine, were immunoprecipitated with antibodies reactive with heavy chains, and part of each immunoprecipitate was treated with

Endo H. The amount of β_2M mRNA used in these experiments was sufficient to ascertain that approximately half of the heavy chains became terminally glycosylated. When translated in the presence of increasing amounts of E19 mRNA, the fraction of Endo H-resistant heavy chains progressively decreased (Fig. 6). These data show that β_2M and E19 have opposite effects on the terminal glycosylation of class I antigen heavy chains. It is also obvious that heavy chains associated with β_2M exhibit abolished terminal glycosylation if the concentration of E19 is sufficiently high.

DISCUSSION

The interaction between E19 and class I transplantation antigens has hitherto been examined mainly in an adenovirus-transformed rat fibroblast cell line (5, 7). These cells produce considerably less E19 protein than do acutely adenovirus-infected human cells. Since the noncovalent interaction between the viral protein and the class I antigens is concentration dependent, we have explored the intracellular interaction between the two proteins by microinjecting purified mRNA fractions into oocytes. This experimental system provides the possibility to vary the concentrations of the mRNA encoding the various polypeptides. Moreover, the phenotypic changes induced by infection and transformation, which may indirectly affect the binding and intracellular behavior of class I antigens and E19, are avoided in the oocyte system.

The intracellular transport of class I antigen heavy chains in *Xenopus laevis* oocytes is regulated by β_2M (17) as is the
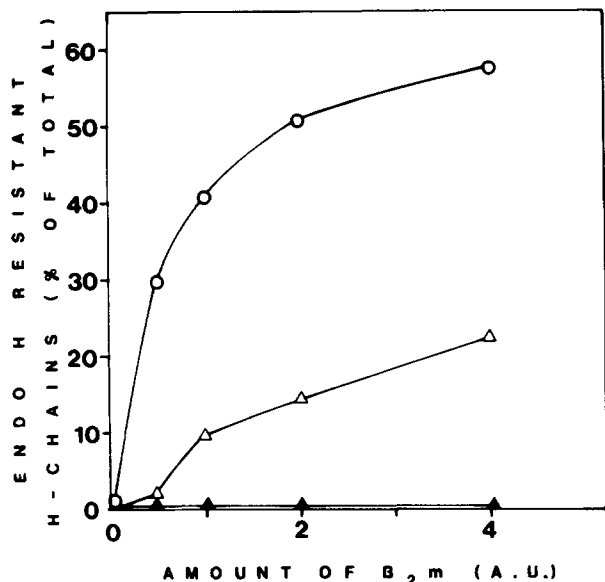
Figure 5 Effect of increasing amounts of β_2M mRNA on the terminal glycosylation of heavy chains in the absence and presence of E19. The fraction enriched for heavy chain mRNA was microinjected into oocytes together with increasing amounts of the fraction enriched for β_2M mRNA alone or together with two concentrations of the fraction enriched for E19 mRNA. Glycoproteins were isolated from oocytes labeled with [^{35}S]methionine for 24 h, and immunoprecipitated with an anti-heavy chain serum. Part of each immunoprecipitate was treated with Endo H before analysis by SDS PAGE. The relative amounts of the heavy chains were determined by densitometric analysis. The percentage of heavy chains resistant to the enzymatic treatment out of the total amount of heavy chains (Endo H sensitive and resistant chains) was calculated for each β_2M mRNA concentration in the absence and presence of the E19 protein. The amounts of β_2M translated were proportional to the amounts of injected mRNA and are expressed in arbitrary units (A.U.). The symbols denote no E19 mRNA (O), 0.03 μg/μl of mRNA (△), and 0.06 μg/μl of mRNA enriched for E19 mRNA (▲), respectively.

case in mammalian cells (15, 16). This was confirmed in the present study, and we could show that in the presence of β_2M, the heavy chains became not only terminally glycosylated but also expressed on the oocyte surface. It seems unlikely that this observation was due to fortuitous contamination by intracellular material, since free heavy chains, which were present intracellularly, could not be discovered on the cell surface and in the presence of β_2M; only terminally glycosylated heavy chains could be detected in the cell surface fraction. The E19 protein was efficiently translated by the oocytes, but we failed to show its presence on the cell surface. In keeping with this negative finding, the E19 protein was susceptible to Endo H digestion throughout the pulse–chase period.

These observations do not formally rule out that minor amounts of E19, too small to detect with the methodology employed, may have appeared on the oocyte cell surface. However, E19 and class I antigens in adenovirus-infected HeLa cells also fail to become terminally glycosylated, as shown in the present study. In fact, the nascent chains do not become integrated into the plasma membrane and as the virus infection progresses, the cells display gradually diminishing quantities of cell surface–expressed class I antigens (Andersson, M., S. Pääbo, T. Nilsson, and P. A. Peterson, manuscript

submitted for publication). Thus, it seems reasonable to conclude that the impaired terminal glycosylation of class I antigens translated together with E19 in oocytes is due to abrogated intracellular transport as is the case in virally infected cells.

The Endo H digestion always gave rise to three electrophoretic species of the E19 protein. These results are consistent with incomplete Endo H digestion of two Asn-linked carbohydrate moieties (13). However, increased concentrations of the enzyme did not promote additional digestion. Since Endo H does not cleave terminally glycosylated or fully trimmed carbohydrate moieties (27), it may be argued that a fraction of the E19 molecules had undergone one or both of these carbohydrate modifications. That E19 undergoes trimming is obvious from the pulse–chase experiments. However, it is less obvious whether the trimming involves mannose residues in the cis-Golgi compartment as well as glucose residues in the endoplasmic reticulum (28). The trimming seems to be facilitated when E19 is translated in the presence of class I antigens, but the resistance of E19 to Endo H digestion is not measurably altered under these circumstances. Moreover, heavy chains, co-precipitated with E19, are fully susceptible to the glycosidase. Therefore, it seems reasonable to suggest that the trimming is confined to the endoplasmic reticulum. If so, the partial resistance of E19 to the action of Endo H remains obscure.

The association between E19 and the class I antigens was recorded by immunological co-precipitations only. The non-covalent interaction is concentration-dependent and may be perturbed by antibodies (7). This observation renders immunological co-precipitations difficult to interpret in quantitative terms. Thus, in the present study, the antibodies used against E19 co-precipitated class I antigen heavy chains when large
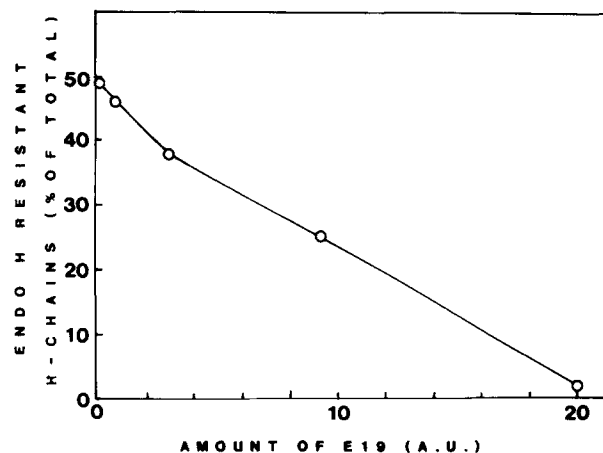

Figure 6 Effect of varying amounts of the E19 mRNA on the terminal glycosylation of heavy chains in the presence of β_2M. Oocytes were microinjected with constant amounts of heavy chain and β_2M mRNA together with varying amounts of E19 mRNA. Glycoprotein fractions isolated from oocytes labeled with [^{35}S]-methionine for 24 h were immunoprecipitated with antiserum against heavy chains. Part of each immunoprecipitate was treated with Endo H before analysis by SDS PAGE. Fluorographs were subjected to densitometric analysis and the percentage of heavy chains resistant to Endo H treatment out of the total amount of heavy chains precipitated (Endo H sensitive and resistant heavy chains), was calculated for each E19 mRNA concentration. The amounts of the E19 protein translated were proportional to the amount of mRNA injected and are expressed in arbitrary units (A.U.).

amounts of E19 were produced (see Fig. 2), whereas little or no co-precipitation could be visualized at lower concentrations of the virus protein (Figs. 1 and 4). In contrast, antibodies against class I antigens generally failed to co-precipitate the viral protein in the oocytes, while substantial amounts of co-precipitated E19 were encountered in virally infected HeLa cells (Fig. 3). Studies aimed at elucidating the particular features of the complex formation between the two types of proteins obviously will require the development of improved techniques to monitor the association. Nonetheless, the present study confirms the finding that the viral protein associates with class I antigens and demonstrates that the interaction occurs intracellularly.

The effect of E19 on the terminal glycosylation of class I antigen heavy chains combined with β_2M is concentration dependent. At relatively high concentrations of E19, the transfer of the heavy chains to the *trans*-Golgi compartment seems to be impeded. Thus, the heavy chains behave as if no β_2M were present inasmuch as they are fully sensitive to Endo H digestion. Likewise, the β_2M-promoted cell surface expression of the heavy chains is abolished. At low concentrations of E19, β_2M partly overcomes the effect of the viral protein, and terminal glycosylation of heavy chains occurs. Although it cannot be ruled out that E19 may affect trimming enzymes and/or glycosyl transferases to account for the abolished terminal glycosylation, it also must impede the intracellular transport of the class I antigens. It is conceivable that the mere association between the viral protein and the heavy chains suffices to abrogate the intracellular transport. The apparently contrasting effects of β_2M and E19 on the intracellular transport of the heavy chain may then be explained on the assumption that heavy chains, upon binding β_2M, attain a conformation that exhibits diminished affinity for E19. Alternatively, the structural homology between E19 and β_2M (29) may suggest that the two proteins compete for the same binding site on the heavy chain. However, apart from the homology being weak at best, β_2M-associated heavy chains in the presence of E19 do not become terminally glycosylated.

Previous studies in transformed and infected mammalian cells have demonstrated the existence of class I antigen–E19 complexes and have shown that E19 becomes degraded in a compartment sensitive to lysosomotropic agents (30). The present study suggests that in oocytes, complex formation is initiated intracellularly and is accompanied by abrogated intracellular transport of the class I antigens. Similar results were recorded in adenovirus-infected HeLa cells as reported here and in more detail elsewhere (Andersson, M., S. Pääbo, T. Nilsson, and P. A. Peterson, manuscript submitted for publication). These observations raise the intriguing possibility that adenovirus-2 may use E19 to escape immune surveillance.

Interestingly, the adenovirus-2 genome does not possess the same type of E1a region as does adenovirus-12, whose E1a impedes the transcription of class I antigen heavy chain genes (31, 32). Thus, two viruses of the same family may have developed different strategies to avoid the same defense mechanism of the host.

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