

NEWCASTLE DISEASE VIRUS-INFECTED SPLENOCYTES
EXPRESS THE PROOPIOMELANOCORTIN GENE

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During the past few years there has been an outgrowth of research designed to elucidate the intricate relationships that appear to exist between the immune and neuroendocrine systems (1–4). The postulated lymphoid-adrenal axis provides the most fascinating example of this concept. Infection with Newcastle disease virus (NDV) augments plasma glucocorticoid concentrations in hypophysectomized mice (5). Indirect immunofluorescence studies with splenocytes from NDV-infected mice showed positive cytoplasmic staining with an antibody directed against adrenocorticotrophic hormone (ACTH 1–13 amide). This finding suggested that splenocytes can synthesize a cytokine that is identical to pituitary-derived ACTH. The lymphocyte-derived ACTH is similar to pituitary-derived ACTH in regard to its biological activity, molecular weight, antigenicity, and retention time on a reverse phase HPLC column (6–8), but its structural identity to pituitary-derived ACTH has not yet been proven.

ACTH is derived from the 31-kD precursor protein proopiomelanocortin (POMC), as is α -, γ -, β -melanotropin, β - and γ -lipotropin and β -endorphin (9). Immunoreactive β -endorphin also has been shown to exist in virus-infected lymphocytes (7). Glucocorticoids inhibit cytoplasmic staining of immunoreactive ACTH and β -endorphin in NDV-infected lymphocytes, while corticotropin releasing factor (CRF) enhances *de novo* synthesis of ACTH and β -endorphin (10). These results suggest that POMC gene products are expressed in lymphoid cells and that they are regulated in a similar manner as POMC gene products in the anterior pituitary gland. If this concept is true, lymphoid cells infected with virus, but not noninfected cells, should synthesize the POMC precursor protein. Demonstration of specific POMC mRNA in virus-infected lymphocytes would provide evidence in support of this hypothesis. We report here that virus-infected murine splenocytes contain poly(A)⁺ cytoplasmic RNA that hybridizes with a cDNA probe that contains the entire coding sequence for murine POMC. These results offer the first evidence using molecular hybridization that lymphoid cells can be activated to express the POMC.

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Materials and Methods

RNA Analysis. Splenocytes were isolated from 4–8-wk-old Balb/c mice and incubated in the absence or presence of NDV (10 hemagglutination U/10⁶ cells) for 18 h at 39°C, 7% CO₂. Total cellular RNA was extracted by the guanidinium/hot phenol method (11), cytoplasmic RNA was isolated (12), and poly(A)⁺ RNA purified by oligo(dT) chromatography (13). Total cellular and poly(A)⁺ cytoplasmic RNA from AtT-20 cells, noninfected splenocytes, and NDV-infected splenocytes were blotted onto Gene Screen membranes (New England Nuclear, Boston, MA). Transfer membranes were baked for 2 h at 80°C. Prehybridization of the membranes was in 50% formamide, 0.2% polyvinyl-pyrrolidone, 0.2% BSA, 0.2% ficoll, 0.05 M Tris-HCl, 1.0 M NaCl, 0.1% sodium pyrophosphate, 1% SDS, 10% dextran sulfate, and denatured salmon sperm DNA (>100 µg/ml) for 16–20 h at 42°C. Hybridization was in 50% formamide, 0.2% polyvinyl-pyrrolidone, 0.2% BSA, 0.2% ficoll, 0.05 M Tris-HCl, 0.1% sodium phosphate, 1% SDS, denatured salmon sperm DNA, and the α-³²PdCTP-labeled pMKSU16 plasmid for 24 h at 42°C. This plasmid contains a 1,026 bp insert that contains the entire coding sequence of murine POMC (14). Gene Screen membranes were washed sequentially with agitation twice in 2× SSC (0.3 M sodium chloride, 0.03 M sodium citrate) for 15 min at room temperature, twice in 2× SSC with 1% SDS for 60 min at 65°C, and twice in 0.1× SSC for 30 min at room temperature. Membranes were dried and exposed to XAR-5 film (Eastman Kodak Co., Rochester, NY) for 48 h at –80°C before development of autoradiographs. The cDNA insert for POMC was not purified from the recombinant plasmid, pMKSU16, since this plasmid (pBR322) without the insert did not hybridize to RNA isolated from either AtT-20 cells, noninfected, or NDV-infected splenocytes (data not shown). For Northern blot analysis, poly(A)⁺ cytoplasmic RNA was denatured with glyoxal and electrophoresed in a 1.2% agarose gel (15) in 0.01 M sodium phosphate (pH 6.5) before transfer onto Gene Screen membranes.

Results

If ACTH is synthesized by splenocytes according to the same process as that which takes place in the pituitary gland, it should be possible to show that infection of lymphoid cells with a virus leads to expression of the POMC gene. To test this possibility, total cellular RNA from NDV-infected splenocytes was isolated and hybridized with the recombinant plasmid, pMKSU16, which contains the POMC insert (Fig. 1, lane 3). Positive hybridization was detected with total cellular RNA from NDV-infected splenocytes and a positive control, the ACTH-secreting AtT-20 pituitary tumor cell line (Fig. 1, lane 1). This hybridization was specific for total cellular RNA from NDV-infected splenocytes since the probe did not hybridize to total cellular RNA from noninfected splenocytes (Fig. 1, lane 2) or from mouse liver (data not shown). Positive hybridization by RNA from NDV-infected splenocytes was not due to crosshybridization of the cDNA probe to the NDV virus because RNA isolated from the NDV virus did not hybridize to pMKSU16 containing the POMC insert (data not shown).

To determine if there was specific mRNA for POMC in the cytoplasm of NDV-infected splenocytes, poly(A)⁺ cytoplasmic RNA was purified. There was positive hybridization of poly(A)⁺ cytoplasmic RNA from NDV-infected splenocytes (Fig. 1, lane 6) and from the positive control, AtT-20 cells (Fig. 1, lane 4). When equal concentrations of RNA were dot blotted onto Gene Screen membranes, there was a greater amount of hybridization of both total cellular and poly(A)⁺ RNA from AtT-20 pituitary cells than from NDV-infected splenocytes (Fig. 1, lanes 1 and 3 vs. lanes 4 and 6, respectively). Noninfected splenocytes did not contain specific mRNA for the POMC gene (Fig. 1, lane 5). Northern

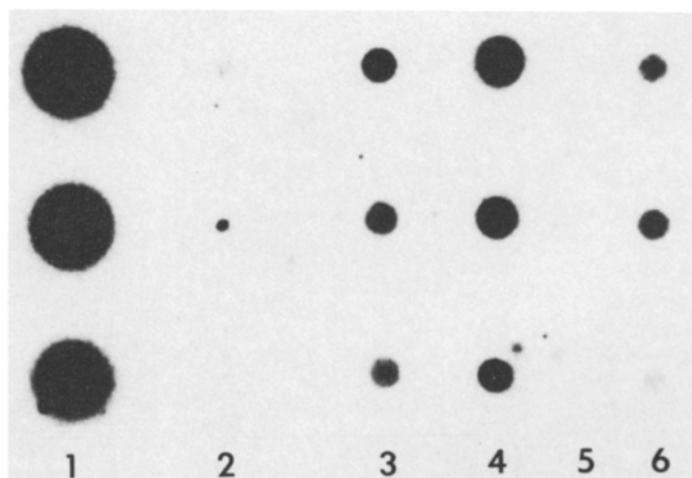


FIGURE 1. Hybridization of the recombinant plasmid, pMKSU16, which contains an insert coding for the entire mouse POMC sequence (16), to decreasing concentrations of total cellular RNA (20 μ g, 10 μ g, 5 μ g) isolated from AtT-20 cells (lane 1), noninfected splenocytes (lane 2) and NDV-infected splenocytes (lane 3), and to poly(A)⁺ cytoplasmic RNA (10 μ g, 5 μ g, 1 μ g) from AtT-20 cells (lane 4), noninfected splenocytes (lane 5), and NDV-infected splenocytes (lane 6).

blot analysis revealed that molecular size of POMC mRNA from NDV-infected splenocytes was similar to mRNA that was isolated from AtT-20 pituitary cells (Fig. 2, lanes 3 and 1). No specific mRNA from splenocytes cultured for 18 h in the absence of virus could be detected by Northern blot analysis (Fig. 2, lane 2).

Discussion

Until now, it has only been assumed that lymphocyte-derived ACTH and β -endorphin are POMC gene products. Since IFN- α is also synthesized by virus-infected lymphoid cells, it could be argued that lymphocyte-derived ACTH is synthesized from a gene sequence within the family of genes that codes for interferon proteins. However, there are no common nucleotide sequences shared by the cloned IFNs and POMC cDNA (14, 16). This finding could indicate that the cDNA probe used in this study hybridized to a POMC-like mRNA and not to an IFN- α mRNA. Therefore, these data provide direct evidence for expression of a POMC-like gene in virus-infected splenocytes.

Earlier studies using indirect immunofluorescence with ACTH and β -endorphin antibodies (7) have shown that nearly all of the human PBMC infected with NDV are positive. Both lymphocytes (7) and macrophages (17) may synthesize immunoreactive ACTH, which suggests that all virus-infected lymphoid cells can express the POMC gene. The percentage of positive cells may be dependent on the number of splenocytes that are actually infected with the virus, which would explain the coordinate expression of the IFN- α genes and the POMC gene (7). We are presently using cloned T, B and macrophage cell lines to determine if different activating signals can induce lymphoid cell subpopulations to express the POMC gene.

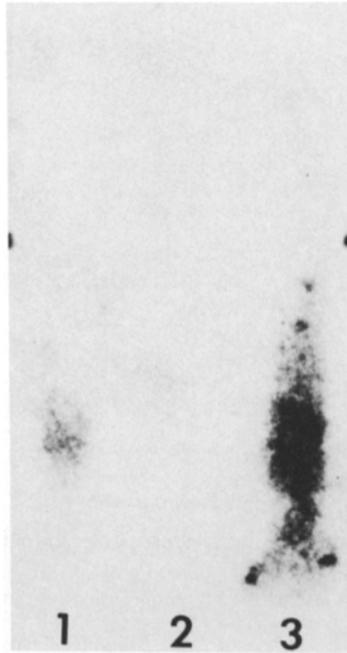


FIGURE 2. Northern blot analysis of poly(A)⁺ cytoplasmic RNA isolated from AtT-20 cells (lane 1, 0.50 μ g), noninfected splenocytes (lane 2, 6.5 μ g), and NDV-infected splenocytes (lane 3, 14 μ g).

The physiological significance of hormone secretion by lymphoid cells may be to serve as an afferent signal to the central nervous system or a target organ during viral or bacterial invasion. Synthesis and secretion of biologically active ACTH from lymphocytes would signal the adrenal gland to synthesize and secrete glucocorticoids. This possibility has been shown *in vivo* with a patient bearing an ectopic, ACTH-secreting lymphocytic tumor (18). Glucocorticoids are well known to play an important physiological role in lymphocyte regulation (19–21). It also is possible that hormones secreted by activated lymphocytes act as paracrine immunomodulators in their own microenvironment. ACTH has been shown to synergize with B cell growth and differentiation factors to augment B cell proliferation and differentiation (22) and to inhibit IFN- γ production (23). β -endorphin increases the proliferative response of lymphocytes to T cell mitogens (24), enhances natural cytotoxicity and IFN production by natural killer cells (25, 26), and stimulates chemotaxis of human PBMC (27). Enkephalins have common amino acid sequences to peptides derived from POMC (28) and these endogenous opioid peptides have also been shown to modulate immune events (29). Furthermore, lymphoid cells possess specific receptors for ACTH and β -endorphin (30, 31). Thus, there is substantial support for the idea that POMC gene products directly affect functional activities of lymphoid cells. Future studies aimed at understanding the process by which lymphocytes may become activated to synthesize mRNA for hormone precursors, translate this message into protein, process the precursor protein into active hormone peptides, and eventually

secrete these peptides will increase the understanding of systems of communication between the nervous, endocrine and immune systems.

Summary

Infection of murine splenocytes with Newcastle disease virus results in expression of the proopiomelanocortin gene as determined by dot and northern blot analysis of total cellular and poly(A)⁺ cytoplasmic RNA. These data provide the first evidence that the precursor protein for adrenocorticotropin and β -endorphin can be synthesized by lymphoid cells.

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