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Relationship Between the Neural Dysgenesis and Increased Production of Class I MHC H-2K^k mRNA and Protein in Neurons of Murine Trisomy 16 Fetuses

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Summary: Neuronal cells from murine trisomy 16 fetuses have increased levels of class I MHC H-2K^k. To determine whether this increased level of H-2K^k protein product resulted from an increased synthesis of mRNA, a 33 base antisense cDNA probe complementary to a region in exon 2 of the H-2K^k sequence (nucleotide 392-424) was synthesized. This probe was used to examine, by in situ hybridization and immunohistochemistry, the neural distribution of H-2K^k mRNA and protein product. A marked elevation of the H-2K^k mRNA and protein were localized in mts16 neuronal populations that were susceptible to dysgenesis. The results implicate the expression of the H-2K^k in the neuropathology of mts16 and its human counterpart, Down syndrome.

Murine trisomy 16 (mts16) has been studied (1) as a model for human trisomy 21 (Down Syndrome) because several common gene products are coded by these chromosomes (2) and several phenotypic anomalies, including endocardial cushion defect (3) and CNS dysgenesis (4-8), are shared by both trisomies. Cells from mts16 fetuses have an elevated expression of the H-2K^k antigen (4,5) and those from mts16 and hts21 have increased sensitivity to beta adrenergic agonists (9,10). Previous studies in cardiac tissue have shown interactions between beta-adrenergic function and the MHC class I H-2K^k antigen (11). This report describes the construction of a cDNA probe for the H-2 K^k mRNA, the appearance of the mRNA as determined by in situ hybridization between day-15 and day-18 of gestation and correlates the

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cellular localization of the increased expression of the mRNA for H-2 K^k with that of the protein product.

Methods and Material

Animals

The mts16 fetuses for our studies were produced by crossing all acrocentric BALB/c females with males carrying two different Robertsonian translocation chromosomes. One of the two translocation chromosomes is composed of autosomes 16 and 17 (Rb 16.17) and the second is composed of autosomes 11 and 16 (Rb 11.16).

Dams were sacrificed by cervical dislocation at gestation day 15, 16, 17 and 18. The appearance of a vaginal plug was marked as day 0 of gestation. For each time point studied, at least two dams were sacrificed and two normal and two trisomic conceptuses were removed and in situ hybridization performed on nervous tissue from each of these conceptuses.

Preparation of the Brain Sections

The heads of the fetuses were removed and placed in O.C.T embedding medium on a platform that was immersed in dry ice. Sections, 12-14 um thick, were then cut in a cryostat at -22° C and placed on glass slides coated with gelatin. One slide from each series of sections was retained for histological staining with thionine. The remainder of the frozen sections were processed for in situ hybridization as described below.

cDNA Probe

The preparation of the cDNA probe for mRNA coding for the H-2K^k MHC marker was based on previously derived data (12,13). A 33 base antisense cDNA probe (5' att ctc cgc gtc gct gtc gaa gcg cac gaa ctg 3') was designed complementary to a region in exon 2. This region extends from nucleotide 392 to 424 and has complete homology only with the H-2K^k mRNA (GenBank, Los Alamos NM). A search of GenBank revealed that the homologies with this region were restricted to class I genes and mRNA fragments. The antisense probe mRNA sequence has a total CG content of 50-60% The cDNA probe was labeled at the 3' end with digoxigenin-11-dUTP using terminal transferase (14; Boehringer Mannheim protocol). The labeled probe was purified by acid/ethanol precipitation cycles.

The method of Sambrook et al. (14) and the protocol of Boehringer Mannheim was used for examination of the reaction between the cells in the tissue and the digoxigenin labeled probe. The labelled sections were dehydrated through graded alcohol solutions, covered with mounting medium (66% Permount, 34% xylene) and a cover slip and examined microscopically. cDNA probes to somatostatin were used on control sections to determine background staining.

Immunohistochemistry

The frozen sections of brains from 17-day gestation fetuses were examined for the presence of the H-2K^k surface marker. Because BALB/c mice have H-2^d as the class I MHC marker, we used antibodies against H-2^b as a control immunoglobulin. The anti H-2K^k and anti H-2^b immunoglobulins were provided by Dr. Robert Auerbach, University of Wisconsin Department of Zoology. The frozen sections were fixed in 90% ethanol, air dried and then processed for immunohistochemical reaction with antibodies directed to the MHC antigens. The sections were incubated first with normal goat serum to block non-specific adsorption of antibody to the neural tissue and then incubated with the murine anti H-2K^k or anti H-2^b antibodies. The sections then were rinsed with phosphate buffered saline (0.01 M, pH 7.3) and incubated with the second antibody, fluorescein labeled goat anti mouse IgG purchased from Kirkegaard and Perry (Gaithersburg, MD). The sections were then rinsed and

covered with Entellan mounting medium. Several sections were incubated with second antibody alone (only with fluorescein labeled goat anti-mouse IgG).

Results and Discussion

The digoxigenin-labeled cDNA probe for H-2K^k reacted extensively with neurons in the central nervous system of the mts16 conceptuses and weakly with control conceptuses. The most reactive neurons were in the trigeminal ganglion and the supraoptic hypothalamic nuclei; extensive reaction also occurred with neurons in the cerebellum, inferior colliculus, thalamus, and brain stem. The maximal reaction of the probe with the neurons in all the examined brain areas occurred at gestation age of 16- and 17-days while the weakest reaction was at day-18.

The 16-day gestation age neural tissue from mts16 (Figs. 1,2) reacted more intensely with the H-2 K^k probe than tissue from younger or older conceptuses. Particular cells of the cerebellar external granule cell (EGC) and Purkinje cell layers were highly reactive with the probe and others were minimally to non-reactive (Fig 1). The pattern of reaction between the probe and the cells was consistent with a cytosolic localization of the reactive mRNA. In the control cerebellums, reaction was observed between cells in the EGC layer and the probe (Fig. 1) but the intensity of reaction product was much reduced compared with the ts 16. The developing Purkinje cells of control fetuses were minimally to non-reactive.

Neurons in the inferior colliculus (Fig. 1) and brain stem of the mts16 reacted more positively with the probe than those in controls (Fig. 1). Positive reaction was observed in neurons (Fig. 2) in the thalamus of mts16 conceptuses. The sections from control fetal thalamus revealed a much weaker reaction between the probe and neurons (Fig. 2).

The most intense reactions between the probe for the H-2K^k mRNA and neural tissue occurred in the sensory neurons of the trigeminal ganglion (Fig. 2) and the supraoptic hypothalamic nuclei. The cytosolic regions of the neurons in these areas were reactive; minimal to no reaction was seen in the nuclei. The intense reaction of the probe with the sensory neurons is consistent with the immunohistochemical studies described below which indicate that these cells have the highest level of the H-2K^k protein product.

The distribution of the protein MHC antigens in the cerebellum, colliculus and sensory ganglia (Figs. 1,2) of 18-day mts16 fetuses is consistent with the pattern of distribution of the cDNA probe for the mRNA of the H-2K^k. Selected cells in the EGC layer of cerebellum were reactive with the

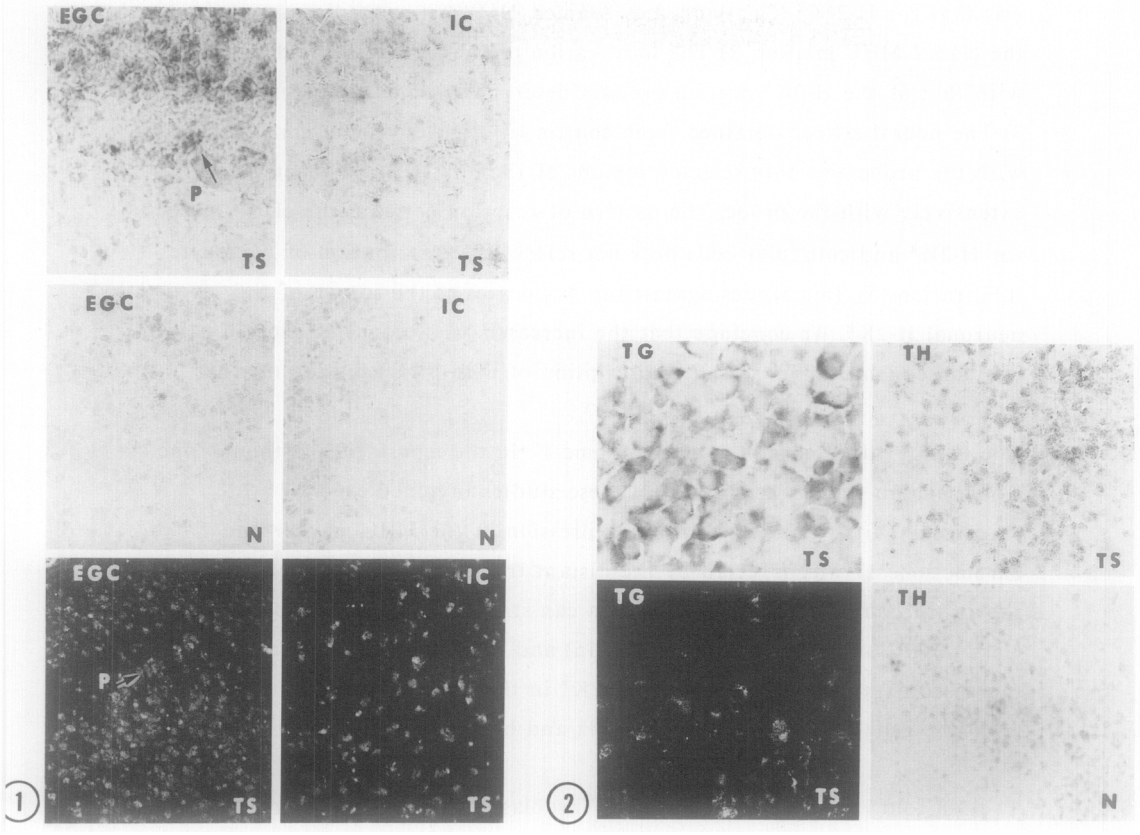

Figure 1. Micrographs of cerebellums (left side) and inferior colliculi (right side) from trisomy 16 (TS) and control (N) mice. The upper set of four micrographs, of tissue from 16-day fetuses, were reacted with the digoxigenin-labeled anti-sense cDNA probe for class I MHC H-2K^k. The dark staining material is indicative of positive labeling with the probe. The bottom set of two micrographs, of tissue from 17-day fetuses, is of fluorescein-labeled antibodies reflecting the presence of the H-2K^k protein product. Many cells in the external granule cell (EGC) layer and some in the developing Purkinje cell region (P) are positively labeled with the cDNA probe and the anti-protein antibody. All images were magnified 220 times.

Figure 2. Micrographs of sections through the trigeminal ganglion (left side) of a trisomy 16 mouse that was reacted with the anti-sense cDNA probe for H-2K^k (16-day gestation, upper frame) or with antibodies to H-2K^k protein product (17-day gestation, lower frame). The right side shows micrographs of thalamic sections from the 16-day gestation trisomy 16 mouse (TS, upper) or control (N, lower) that were reacted with digoxigenin-labeled antisense cDNA probe for class I MHC H-2K^k. The trigeminal ganglion images were magnified 350 times and the thalamic images 220 times.

immunoglobulin as were a subset of the Purkinje cells. Subsets of neurons distributed throughout the colliculus were H-2K^k positive. The strongest reaction was observed between the antibodies and the sensory neurons (Fig. 2).

The specificity of the probe for the class I MHC H-2 K^k marker was verified by several observations: 1) The only sequence with total homology identified

was that for H-2 K^k (GenBank, Los Alamos, NM). All related sequences were of the class I MHC marker; 2) The localization of the mRNA coincided very well with that of the H-$2K^k$ protein product determined immunohistochemically; 3) The neural tissues obtained from control littermates reacted only weakly with the probe and only selected regions of the mts 16 brain reacted extensively with the probe. The pattern of reaction between the cDNA probe for H-$2K^k$ and collicular cells does not reflect the distribution of monocyte infiltration (5); this argues against the monocytes as the source of the neuronal H-$2K^k$. We conclude that the increased MHC concentration in neurons is a consequence of increased transcription of this mRNA by the neurons.

The H-$2K^k$ MHC locus is on chromosome 17 in the mouse (13). Although one Robertsonian translocation used in these studies involved a no. 17 chromosome, it is unlikely that the expression of the MHC marker on chromosome 17 was affected by the distant translocation. Because it was shown in tissue culture that interferon can stimulate the expression of class I MHC antigens on neuroblasts (15) and trophoblasts (16), the increased expression of the class I H-$2K^k$ in the mts16 fetus could result from the gene dose effects of the alpha and beta IFNR (2).

The 3-8 fold increased sensitivity of fibroblasts from Down Syndrome patients to interferon (17), with regard to inhibition of viral replication, may be related to the increased level of the IFNR (2) and their increased sensitivity to beta adrenergic agonists (9). The interaction between the beta adrenergic receptor and the MHC complex was shown by Cremaschi et al. (11) who observed that antibodies to the H-$2K^k$ complex increase contractility in heart auricles and that beta blockers inhibited the increased contractility induced by the antibodies.

Since the level of the MHC H-$2K^k$ is markedly increased in developing neurons of mts16 and anti- H-$2K^k$ antibodies perturb the ligand binding site on beta adrenergic receptors, the MHC molecules may act synergistically with the adrenergic receptor. The increased expression of the H-$2K^k$ on neurons in the mts16 conceptus and the interaction between this antigen and beta adrenergic receptors may cause the mts16 cells to be unusually sensitive to beta adrenergic agonists. Indeed the observations of Iwasaki et al. (18), that the administration of beta adrenergic agonists to pregnant dams induces endocardial cushion defects in fetuses, suggest that altered expression of beta adrenergic receptors in the Down patient or in mts16 animals may be involved in the genesis of this defect. Our observations that the mts16 fetuses exhibit 1) an altered organization of the cerebellum, thalamus and inferior colliculus (4,5), 2) delayed synaptogenesis in the cerebellum (4),

3) abnormal dimensions and structure of the labyrinth and junctional zone of placenta (19) is relevant since we have shown found that these regions express high levels of H-2K^k. Because the H-2K^k marker and the adrenergic receptors are cell surface proteins, an overexpression of these cell surface molecules may affect cell function through transmitter mediated mechanisms and/or by mechanically limiting the normal distribution of other surface molecules involved in organogenesis.

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