Immunohistochemical Study of Foci of Recent Cell Death in Huntington's Disease

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SUMMARY: *Foci of recent cell death were identified in 10 consecutive cases of Huntington's disease in the caudate nucleus and putamen. Indirect immunohistochemical staining procedures were performed on these areas and controls using fluorescein conjugated anti-globulin and peroxidase-conjugated anti-globulin. Positive results were found when human antiserum to Cytomegalovirus was used. The possibility of abnormal antigen presence within the dying neurons in this disease is discussed.*

RÉSUMÉ: *Dans le noyeau caudé et le putamen de 10 cas consécutifs de Chorée de Huntington nous avons identifié des foyers de mort cellulaire récente. Des procédures de coloration immunohistochimiques indirectes furent faites sur ces zones, ainsi que chez des témoins, en employant les antiglobulines conjugués à la fluoresceine ou au peroxidase. Des résultats positifs furent obtenus avec l'emploi d'antiserum humain au cytomegalovirus. Nous discutons de la présence possible d'antigène anormal à l'intérieur des neurones.*

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The principal morbid anatomic finding in Huntington's disease (H.D.) is atrophy of the brain involving many areas, but most noticeably the striatum and cerebral cortex (Bruyn, 1973; Corsellis, 1976; Lange et al, 1976). Microscopically the shrunken areas show nerve cell loss and gliosis. While the latter has generally been assumed to represent the end-stage of the process of nerve cell depletion, descriptions of acute cell loss in this disease have been lacking. The frequent presence of regions of normal appearance within the known abnormal regions of the brain in this disease suggest that the pathological process is not global ("holotopistic"). The recent finding of recent cell death in the involved regions (Averback, 1979) has suggested that the underlying abnormality may progress in piecemeal fashion. In order to further explore this possible mechanism, immunohistochemical study of these foci has been carried out. In this report evidence is presented from immunofluorescence and immunoperoxidase studies suggesting that abnormal antigen and perhaps virus may be related to these foci of cell death.

MATERIAL AND METHODS

From Nissl stained paraffin embedded sections of 10 cases areas of interest were demarcated and further sections were examined by indirect immunofluorescence techniques. The areas were noted on the slides by etching the glass with a diamond marker. Sections were brought to water, incubated with antisera in a moist box at 37°C. for 30 minutes, washed in phosphate buffered saline, rinsed in distilled water, air dried, incubated with fluorecein conjugated anti-immunoglobulin (diluted 1 in 20 in buffered saline) for 30 minutes, washed in buffered saline, rinsed in distilled water, air dried and mounted with glycerol adjusted to pH 8.7. Rabbit anti-Herpes simplex (HSV), human anti-HSV, human anti-Cytomegalovirus (CMV), and human anti-Varicella-Zoster (VZ) were used as antisera. Human antisera contained no cross-reactivity, as determined by initial complement fixation titers against all three viruses. Each test was done on each case (in some instances the foci of cell death were not present on subsequent sections) as well as positive and negative controls. When sections were available procedures were repeated twice and specific antisera were used from 2-3 different sources for each virus. The tests were controlled at several levels: 1) Background autofluorescence and staining of H.D. brain was monitored, 2) Fluorescein conjugated anti-human globulin alone was tested on H.D. brain sections, 3) Normal human brain sections (six) from the basal ganglia were tested, 4) Histologically unaffected areas in sections from H.D. brain, as determined from adjacent Nissl stained sections, were tested, 5) A limited number (eleven) of sections of striata from various chronic neurological diseases (including Alzheimer's disease, multiple sclerosis, stroke) were also compared, as well as much larger numbers (over 200, detailed elsewhere) initially screened for the small foci of acute cell death as seen in H.D. striata, 6) A final form of control was provided by variation of viral specific antisera. Fluorescein conjugated antisera used were swine anti-rabbit and rabbit anti-human. Immunoperoxidase staining was done on sections, using anti-HSV, and anti-CMV. Diaminobenzidine, peroxidase-antiperoxidase and peroxidase conjugated anti-IgG were used in this procedure. In all of the above tests

https://doi.org/10.1017/S0317167100023441 Published online by Cambridge University Press

selection of cases was consecutive except when remaining sections were exhausted.

RESULTS

In the material thus far studied, positive staining with the immunoperoxidase method and immunofluorescence (Figure 1) have been found with the use of anti-CMV and the above methods. Positive results have seemed to be limited to the regions showing acute cell loss histologically, although strict correlation has not always been evident. Control normal brain and chronic neurological diseases have been negative.

DISCUSSION

Reservation is necessary in the interpretation of findings based on indirect immunohistochemical techniques on postmortem tissues. This study will require followup work using absorbed antisera, exposed to virus or virus infected tissue, as well as continued efforts at culture. If indeed the foci of immunofluorescence and positive immunoperoxidase staining prove not to be due to presence of virus within the neurons in these areas, then some alternative explanation needs to be sought. Slight autofluorescence and the amount of visible pigment seen with conventional stains both suggest that this phenomenon is not due to lipofuscin, and negative findings when fluorescein conjugated antiglobulin alone was used argues against the simple presence of spurious antibody in areas of recent cell death. It is tempting to speculate that CMV is responsible for the fluorescence and cell death, but much carefully controlled further work (to rule out or perhaps identify other serum antibodies and/or cellular antigens potentially responsible for the positive tests) will be necessary before firm conclusions can be drawn.

ACKNOWLEDGEMENTS

This work was supported by the Medical Research Council of Canada. The author is grateful to Mr. B. Potter, Mrs. R. Brown, and Mr. D. Smith for technical assistance, and to Dr. J. Nagington for providing antisera.

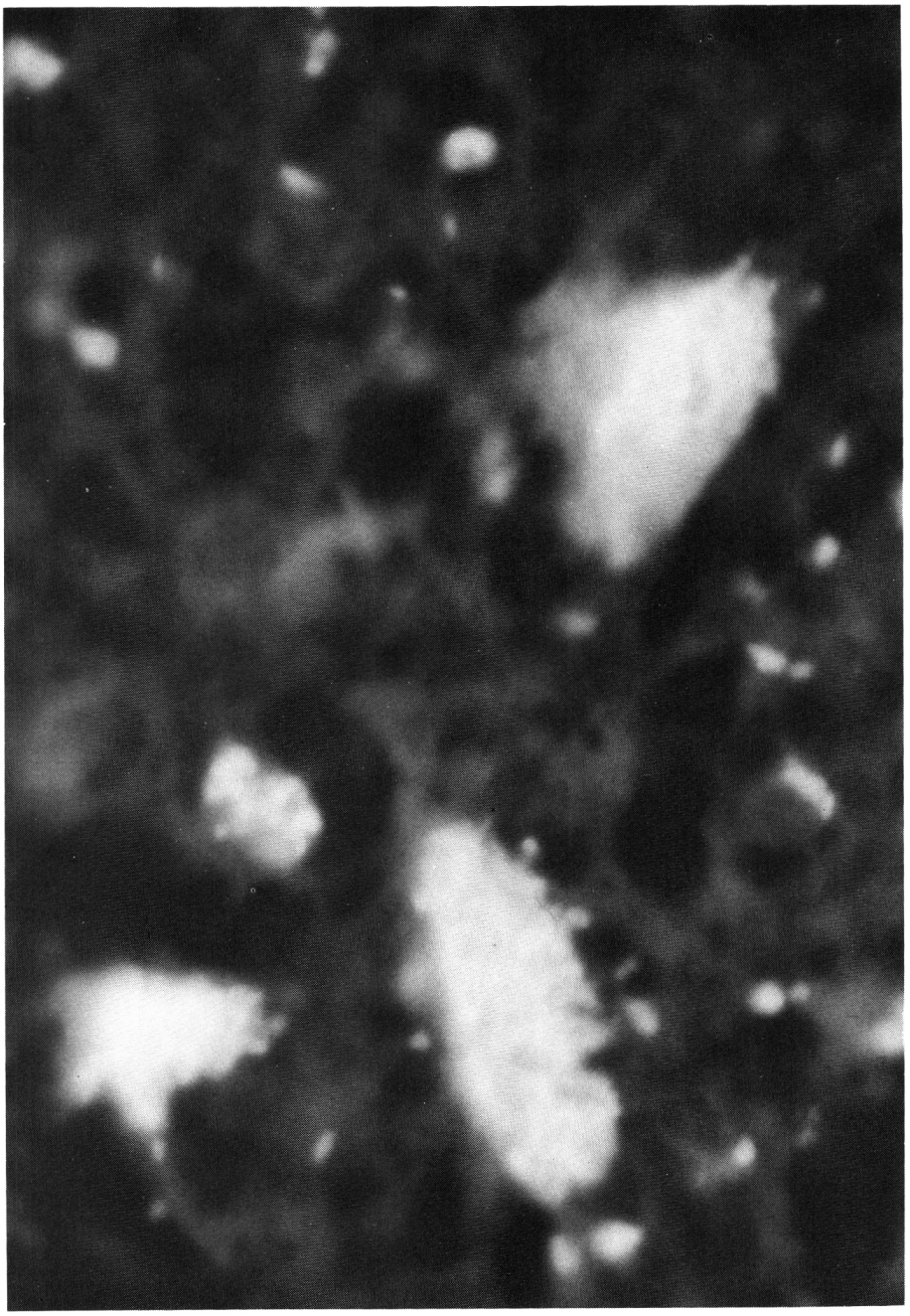

Figure 1 — Positive immunofluorescence of striatal neurons in Huntington's chorea, using anti-CMV in indirect technique. X 1800.

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