References and Notes

1. I. Rock, *Sci. Am.* **230**, 78 (January 1974).
2. R. K. Yin, *Neuropsychologia* **8**, 395 (1970).
3. A. G. Goldstein [*Psychonom. Sci.* **3**, 447 (1965)] reported that 8-year-olds, unlike adults, learned to pair letters with inverted faces almost as easily as with upright faces. He did not specify the age at which the adult susceptibility to inversion appears. The full reports of experiments 1 and 2 (*4*, *8*) indicate subtle changes in face perception at least until age 16. See also A. G. Goldstein [*J. Genet. Psychol.* **127**, 109 (1975)].
4. S. Carey, R. Diamond, B. Woods, in preparation.
5. A. Lévy-Schoen, *Image d'Autrui Chez l'Enfant* (Presses Universitaires de France, Paris, 1964).
6. On paraphernalia-to-fool items, if the child bases his judgment of identity on paraphernalia, he will make the wrong choice. On paraphernalia-equal items, reliance on paraphernalia cannot lead to a choice since all three photographs show the same paraphernalia. On paraphernalia-to-help items, reliance on paraphernalia leads to a correct choice. There are also expression-to-fool (types 3 and 4), expression-equal (type 1) and expression-to-help (type 2) items (Fig. 1).
7. That is, the 6- and 8-year-olds gave the pattern of errors expected if paraphernalia alone were the source of confounding while the 10-year-olds have moved toward equal difficulty of all four problem types. At age 10 expression does not replace paraphernalia as the sole source of confounding; expression-to-fool problems are not harder than expression-to-help problems.
8. R. Diamond and S. Carey, *J. Exp. Child Psych.*, in press.
9. Face recognition deficits are associated with right hemisphere injury [for example, E. De Renzi and H. Spinnler, *Neurology* **16**, 145 (1966), E. Warrington and M. James, *Cortex* **3**, 317 (1967), B. Milner, *Neuropsychologia* **6**, 191 (1968), A. L. Benton and M. W. Van Allen, *Cortex* **4**, 344 (1968)]. A privileged role for input to the right hemisphere in nonverbal face recognition tasks has been demonstrated for patients in whom the two hemispheres have been surgically disconnected [J. Levy, C. Trevarthen, R. W. Sperry, *Brain* **95**, 61 (1972)] and for normal adults [for instance, G. Rizzolatti, C. Umiltà, G. Berlucchi, *Brain* **94**, 431 (1971); R. D. Hilliard, *Cortex* **9**, 246 (1973)].
10. B. Woods and H.-L. Teuber, in preparation.
11. B. Kohn and M. Dennis [*Neuropsychologia* **12**, 505 (1974)] found that young adults with early right hemidecortication were unimpaired on a number of visual and spatial tasks in which patients with right hemisphere lesions incurred during adulthood are severely deficient. Sparing was limited to tasks on which normal children become proficient before age 10. This suggests that the early-maturing skills can be mediated by either hemisphere but that functions for which the right hemisphere is specialized by age 10 cannot be assumed by the left.
12. For example, D. Kumura, *J. Comp. Physiol. Psychol.* **56**, 899 (1963); D. S. Geffner and I. Hochberg, *Cortex* **7**, 193 (1971); but see also D. Ingram, *Neuropsychologia* **13**, 103 (1975); P. Satz, D. Bukker, J. Tenunissen, R. Goebel, H. Van der Vlugt, *Brain Lang.* **2**, 171 (1975).
13. S. Leehey, thesis, Massachusetts Institute of Technology (1976). With tachistoscopic presentation of words and faces, 8-and 10-year-olds both show a right visual field advantage for words, but only at age 10 is the left visual field advantage for faces present. Leehey used unfamiliar faces, each presented only once. In young children the right hemisphere might be differentially involved in the processing of familiar faces, especially if represented configurationally. Leehey found a left visual field advantage in the recognition of familiar faces as young as age 8. Thus it seems that maturation of the right hemisphere may be directly implicated in efficient configurational encoding of new faces, the ability tapped in experiments 1 and 2.

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Huntington's Disease: Delayed Hypersensitivity in vitro to Human Central Nervous System Antigens

Abstract. *Huntington's disease is a hereditary, chronic, degenerative disease of the brain which is transmitted by an autosomal dominant gene. We have discovered that lymphocytes from patients with Huntington's disease respond to the presence of brain tissue from patients with the disease by producing migration inhibition factor, a correlate of the cellular immune response. Lymphocytes from donors without the disease do not respond to the diseased brain tissue, and lymphocytes from patients with Huntington's disease respond only rarely to brain tissue from donors without the disease.*

Huntington's disease (HD) is a chronic, degenerative disease of the brain most often characterized by progressively increasing choreiform movements and dementia. It is hereditary and is transmitted by an autosomal dominant gene. Pathologically it is marked by neuronal dropout principally in the striatum and cerebral cortex, but with diffuse and variable involvement of other brain regions as well. Other characteristic features include an intense gliosis in regions of neuronal loss, and accumulations of lipofuscin in both neurons and glia (*1*, *2*). Although brain macrophages are often found in devastated regions, no infiltration of lymphocytes or other signs of inflammation have been seen (*2*). Nothing is known about the manner in which the HD gene causes cell death, and management of the disease is largely confined to the use of tranquilizers and dopamine antagonists which reduce choreiform movements (*1*).

We have discovered that lymphocytes from patients with HD respond to the presence of HD brain tissue with the production of migration inhibition factor (MIF), a correlate in vitro of delayed hypersensitivity. Lymphocytes from donors without HD do not respond to HD brain, and HD lymphocytes respond only rarely to brain tissue from donors without the disease.

Brain antigen was prepared by homogenizing frontal lobe gray matter in Ca- and Mg-free Hanks balanced salt solution (CMF Hanks) in a Teflon tissue grinder at 4°C. The homogenate was centrifuged at 1000*g* for 20 minutes at 4°C, and sterilized by passage through a 0.45-μm filter. The brains used are identified in Table 1 with the catalog number of the Human Specimen Bank (*3*). Specimens 201 (from a female, age 69), 203 (from a female, age 55), and 204 (from a male, age 60) were all removed postmortem from patients with HD. Pneumonia was the immediate cause of death in all cases. Specimens 208 (from a female, age 61) and 154 (from a female, age 63) did not have HD and died of pulmonary tuberculosis and a myocardial infarction, respectively. Specimens 209 (from a male, age 62) and 193 (from a male, age 65) both had Alzheimer's disease and died of renal failure and pneumonitis, respectively. Specimen 180 (from a male, age 60) had Parkinson's disease and died of a pulmonary embolism with infarction.

Lymphocytes were obtained from patients with HD and from individuals without degenerative disease of the central nervous system or other serious medical problems. One donor of control lymphocytes (C4) had tardive dyskinesia. Venous blood (30 ml) was drawn in heparin or ethylenediaminetetraacetate (EDTA) and lymphocytes were purified by centrifugation in a discontinuous Ficoll-Hypaque gradient (*4*). The cells were washed twice in CMF Hanks and suspended in Medium 199 (GIBCO) supplemented with 15 percent horse serum and 25 m*M* Hepes buffer (complete medium) (*5*) at a final concentration of 10^6 cells per milliliter.

Lymphocyte cultures were prepared by placing 2 ml of cell suspension in 15-ml plastic culture tubes (loosely capped) with and without antigen (100 to 300 μg of protein). All cultures were made in duplicate and incubated for 24 hours at 36°C in 5 percent CO_2 and 95 percent air. Better responses were obtained in a CO_2 incubator despite the presence of Hepes buffer. The conditioned medium was centrifuged at 1000*g* for 10 minutes, and the supernatants were stored at −70°C prior to assay for MIF activity.

Migration inhibition factor was assayed with guinea pig peritoneal exudate elicited with an intraperitoneal injection of 25 ml of light mineral oil. Four days after the injection the peritoneal cavity was washed with CMF Hanks and the exudate separated from oil. The cells were washed twice in CMF Hanks and suspended in complete medium (approximately 2×10^6 cells per milliliter). Microcaps (50 μl) containing the cell suspension were heat-sealed and centrifuged at 400*g* for 5 minutes. The

capillary tips were broken at the interface between the cells and the medium and were fixed to the bottoms of Dispotray culture wells (Linbro Chemical) with silicone grease. Test medium (1 ml) was added and the cultures incubated at 36°C in 5 percent CO_2 and 95 percent air. After 24 hours the cultures were fixed in glutaraldehyde, and the halo of cells migrating from the capillary tip projected onto integration paper with an overhead projector. The weight of the paper over which the halo projected was used as a measure of the area of migration.

The activity of MIF was recorded as the percentage migration, calculated from the formula:

$$\text{Percentage migration} = (X/Y) \times 100$$

where X is the migration area in conditioned medium (lymphocytes plus antigen) divided by the migration area in unconditioned medium (plus antigen), and Y is the migration area in conditioned medium (lymphocytes minus antigen) divided by the migration area in unconditioned medium (lacking antigen). The value for Y was not permitted to exceed 1. Four measurements were obtained for each lymphocyte preparation and the results averaged.

Lymphocytes from both the HD patients and the control donors were confronted with a total of eight different antigen preparations, and the MIF activities calculated as the percentage migration (Table 1). Three brains from HD patients, two brains from patients with Alzheimer's disease, the brain from a patient with Parkinson's disease, and two brains from patients without known neurological disease were used. Activity of MIF was detected only when HD lymphocytes were confronted with HD brain (Table 1 and Fig. 1). An average of 108 percent migration was obtained for HD lymphocytes confronted with control brains without neurological disease (specimens 208 and 154). An average percentage migration was obtained for each of the other test antigens and compared with the mean value for normal brains in a t-test (unpaired). All three HD brains were significantly different from normal ($P < .01$). None of the other brains with neurological disease, separately or pooled in any combination, were significantly different from normal.

Data from control lymphocytes confronted with test antigens were treated similarly. Neither HD nor other diseased brains were significantly different from normal brain when confronted with control lymphocytes. The observed enhancement was probably artifactual, and has not been seen in subsequent controls.

Equal portions of lymphocyte preparations from five HD patients were confronted simultaneously with either HD or non-HD brain (Table 1). Lymphocyte preparations from three out of five patients yielded MIF activity only when confronted with HD brain. One showed MIF activity with both normal and HD brains.

Migration inhibition factor can appear in response to virus infection without activation of the immune system (*6*), and possibly other nonimmune mechanisms exist which could elicit its appearance. It is unlikely that some agent present in HD brain induced secretion of MIF in lymphocytes in any reaction other than an immune reaction, because control lymphocytes did not secrete MIF in the pres-

Table 1. Lymphocytes from patients with HD (HD1 to HD16) and from donors without the disease (C1 to C17) were cultured with extracts of three HD brains (201, 203, 204), three brains with other neurological disease (209, 193, 180), and two brains without neurological disease (208, 154). The conditioned medium was assayed for MIF activity and the responses were recorded as percentage migration. Low values indicate high MIF activity. A mean percentage migration for both HD and control lymphocytes was obtained from pooled data for undiseased, control brains (208, 154). Mean percentage migrations for each of the other brain preparations were compared with the pooled values in a t-test (unpaired). HD lymphocyte preparations showed significant MIF activity only when confronted with HD brain tissue. No differences were seen with control lymphocytes.

Lymphocyte donors			HD antigens			Control antigens				
Name	Age	Sex	201	203	204	208	154	209	193	180
HD1	51	F			78					
HD2	45	M			67					
HD3	51	M	75*	89	125		68*		95	105
							125			
HD4	42	M			78					
HD5	43	M	101	90	81		101			
HD6	44	M	100	88	92					94
HD7	47	F	82	67	85					75
										98
HD8	49	F				160		150		
HD9	43	F				86		85		
HD10	54	F				114		110		
HD11	60	M	76*	93	65		113*		109	93
				65						
HD12	50	M	76		69		92			
					108					
HD13	49	F	83	100	78*	100*			104	
				70	92					
HD14	54	M	72	80	76*	100*	107		99	97
			75	70	92*	100*	109			
HD15	66	M			92*	100*	150	100		
						100	108			
HD16	49	M	120		88					
					78					
Means			86†	81†	85†	108	108	111	102	94
C1	39	F			175					
C2	30	M			151					
C3	47	F			166		118			
C4	51	M			100					
C5	53	M			113					
C6	35	M			113					
C7	47	M				120		140		
							116			
C8	27	M				71		92		
C9	24	M				100		115		
C10	48	F				115		160		
C11	44	M	140*				115*			
C12	54	M	155*		150		150*			
C13	51	M	117*		120		166*			
C14	52	M	147*		135		166*			
C15	32	M	115		110		100			
					125		100			
					125					
					171					
C16	35	M					95			
C17	58	M	100				112			
Means			129		135	102	124	127		

*Lymphocyte preparations were confronted simultaneously with both HD brain and control brain. †Significantly different, $P < .01$.

ence of HD brain. Likewise, the specificity of the response dictates against lymphocyte "processing" of an agent unique to HD brain such that it inhibited macrophage migration. While it is necessary to confirm the immunological nature of the response with other assays of delayed hypersensitivity in vitro, response specificities strongly implicate the immune system in the mediation of the reaction described here.

The immune response of HD patients might have several causes. The brain is an immunologically privileged site, and degeneration of the brain can lead to the release of previously sequestered macromolecules. Rocklin *et al.* (*7*), for example, have shown that six out of nine patients with stroke had a cellular immune response to myelin basic protein. The double specificity of the HD immune response does not support such an explanation in this instance, however. While the data suggest that a nonspecific response may occur in a few instances, the principal component of the response appears directed against something present only in HD brain. In a recent double-blind study with nine coded samples of brain tissue, the MIF assay selected out all of the HD brains with only one significant error (*8*).

Although the sample size is smaller, it is probable that the brains from patients with Alzheimer's disease did not contain the HD antigen. The mean percentage migration from the Parkinson's brain, while somewhat low, was not significantly different from normal. Degeneration per se does not, therefore, seem to modify brain to yield an immunologically active product.

Another possibility would be that degeneration, and subsequent reactive gliosis, could create a brain with substantially different antigenic properties. For example, a sequestered glial antigen released during brain degeneration might elicit an immune response detectable only when the glial scar of an HD brain was used as antigen. However, in an examination (*9*) of portions of HD brain (specimen 201) it was found that while the striatum was devastated, the frontal cortex showed minimal cell loss and no reactive change. Lymphocytes from patients with HD responded positively to this brain with MIF production.

The HD immune response does not, therefore, appear to be directed against an agent common to all human brains, a common degeneration product of brain, or a constituent amplified in HD brain because of changes in proportions of cell types.

It is important to emphasize that the presence of an immune response in HD patients does not suggest that HD is an autoimmune disease. Huntington's disease lacks any of the pathological features associated with autoimmunity. Rather, the response seems to be directed against an agent unique to the HD brain. The nature of this agent and its relation to the HD gene remains to be determined.

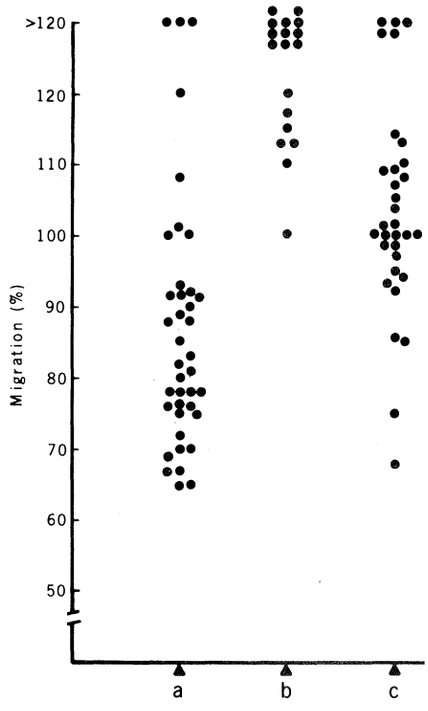

Fig. 1. Pooled results for all of the HD brains and both of the undiseased, control brains (from Table 1). (a) HD lymphocytes, HD brain. (b) Control lymphocytes, HD brain. (c) HD lymphocytes, control brain. The mean percentage migration for HD lymphocytes confronted with HD brain is significantly different from both HD lymphocytes plus control brain and control lymphocytes plus HD brain ($P < .001$). Although enhancement is seen in control lymphocytes plus HD brain the mean value is not significantly different from HD lymphocytes plus control brain.

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References and Notes

1. A. Barbeau, T. N. Chase, G. W. Paulson, *Advances in Neurology*, vol. 1, *Huntington's Chorea, 1872–1972* (Raven, New York, 1973).
2. I. Tellez-Nagel, A. B. Johnson, R. D. Terry, *J. Neuropathol. Exp. Neurol.* **33**, 308 (1974).
3. Brains were obtained from the Human Specimen Bank, Department of Neurology, Wadsworth V.A. Hospital, Los Angeles, Calif.
4. A. Boyum, *Scand. J. Clin. Lab. Invest.* **21**, 77 (1968).
5. Hepes buffer: 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.
6. Y. Takeshi, P. Bigazzi, S. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1641 (1975).
7. R. E. Rocklin, W. A. Sheremata, R. G. Feld man, M. W. Kies, J. R. David, *N. Engl. J. Med.* **284**, 803 (1971).
8. D. S. Barkley and S. Hardiwidjaja, in preparation.
9. Specimen 201 was examined by Hideo H. Itabashi, Harbour General Hospital, Long Beach, Calif.

10. We thank G. Ellison, L. Myers, D. Baumann, E. Martz, and S. Matthysse for their advice and assistance. W. W. Tourtellotte and R. F. Goodlett at the Human Specimen Bank, Wadsworth V.A. Hospital, Los Angeles, assisted us in acquiring both bloods and brains for this study. The research was supported by grants from the Foundation for Research in Hereditary Disease, NIH (NS12256-01), and the Medical Research Service, Veterans Administration. Reprint requests should be addressed to D.S.B., Department of Pathology, University of California, Los Angeles, Center for the Health Sciences, Los Angeles 90024.

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