Expression of Intracisternal A-Type Particles Is Increased When a B-Cell Lymphoma Differentiates into an Immunoglobulin-Secreting Cell

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The murine B-cell lymphoma CH12, like many other murine cells, expresses intracisternal A-type particles (IAPs). When CH12 cells are treated with lipopolysaccharide, the cells differentiate and secrete immunoglobulin M. The expression of IAP genes was also increased, in parallel with the increased expression of immunoglobulin genes. The amount of IAP mRNA increased within 48 h of lipopolysaccharide treatment and reached a level fivefold higher than that in unactivated CH12 cells. The two major IAP transcripts (7.2 and 5.4 kilobases) were induced to similar extents. The increased level of mRNA was reflected in higher levels of the major IAP structural protein p70, whose abundance increased 3.6-fold. The number of IAP particles per cell also increased upon activation of CH12, from 67 in nonsecreting CH12 to 290 in secreting cells. All of the IAPs were confined to the cisternae of the endoplasmic reticulum (ER), regardless of the differentiation state of the cell. Accompanying the induction of p70 was the induction of the related IAP polypeptide p102. A third viral polypeptide, p120, was also made in CH12; its abundance was almost unchanged. Localization of the IAP proteins on ultrathin frozen sections showed that most were assembled into particles in the ER. However, there were small pools of unassembled proteins both in the ER and on the plasma membrane. p70 and p120 could be detected, by iodination, on the surfaces of both secreting and nonsecreting CH12 cells. Unlike p70 and p120, p102 did not seem to be membrane associated. Taken together, these observations show that IAP expression is regulated developmentally in B lymphocytes. Also, these observations point to possible interactions between IAP genes and other cellular genes.

Most mouse myelomas harbor numerous intracisternal A-type particles (IAPs) in the cisternae of their endoplasmic reticulum (ER). These particles are noninfectious retrovirus-like structures (11, 24, 53), which contain specific high-molecular-weight RNAs associated with a major IAP-encoded structural protein, p73 (37). p73 is not glycosylated and seems to interact with the ER membrane by virtue of a hydrophobic N terminus that mimicks normal signal sequences (39).

IAP proviruses are found in many rodent species and are present at about 1,000 copies per haploid genome in *Mus musculus* and Syrian hamster (34, 42). The IAP provirus in both species has significant nucleotide homology with the type B mouse mammary tumor virus and the type D Mason-Pfizer monkey virus (7, 39, 43). Several cloned mouse IAP long terminal repeats (LTRs) have been shown to be active promoters of linked genes when transfected into mouse, monkey, and human cells (8, 17, 32). IAPs can also act as movable genetic elements and influence the activity of genes at target sites (22). For example, IAP elements have been found translocated into the *c-mos* oncogene in two plasmacytomas (5, 9), and in one instance, the rearranged *c-mos* gene was shown to be transcriptionally active and transformation competent (47).

IAP expression exhibits tissue and developmental specificities, as well as inducibility in vitro. In adult mouse tissues, IAP transcripts are barely detectable in spleen, brain, liver, kidney, and epididymus but are more abundant in cortical thymocytes (23). High constitutive expression of IAPs is found in many types of murine tumor cells, such as plasmacytomas and neuroblastomas (11, 23, 50). During

normal mouse embryogenesis, IAPs are expressed transiently in oocytes and in preimplantation embryos (19, 45). IAP expression is also inducible in some situations. In pancreatic beta cells of certain inbred mouse strains, IAP expression is increased when insulin secretion is induced by high glucose levels (27, 28). In addition, IAP expression is induced when teratocarcinoma cells are stimulated to differentiate with retinoic acid (18). In the majority of cells and tissues which have been examined, the IAP transcripts seem to be derived only from very few proviruses (13, 16, 18, 40). The cellular mechanisms underlying the specificity of IAP expression are unclear.

The present investigation into IAP expression stems from our interest in the differentiation of the CH12 lymphoma into an immunoglobulin-secreting cell. This lymphoma line arose spontaneously in a B10.H-2^aH-4^bp/Wts mouse that had been hyperimmunized with sheep erythrocytes (2). CH12 expresses surface immunoglobulin M specific for a determinant on autologous senescent erythrocytes (2, 30, 51) and can be induced to differentiate into an immunoglobulin M-secreting cell line in response to several stimuli (10, 29). During this differentiation, the ER of CH12 is expanded three- to fourfold and the expression of certain ER proteins is preferentially increased (Wiest et al., manuscript submitted). Our search to define these ER proteins resulted in the identification of one protein as p70, the major IAP structural protein in CH12.

The results presented here show that IAP expression was induced during the in vitro differentiation of CH12 in response to bacterial lipopolysaccharide (LPS). Both the IAP RNA and protein levels were increased, leading to higher numbers of viral particles in the rough ER.

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MATERIALS AND METHODS

Cell culture. CH12 cells were passaged as an ascitic tumor in B10.A mice (from our breeding colony). After the cells were removed from the mouse peritoneum, this population was shown to contain greater than 94% CH12 cells (2, 30). Cells harvested from the mouse peritoneal cavity were plated at 1.3×10^5 per ml in Dulbecco modified Eagle medium (Hazelton Laboratories), supplemented as described by Stockdale et al. (51). These cultures were then incubated for 48 h either with or without 50 μ g of LPS per ml (from *Escherichia coli* B [O55:B5]; Difco Laboratories). At the end of 48 h, the differentiation of CH12 was evaluated by enumerating the immunoglobulin-secreting cells in the cultures. Since CH12 produces an immunoglobulin M (κ) specific for a determinant on senescent erythrocytes (2), the number of secreting cells was measured by a direct plaque assay on bromelain-treated sheep erythrocytes (10, 51). The B1-88.1 hybridoma was cultured in the same medium (without LPS) as was used for the CH12 cells. For experiments involving modulation of glucose levels, we cultured CH12 cells in Dulbecco modified Eagle medium containing either 1 or 4.5 g of glucose (Hazelton) per liter and the other additions previously described (51).

RNA analysis. RNA was purified either by the lithium-urea or the detergent lysis method (3, 23). Poly(A) RNA was isolated by chromatography on poly(U)-Sepharose (Pharmacia) according to the recommendations of the manufacturer. Fractionation of RNA in 1% formaldehyde-agarose gels, transfer to nitrocellulose (BA85; Schleicher & Schuell, Inc.) and hybridization were all done as described previously (23, 36). The IAP probe, pMIA14.10 (23), is a full-length genomic clone of a mouse IAP which contains the 5' LTR repeat but not the 3' LTR (a generous gift from E. L. Kuff and J. W. Fewell, National Cancer Institute, Bethesda, Md.). The probe was labeled by nick translation (48).

Radiolabeling and immunoprecipitations. Metabolic labeling of the cells was done as previously described (1), with [35 S]methionine from Amersham Corp. Surface proteins were labeled by lactoperoxidase-catalyzed iodination (26) with 125 I from Amersham. The rabbit anti-IAP antiserum A3607.4 was raised against p73 which had been excised from sodium dodecyl sulfate (SDS) gels (a generous gift of E. L. Kuff and J. W. Fewell). Three microliters of the antiserum was used to isolate p73 from lysates of 5×10^6 labeled cells, after these lysates were denatured by heating in an SDS-Nonidet P-40 solution as previously described (23). Immune complexes were isolated with 50 μ l of packed protein A-Sepharose beads (Pharmacia).

Gel electrophoresis and immunoblots. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% polyacrylamide gels was used to separate the proteins. Gels intended for immunoblotting were transferred electrophoretically to nitrocellulose at a current of 1 A for 90 min. The blots were first blocked overnight at 4°C with Tris-saline-Blotto (50 mM Tris [pH 8.5], 150 mM NaCl, 0.05% Tween 20, 5% powdered milk, 0.02% NaN_3). Immunoblots were probed for 2 h at room temperature with the rabbit antibodies diluted 1:1,000 in a solution of 1% bovine serum albumin in phosphate-buffered saline (PBS) and then washed three times with Tris-saline-Blotto for 15 min at 4°C. The blots were then reacted with iodinated protein A at a concentration of 10^6 cpm/ml. After four additional washes with Tris-saline-Blotto for 30 min each at 4°C, the blots were exposed to XAR-2 film (Eastman Kodak Co.) at -70°C.

Cell fractionation. A total of 10^9 cells were lysed by

nitrogen cavitation at 750 lb/in², and the postnuclear supernatant was subjected to the sucrose flotation centrifugation of Mechler and Vassalli (38) for 5 h at 24,000 rpm in a Beckman SW28.1 rotor. The rough microsomal fraction is defined as the material banding at the 2.0 and 1.3 M sucrose interface, the smooth microsomal fraction is the material banding above the 1.3 M sucrose layer, and the load zone is the 2.05 M sucrose layer. The rough microsomal fraction contained 6.5% of the total cell protein. It was enriched threefold in immunoglobulin kappa chain, a marker for the contents of secretory apparatus, and 4.5-fold in ribophorin II, a marker for the rough ER membrane. The anti-ribophorin II monoclonal antibody was a generous gift of M. Hortsch and D. Meyer (European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany). The rough microsomal fraction contained negligible amounts of wheat germ agglutinin-binding glycoproteins, indicating that it was not contaminated significantly by plasma membrane, Golgi complex, or lysosomal glycoproteins. A more extensive characterization of these subcellular fractions is given elsewhere (Wiest et al., manuscript submitted).

Electron microscopy. For conventional electron microscopy, cells were washed with a balanced salt solution and fixed in suspension at room temperature with 2% glutaraldehyde in 0.1 M sodium phosphate [pH 7.4]-1.8% sucrose-0.01% CaCl_2 . After 3 to 5 min, cells were centrifuged and the resulting pellets were fixed an additional hour. The fixed cell pellets were embedded in 1% agar and postfixed for 1 h at room temperature with 1% OsO_4 in 0.1 M sodium phosphate [pH 7.4]. The agar blocks were washed with 0.1 M Veronal acetate [pH 7.4], stained en bloc with 1% uranyl acetate-0.1 M Veronal acetate for 1 h at room temperature, dehydrated through graded ethanol solutions, and embedded in EMBED 812 (EM Sciences). Silver sections were contrasted with uranyl acetate and lead citrate and observed with a Philips EM300 electron microscope at 80 kV.

The number of virions per cell was determined stereologically, by the methods described by Weibel (52). Briefly, the cell volume served as the reference space and was derived from the mean diameter of the cell, as measured with a caliper on electron micrographs magnified $\times 8,000$. Quantitation of the number of virions per cell was accomplished by multiplying their numerical density by the cell volume. Numerical density was determined by counting the total number of virions in the sample ($n = 30$ micrographs) and normalizing this number to the volume in which the sample is contained. Virion diameters were measured on micrographs magnified $\times 27,000$, using a graduated reticle. A detailed description of the entire stereological study is presented in Wiest et al. (manuscript submitted).

Immunoelectron microscopy was performed essentially as described by Griffiths et al. (15). Cells were fixed for 10 min at room temperature with 4% paraformaldehyde in 200 mM PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid) (pH 7.0)], followed by overnight fixation in 8% paraformaldehyde in the same buffer. The cell pellets were cryoprotected by infiltrating with 2.1 M sucrose in PBS, mounted on a specimen pin, and frozen in liquid nitrogen. Ultrathin sections were cut at -100°C with a Reichert Ultracut E equipped with an FC4E cryounit, collected on a drop of 2.3 M sucrose in PBS, and transferred to grids coated with Formvar and carbon. The grids were floated on 5% fetal total calf serum in PBS, washed three times with PBS for 15 min, and incubated with anti-p73 diluted 1/300 for 30 min. After being washed six times in PBS for 15 min total, the grids were incubated for 20 min with 9-nm-diameter colloidal gold

FIG. 1. Expression of IAP-encoded RNA during CH12 differentiation. Poly(A)⁺ RNA was isolated from CH12 cells cultured for 48 h without LPS (lane 1), from ascitic CH12 cells (lane 2), and from CH12 cells cultured with 50 µg of LPS per ml for 48 h (lane 3). Equal amounts (1 µg each) of RNA were fractionated by electrophoresis in a 1% formaldehyde-agarose gel and transferred to nitrocellulose. The blot was hybridized simultaneously with ³²P-labeled plasmids pMIA14.10, which contains an entire IAP genome, and pK(11)²⁴, which contains a full-length mouse kappa cDNA. Equal loading of RNA in each lane of the blot was verified by subsequent probing with pHF1, an actin probe (data not shown). Molecular size standards (in kilobases) are shown to the left of the gel.

particles conjugated to protein A (gift of G. Griffiths, European Molecular Biology Laboratory). Grids were then washed again six times with PBS for a total of 30 minutes and stained for 10 minutes on ice with 0.3% uranyl acetate in 2% methylcellulose. Excess methylcellulose was removed, and the remainder was air dried to a film. The grids were observed as described above.

RESULTS

We have previously quantified the changes in expression of immunoglobulin genes during the *in vitro* differentiation of CH12 in response to several mitogenic stimuli (51). The levels of mRNA encoding the secretory form of μ (μ_s) and kappa (κ) light chains increased during culture with either LPS or the mitogenic supernatant of the mouse thymoma EL-4. Cultures of LPS-stimulated CH12 cells typically contain 55 to 80% immunoglobulin-secreting cells and express 10 to 20 times more immunoglobulin RNA than do nonstimulated cells. The kinetics of accumulation of μ_s and κ RNA indicate that they are regulated coordinately (51).

We used RNA blots of poly(A) RNA to quantify the level of IAP RNA during CH12 differentiation. The probe used, pMIA14.10, detects both type I and type II IAP sequences (50). A representative blot of poly(A) RNA is shown in Fig. 1. CH12 cells express the pattern of IAP transcripts that is commonly observed in myelomas and plasmacytomas (23, 49). There are two major species, 7.2 and 5.4 kilobases (kb), and several minor transcripts of intermediate sizes. The abundance of all IAP-related RNAs increased after culture with LPS for 48 h (Fig. 1, compare lanes 2 and 3). Densitometric quantitation of blots from two different RNA prepa-

TABLE 1. Quantitation of induction of IAP RNA

RNA species (size)	Incubation time (h)	LPS	Relative abundance ^a
IAP (7.2 kb)	0		1.0
	48	—	1.7
	48	+	5.8
IAP (5.4 kb)	0		1.0
	48	—	1.9
	48	+	4.8
γ -Actin	0		1.0
	48	—	1.1
	48	+	0.9
Immunoglobulin, kappa ^b	0		1.0
	48	—	2.6
	48	+	12.3

^a Densitometric signal in arbitrary units per microgram of RNA ($n = 4$).

^b Data recalculated from those reported by Stockdale et al. (51).

rations is given in Table 1. Relative to the abundance of IAP transcripts in freshly harvested ascitic CH12 cells, the 7.2-kb species increased 5.8-fold, while the 5.4-kb species increased 4.8-fold in LPS-stimulated cells. Although both major IAP RNAs were increased, induction of the 7.2-kb RNA was reproducibly greater than that of 5.4-kb RNA. This five- to sixfold increase in IAP RNA during differentiation was derived by comparing equal amounts of RNA. Since total poly(A)⁺ RNA per cell increases twofold (51), the total induction of IAP RNA per cell was about 10-fold. CH12 cells that were cultured for 48 h in LPS-free medium showed a slightly enhanced expression of IAP RNA when compared with ascitic CH12 cells (Fig. 1., compare lanes 1 and 2). This slight induction was presumably due to mitogens endogenous to the growth medium. This phenomenon is also observed with immunoglobulin transcripts (51).

IAP RNA was induced to a slightly lesser extent than immunoglobulin RNA. The blot shown in Fig. 1 was hybridized simultaneously with a κ probe, to provide an internal standard for comparison. The changes in IAP transcripts resembled the changes in the 1.3-kb κ transcript. However, the kinetics of IAP RNA accumulation did not exactly parallel the kinetics of immunoglobulin RNA accumulation. Whereas half-maximal induction of immunoglobulin RNA was observed after 24 h, half-maximal induction of the 7.2-kb IAP transcript occurred slightly later, at 30 to 36 h after the initial LPS stimulus (A. M. Stockdale, unpublished data). Levels of both κ and IAP RNAs were maximal by 48 h. The similarity in kinetics was not simply due to the metabolic state of the cells, because another RNA, the *env* transcript from an endogenous mammary tumor virus, showed half-maximal induction after 12 h (R. B. Corley, personal communication; our own preliminary data). At that time immunoglobulin secretion was only at 10 to 20% of its maximal value (51).

Mouse myelomas and plasmacytomas may express two types of IAP genes: type I transcripts are RNA species of 7.2 and 5.4 kb (23, 44), and type II transcripts are 3.8 kb or smaller (50). CH12 cells express only type I transcripts; no type II transcripts were detected either before or after stimulation with LPS.

Since IAP RNA was induced, we wished to examine the synthesis of the major structural IAP protein p73. For this purpose, cells which had been cultured for 48 h either with or without LPS were metabolically labeled for 1 h with

FIG. 4. Electron micrographs showing representative areas of the ER from secreting CH12 cells (a) and from the hybridoma B1-88.1 (b). The arrowheads point to the intracisternal particles. Bar, 0.2 μ m.

(Fig. 3, lanes 3 and 4, respectively) contained a minimum of five times more p70 than did secreting CH12 cells (lane 1). This was also true concerning the number of virus particles. Comparison at the electron microscopic level showed that B1-88.1 cells (Fig. 4b) contained many more particles than did secreting CH12 cells (Fig. 4a). These comparisons demonstrate that the induction of IAP in secreting CH12 cells was far from the maximal expression possible.

The major IAP structural protein, p73, has been shown to be synthesized by membrane-bound polysomes (31) and to be limited in distribution to the ER membrane (11, 24). Leiter et al. (27) have previously hypothesized that in juvenile-onset diabetes, a glucose-mediated translocation of IAP proteins to the plasma membrane may be involved in the autoimmune destruction of pancreatic β islet cells. We therefore asked if the cellular location of p70 changes when CH12 is induced to differentiate. To examine this question, we used a subcellular fractionation method which results in good enrichment of the rough ER membranes (38). The protein contents of three distinct areas of the sucrose gradient were compared with the protein contents of the cell lysate before fractionation (Fig. 5). Immunoblots of these fractions with anti-p73 show that the rough microsomal membranes (RM) were enriched in p70. The specific immunoreactivity of p70 in the rough microsomal fraction was 3.1 times that of the smooth membrane fraction (SM) and 3.6 times higher than that in the total cell lysate (TOTAL) (Fig. 5). The small amount of p70 that copurified with smooth microsomes can be accounted for by contamination of this fraction with stripped rough microsomal membranes. This contamination was measured by probing the same blots with anti-ribophorin II, an integral rough ER protein that is present in low levels in our smooth microsomal fraction (data not shown). The distribution of p70 in the sucrose gradient was unaffected by the secretory status of the cells (Fig. 5). We conclude that the retention of p70 in the rough ER is an intrinsic property of this protein, regardless of its concentration.

The anti-IAP serum also detected other IAP proteins present in CH12 (note the two smaller proteins which

cofractionate with p70 [Fig. 2]). These are presumably the processed polypeptides p46 and p30 (37). The antibody also reacted with p102. It is interesting to note that the subcellular distribution of p102 did not mirror that of p70. p102 remained in the load zone (LZ) of the gradient (Fig. 5) which contains mostly cytoplasmic proteins. This subcellular distribution of p102 was also not changed during CH12 differentiation.

The cellular fractionation experiments were limited in resolution and provided information only about the bulk of the protein molecules. We wished to determine, using a more sensitive measure, whether p70, p102, or p120 is

FIG. 5. Autoradiogram of subcellular location of IAP-encoded proteins. CH12 cells (10^9) that were either induced with LPS (+) or not (-) were lysed by nitrogen cavitation and fractionated on discontinuous sucrose gradients, as described in Materials and Methods. Protein samples (30 μ g) from each pooled fraction were loaded on 10% polyacrylamide-SDS gels. The proteins were blotted electrophoretically onto nitrocellulose, probed with rabbit anti-IAP, and developed with radioactive protein A. Abbreviations for fractions: TOTAL, the cell lysate which was loaded on the gradients; LZ, protein remaining in the load zone after centrifugation; RM, rough microsomal membranes; SM, smooth microsomes.

FIG. 6. Cell surface expression of IAP proteins. Secreting (+) and nonsecreting (–) CH12 cells were labeled by surface iodination with lactoperoxidase, lysed, and immunoprecipitated with the indicated rabbit antiserum. Immunoprecipitated material from 1.5×10^6 cells from each treatment was analyzed by SDS-PAGE. The material in the rightmost lane is [35 S]methionine-labeled intracellular IgM and is included for the identification of the bands in the other lanes. The membrane form of the immunoglobulin heavy chain (μ_m), the secreted form of the heavy chain (μ_s), and the kappa light chain (κ) are shown. Ab, Antibody; 120, p120; 70, p70.

translocated to the plasma membrane of CH12 during its differentiation. To accomplish this, we iodinated the plasma membranes of secreting and nonsecreting CH12 cells and subsequently immunoprecipitated the cell lysates with anti-IAP serum. The immunoprecipitated polypeptides were separated by SDS-PAGE (Fig. 6). Both p70 and p120, but not p102, were detectable on the plasma membrane of CH12 cells (Fig. 6). The band between p70 and p120 is the membrane-bound form of μ (μ_m): it comigrates with the μ_m band in the anti- κ precipitation and its abundance decreases with activation, as described by Stockdale et al. (51). The rightmost lane in Fig. 6 contains the secretory form of μ (μ_s). The absence of intracellular μ_s , a very abundant protein, in the anti- κ precipitation demonstrates that the cells were intact during iodination and that the labeled proteins were indeed expressed on the cell surface. The inability to detect p102 by this assay is consistent with its cytoplasmic localization, although we do not know if it can be labeled by the lactoperoxidase technique. The increase in the levels of p70 and p120 on the plasma membranes of secreting CH12 cells did not exceed the increase in synthesis of these two proteins. This result indicates that, at least for LPS-induced differentiation of B cells, there was no preferential translocation of IAP proteins to the cell surface.

We used immunoelectron microscopy as an independent method to localize the IAP proteins. Secreting and nonsecreting CH12 cells were fixed, cryoprotected, and frozen in liquid nitrogen. Ultrathin frozen sections were labeled with anti-IAP, followed by protein A conjugated to colloidal gold. Specific labeling was observed over viral particles, the ER membrane, and the plasma membrane. The amounts of

labeling over the cytoplasm, the nucleus, and mitochondria were negligible. This is shown in Fig. 7a at a relatively low magnification and in Fig. 7b through k at higher magnifications. Protein A alone or irrelevant rabbit antisera caused no labeling, and anti-IAP did not label a rat leukemia cell line that did not express IAP RNA (controls not shown).

In both secreting and nonsecreting CH12, more than 90% of the gold was over viral particles, and usually a cluster of gold particles was seen (Fig. 7a through e). As expected from conventional electron microscopy, the IAPs were always confined to the cisternae of the ER. A small fraction of the gold particles consistently decorated the ER membrane at locations where no complete or budding virus could be seen. In these cases, the label was in the form of one or two isolated gold particles along a track of membrane (Fig. 7f through i). Two observations show that these tracks are indeed ER membranes. First, in some sections they are clearly studded with ribosomes (arrows in Fig. 7f). Second, the outer membrane of the nuclear envelope was similarly labeled (Fig. 7h). Frequently, single gold particles decorate the ER membrane in the vicinity of labeled virions (Fig. 7g). Although the antiserum we used detected several IAP polypeptides, the cellular fractionation experiments (Fig. 5) indicate that the labeling over the ER was due to p70. The pattern of ER labeling suggests that a small pool of p70 exists in the rough ER membrane of CH12 cells in a form that is membrane-bound but not associated with visible particles.

In addition to labeling free and assembled p70 in the ER, the antiserum consistently recognized a small pool of IAP polypeptides on the cell surface of CH12 (Fig. 7j). This labeling was reproducibly found on the plasma membrane in areas devoid of any budding virus particles and was present as a small percentage of the total label (estimated at 2%) at all antibody dilutions. The plasma membrane labeling was in agreement with the surface iodination experiments described above and probably represents labeling of both p70 and p120.

When secreting and nonsecreting CH12 cells were compared by immunoelectron microscopy, no obvious differences in the distribution of IAP antigens were observed. More gold particles labeled secreting cells, as expected from the increases in p70 and p120 levels. However, the proportions of label over viral particles, ER membranes, and plasma membranes were similar in both populations (data not shown).

During our EM studies, we observed retroviruslike structures associated with the cell surface of CH12. The virions were clearly extracellular, and occasionally budding structures were observed at the plasma membrane. By definition, these extracellular and budding virions are not IAPs, and indeed they were not labeled with anti-p73 (Fig. 7k). This data demonstrates that CH12 cells harbor a second endogenous virus. Like IAPs, these other virions were much more numerous in LPS-stimulated cells. Preliminary data suggest that this retrovirus is a murine leukemia virus. Detailed studies of the expression of this virus will be published elsewhere.

DISCUSSION

The main conclusion from this study is that IAP expression in the B-lymphoma CH12 can be induced when this cell line differentiates into an antibody-secreting cell. The expression of the major IAP structural protein (p70 in CH12) and of a related protein, p102, were both induced, while expression of another protein, p120, was almost unchanged.

FIG. 7. Immunolocalization of IAP antigens in electron microscopic sections of CH12 cells. Secreting CH12 cells were processed for cryomicrotomy as described in Materials and Methods. The sections were labeled with rabbit anti-IAP at a 1:300 dilution, washed, and reacted with protein A adsorbed to 9-nm-diameter-colloidal gold particles. The concentrations of both reagents were chosen so as to avoid clusters of gold particles, which are often artifactual. (a) A lower-magnification micrograph showing about a quarter of a CH12 cell. Note that the label is confined almost exclusively to ER profiles. (b through k) Selected fields at higher magnification. Labeled particles are shown in panels b through e. Panels f, h, and i, as well as one gold particle in panel g (arrow), show label over ER membrane in the absence of obvious underlying particles. Panel j shows label over the plasma membrane. Panel k shows unlabeled extracellular virions. Arrowheads in panel f mark membrane-bound ribosomes. N, Nucleus; M, mitochondrion. Bar, 0.1 μ m.

The increased abundance of the mRNA and the increased synthesis of p70 resulted in a proportional increase in viral particle number. The viral particles were confined to the cisternae of the ER, and this localization was not altered in LPS-stimulated cells.

The induction of IAP in CH12 correlates with the increased rate of immunoglobulin production, which characterizes late-stage B-cell differentiation. If this is a general phenomenon, it may explain the high levels of type A particles expressed by tumors representing these late stages of the B-cell lineage. In fact, the correlation of high rates of immunoglobulin production and IAP expression is not restricted to transformed cells; it was also observed in normal mouse spleen cells stimulated with LPS (D. Wiest, unpublished data; E. Kuff, personal communication). Therefore, CH12 cells can serve as a useful system in which to elucidate the mechanism of coordinate expression of immunoglobulin and IAPs.

Relatively little is known about the mechanisms responsible for IAP gene regulation. The results of this regulation, however, have been observed at several different levels. First, many observations show that IAPs are expressed in a cell-specific manner. In normal mice, IAP RNA is expressed at significant levels only in cortical thymocytes (24). In certain inbred mouse strains, such as *db/db* mice, IAPs are also found in the pancreatic β islet cells (27, 28). IAP expression is also regulated developmentally, as shown in two systems. Mouse oocytes and preimplantation embryos express both the IAP mRNAs and the p73 and p120 polypeptides (4, 6, 19, 45). At later embryonal stages, however, IAPs are not detected (45). When murine teratocarcinoma cell lines are analyzed, IAP expression is inversely correlated with the state of differentiation of these cells (16). Finally, IAP expression can be modulated in response to external stimuli. Two drugs which cause DNA hypomethylation, iododeoxyuridine and 5-azacytidine, also cause increased expression of IAPs in murine fibroblasts (25). Perhaps the most interesting case of regulated IAP expression is the induction of both mRNAs and proteins in the β islet cells of *db/db* mice when these cells are exposed to elevated external glucose levels (27).

IAP induction in islet cells by glucose is coupled to changes in the expression of specific cellular genes. In this paper, we show that IAP expression is induced in a different cell type, a B lymphocyte, by the mitogen LPS. Several interesting parallels exist between the induction of IAP expression by glucose in β islet cells and by LPS in CH12. In both cases, IAP expression is induced coordinately with the major secretory product of the cell, insulin in β islet cells and immunoglobulin in B lymphocytes. In both cases, the IAP RNA is approximately fivefold more abundant in the induced cells, and the total increase in IAP RNA is 10 to 20-fold (27). The induction of IAP expression, in both cases, is maximal after 48 h in culture. Furthermore, the regulation of protein expression and particle formation is similar in CH12 cells and in β islet cells of genetically diabetic mice (27). Particles are present prior to stimulation, and there is coordinate induction of the major structural protein and a higher-molecular-weight species (27).

Because of these parallels, we asked if IAP expression in CH12 cells can be induced by high glucose levels. Culture of CH12 cells in medium containing 4.5 g of glucose per liter caused no differentiation to immunoglobulin secretion above the spontaneous levels of cells cultured in low-glucose medium (1 g/liter). Both populations showed the same levels of p70 synthesis and expression on the cell surface. In

addition, glucose showed no synergism with LPS (D. Wiest, unpublished data). We do not think that the failure of glucose to augment the effects of LPS is because IAP expression in CH12 is already maximal. The levels of IAP proteins and the number of particles in induced CH12 cells are still much lower than the corresponding levels in other B lineage tumors. Thus, other agonists which stimulate CH12 may be able to induce IAP expression even further.

Leiter et al. (27) have proposed a model in which the induction of p73 and p120 in β islet cells of genetically diabetic mice marks these cells for autoimmune destruction. This model predicts the specific translocation of IAP proteins to the cell surface in response to elevated glucose levels. With this in mind, we examined the distribution of the IAP proteins before and after stimulation of the CH12 cells with LPS. Using both surface iodination and immunoelectron microscopy, we detect the presence of both p70 and p120 on the plasma membrane. Although the levels of both proteins on the plasma membrane increased as a result of LPS stimulation, this increase did not exceed the increase in synthesis of these proteins. There is therefore no evidence for a specific translocation to the cell surface in this system.

The vast majority of p70 in CH12 cells, as in neuroblastoma cells (33), exists in the form of viral particles confined to the ER cisternae. This finding is in accord with the tendency of p70 to self-assemble tenaciously and the requirement of strong denaturants to dissociate IAPs (37, 53). There was only a small pool of unassembled p70 in the ER, whose size did not change with LPS stimulation. Instead, the increased synthesis of p70 resulted directly in increased assembly of particles. It is likely that the rapid packaging into particles also protects p70 from degradation, resulting in higher accumulation of p70 relative to the nonstructural proteins p102 and p120.

The expression of endogenous retroviral genes can be induced by several modes. The best studied of these is mammary tumor virus induction by glucocorticoids, which cross the plasma membrane and complex with a cytoplasmic receptor (54). Another mode of induction involves binding of a ligand to a cell surface receptor. For example, epidermal growth factor binding to its receptor on mouse embryo cells causes induction of VL30 RNA (14). This mode of induction is specific to VL30, since a different retrovirus in the same cells is not induced by epidermal growth factor binding (14). Since LPS is thought to integrate directly into the plasma membrane, rather than bind to a specific receptor (12), this study introduces a third mechanism of retroviral induction. Some of the intracellular events that follow LPS stimulation resemble those resulting from activation of the epidermal growth factor receptor (12). Therefore, it is possible that IAP and VL30 genes are activated by similar components of the activation cascade.

The effects of the activation cascade generated by LPS stimulation are likely to be limited to a few IAP genes. In the majority of cells and tissues examined, only a few of the 1,000 IAP copies per haploid mouse genome (34, 49, 50) seem to be expressed (13, 16, 18, 21, 40). We do not yet know how many different IAP proviruses are expressed by unstimulated CH12 cells, nor do we know how the number is affected by LPS stimulation. Identification of the IAP species expressed in CH12 will aid in understanding the mechanism of IAP regulation.

One possible model for IAP induction in B cells is that IAP genes are regulated coordinately with cellular genes that are important for the differentiation to antibody secretion. This coordinate expression may be mediated by homologous

control elements of IAP proviruses and those of the other genes. Studies with the LTRs of IAP support this idea. The promoter activity of an IAP LTR can be induced by the presence of oncogene-encoded nuclear proteins, such as E1A, simian virus 40 large T, *myc*, and p53 (8, 35). At least some of these gene products are known to be expressed at high levels during B-cell differentiation (20).

An alternative model is that specific IAP copies are induced by virtue of their proximity to transcriptionally active loci, such as the immunoglobulin κ and insulin loci. Both the κ and insulin genes are regulated by specific enhancers (41, 46). The predominant insulin gene expressed in the mouse (insulin I [27]) is located on chromosome 6, along with the κ locus. One intriguing scenario, therefore, is that the IAP sequences induced in both B lymphocytes and β islet cells are those copies located on chromosome 6, which may be subject to the effects of the immunoglobulin or insulin enhancers.

Both models above postulate increased IAP transcription during B-cell differentiation. However, a posttranscriptional model is equally plausible. As a result of LPS stimulation, the ER membrane area increases three- to fourfold (Wiest et al., manuscript submitted), providing increased capacity for translating p70. Consequently, the number of particles increases, providing stabilization of RNA in IAP-packaged form. These three models can be distinguished by determining the stability of IAP RNA and the number and location of IAP copies that are expressed in CH12 cells.

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