that ensues allows the comparisons to be made before high hormone concentrations within the carotid artery limit the validity of the method. Since anesthesia modifies both seizure activity and pituitary function, these studies were performed in awake animals.

The high baseline pre-seizure ACTH concentrations of each of the nonhypophysectomized animals provide indirect evidence of stress from the experimental conditions alone. In one animal, Bathsheba, sagittal sinus ACTH concentrations were much higher than carotid artery concentrations prior to the seizure, suggesting that ACTH was carried from the pituitary directly to the brain prior to the convulsion. However, in each animal the convulsion caused further elevations of plasma ACTH.

The elevation of plasma ACTH concentrations noted in sagittal sinus venous blood in all of these animals indicates that this hormone was added to blood at some intracranial site. Venous blood from the frontal lobes, the parietal lobes, and the diencephalon (via the vein of Galen) converges in the sagittal sinus near the spot where our catheter tip was positioned. The high venous ACTH concentrations at this site indicate that hormone-enriched blood flowed through the capillary beds of these regions of the brain. ACTH is produced not only by the pars intermedia and the adenohypophysis, but by the brain itself (*9*), and this particular hormone may have come into sagittal sinus venous blood from either the brain or the pituitary. Since plasma ACTH was markedly reduced after hypophysectomy, most of the ACTH found within the sagittal sinus must have come from the pituitary.

The presence of substantial concentrations of ACTH in the post-seizure sagittal sinus samples after hypophysectomy verifies that ACTH (or ACTH-like material) was released from the brain as well as the pituitary by this stimulus. This demonstrates that the brain has access to two kinds of ACTH: *brain* ACTH produced locally within the brain itself (*9*), and *pituitary* ACTH carried directly to the brain from the pituitary. These may be identical molecules, but they also could by similar molecules recognized by the same antibody.

In a study of four other sheep we have confirmed the reports of others (*12*) by noting that the blood brain barrier becomes permeable to Patent Blue dye injected intravenously immediately before an electrically induced seizure. Presumably ACTH released from the pituitary by this unusual stimulas would pass into the brain. Brain ACTH carried to the sagittal sinus more certainly must have passed through a transiently open blood brain barrier.

Two vascular routes have been described (*3*) that have the potential to carry hormone-enriched pituitary blood directly to the brain. Which of these routes conveys ACTH to the brain cannot be established by this kind of study, yet these pilot physiological studies have demonstrated that, under certain circumstances, vascular mechanisms may transport a hormone from the pituitary directly to the brain. This may be true for many more of the hormones that are found commonly in the brain (*10*) and the pituitary.

R. Bergland, H. Blume
A. Hamilton, P. Monica
R. Paterson

Harvard Medical School
and *Beth Israel Hospital,*
Boston, Massachusetts 02215

References and Notes

1. G. B. Wislocki and L. S. King, *Am. J. Anat.* **58**, 428 (1936); G. W. Harris, *Neural Control of the Pituitary Gland* (Arnold, London, 1955).
2. R. M. Bergland, S. L. Davis, R. B. Page, *Lancet* **1977-II**, 276 (1977).
3. R. B. Page and R. M. Bergland, *Am. J. Anat.* **148**, 345 (1977); R. M. Bergland and R. B. Page, *Endocrinology* **102**, 1325 (1978); R. B. Page, A. E. Leure-duPree, R. M. Bergland, *Am. J. Anat.* **153**, 33 (1978); R. M. Bergland and R. B. Page, *Science* **204**, 18 (1979).
4. B. Torok, *Acta Morphol. Acad. Sci. Hung.* **4**, 83 (1954); J. Szentagothai, B. Flerko, B. Mess, B. Halasz, in *Hypothalamic Control of the Anterior Pituitary* (Akademiai Kiado, Budapest, 1962), p. 81; B. Torok, *Acta Anat.* **59**, 84 (1964); R. Yalow, *Science* **200**, 1236 (1978); E. Mezey, M. Palkovits, E. R. de Kloet, J. Verhoef, D. de Wied, *Life Sci.* **22**, 831 (1978); P. D. Pezalla, M. Lis, N. G. Seidah, M. Chretien, *Can. J. Neurol. Sci,* **5**, 183 (1978); R. Moldow and R. Yalow, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 944 (1978); D. M. Dorsa, E. R. de Kloet, E. Mezey, D. de Wied, *Endocrinology* **104**, 1663 (1979).
5. In the sheep, the vein of Galen enters the sagittal sinus at the vertex of the skull between the occipital lobes of the brain. Thus, the tip of our sagittal sinus catheter was positioned between the parietal lobes.
6. Seizures were induced with the Reiter Electrostimulator (model CW 47C #551). The measured current intensity of the 3-second, 8-Hz stimulus was 25 ma.
7. Hypophysectomy was performed via the transpharyngeal route and included the removal of the median eminence, the entire neurohypophysis, and the entire adenohypophysis. The animal received 10 mg of dexamethasone immediately after hypophysectomy.
8. A. Liotta and D. T. Krieger, *J. Clin. Endocrinol. Metab.* **40**, 268 (1975).
9. D. T. Krieger, A. Liotta, M. J. Brownstein, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 648 (1977); A. S. Liotta, D. Gildersleeve, M. J. Brownstein, D. T. Krieger, *ibid.* **76**, 1448 (1979).
10. R. Guillemin, *Science* **202**, 390 (1978); J. Hughes, *Brain Res.* **88**, 295 (1975); S. T. Pacold, L. Kirsteins, S. Hojvat, A. M. Lawrence, *Science* **199**, 804 (1978); T. Hokfelt *et al.*, *Nature (London)* **284**, 515 (1980).
11. In our laboratory insulin infusions, thyroid-releasing hormone infusions, antidiuretic hormone infusions, hypertonic saline infusions, pyrogen infusions, and intraventricular carbachol have all been used to stimulate pituitary secretion. None of these can be timed as precisely as electrically induced seizures.
12. C. Angel, A. M. Hartman, M. C. Burkett, A. J. Roberts, *J. Nerv. Ment. Dis.* **140**, 405 (1965).
13. Funded by a Beth Israel Hospital Institutional Biomedical Research Grant and gifts from the Kleberg Foundation and the June Rockwell Levy Foundation. We thank A. Liotta and D. Krieger in New York for performing the ACTH assays.

8 September 1979; revised 19 June 1980

Deficient Natural Killer Cell Activity in X-Linked Lymphoproliferative Syndrome

Abstract. *The activity of natural killer cells was found to be deficient in 10 of 12 males with X-linked lymphoproliferative syndrome, a life-threatening proliferation of lymphocytes after infection by Epstein-Barr virus. The activity levels of natural killer cells from affected males were increased after treatment with interferon in vitro, but normal levels of killing were not obtained. Deficient activity of killer cells in individuals with immunodeficiency and chronic infection by Epstein-Barr virus may contribute to the development of lymphoproliferative disorders.*

In 1975 it was found that normal spleen cells of some strains of mice are selectively cytotoxic to neoplastic transformed cells in vitro (*1*). These cells were designated natural killer (NK) cells. Subsequent studies demonstrated increased activity of NK cells in the athymic nude mouse (*2*) and deficient activity in the beige mouse (*3*). The nude mouse is relatively resistant to spontaneous leukemias and lymphomas, while the beige mouse is highly susceptible to viral and chemically induced transplantable leukemias (*4*). A role for NK cells in recovery from certain viral infections was suggested by Welsh *et al.* (*5*), and recent evidence supports this concept in murine cytomegalovirus infections (*6*). It appears that NK activity is regulated by interferon (*7, 8*). Type I leukocyte or fibroblast interferon augments NK cell activity against tumor cells and virus-infected target cells.

Natural killer cells in man are Fc receptor-bearing lymphocytes with a low affinity for binding sheep erythrocyte rosettes. They effect spontaneous lysis of malignant and virus-infected target cells (*1*). Little is known about the role of the NK-interferon system during infection and malignancy in man. The X-linked lymphoproliferative (XLP) syndrome is characterized by immunodeficiency to Epstein-Barr virus (EBV) [a

0036-8075/80/1031-0543$00.50/0

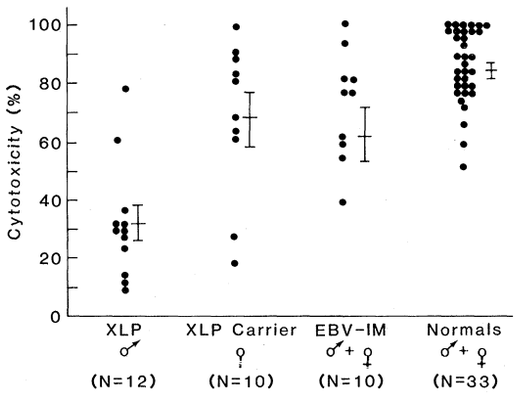

Fig. 1. Activity of NK cells against K562 target cells in XLP-affected males, carrier females, individuals with acute EBV-induced infectious mononucleosis, and normal controls. Peripheral blood leukocytes (5×10^5) were cultured in round-bottom microtissue dishes for 4 hours at 37°C with K562 target cells (1×10^4) prelabeled with ^{51}Cr in a standardized chromium release assay at lymphocyte-to-target cell ratios of 10:1, 25:1, and 50:1. The reaction was terminated by centrifugation for 10 minutes at *250g*, and 100 μl of supernatant was removed from all wells for gamma counting. The percentage of cytotoxicity was calculated as $[(E - SR)/\text{max}] \times 100$, where E is counts per minute per well, SR is spontaneous release from targets incubated in medium alone, and max is maximal incorporation of ^{51}Cr by target cells. Cytotoxicity indices expressed are those obtained at a lymphocyte-to-target ratio of 50:1. Each experiment was performed three times; each data point represents the mean index. Seven XLP-affected males were studied on more than one occasion; the average response is given for each of these individuals. Means ± standard errors are shown for each group.

herpes virus that infects and replicates only in human B lymphocytes (*9*)] that is manifested as fatal infectious mononucleosis (40 to 50 percent of cases) and acquired varied immunodeficiency (15 to 30 percent of cases) (*9*). Approximately 40 percent of patients with XLP syndrome develop lymphoreticular malignancies (*10*). Because NK cells have been implicated in the immune response during herpes virus infection (*6*) and as an antitumor effector mechanism (*1*), we studied NK cell activity in patients with the XLP syndrome.

Our studies were conducted on 10 carrier females and 12 affected males (5 to 19 years old) from kindreds fulfilling the criteria for the diagnosis of the XLP syndrome (*10*). We also studied young adults with acute EBV-induced infectious mononucleosis positive for heterophile antibody. Twenty-eight normal controls were selected from a population consisting mainly of young adults. Five children (4 to 10 years old) also served as controls for age-related differences in NK cell activity. Peripheral blood leukocytes were obtained by Ficoll-Hypaque separation of heparinized blood. Activity of NK cells was measured by incubating effector lymphocytes with ^{51}Cr-labeled human myeloid cells (line K562) for 4 hours (*11*). Lytic units were calculated from effector titration curves, with one unit defined as the number of effector cells required to achieve 50 percent lysis of the standard number of K562 target cells (1×10^4).

In Fig. 1, cytotoxicity indices for XLP carrier females, affected males, and individuals with acute infectious mononucleosis are compared to controls. Males with XLP had significantly lower ($P < .001$, Student's t-test) spontaneous activity of NK cells against the target cells (a ratio of effector cells to target cells of 50:1) than carrier females. individuals with acute infectious mononucleosis, and normal controls. There were no differences among the latter three groups. Ten of the 12 males with XLP had cytotoxicity indices below the lowest value measured for a pool of 33 normal control individuals that included several young children. Seven of the 12 males were studied on two or three occasions and, in each case, abnormally low NK activity was reproducible. Carrier females were generally in the normal range with the exception of two individuals. Except for one individual, the cytotoxicity indices of all persons with acute infectious mononucleosis were in the normal range. Of the ten affected males with deficient NK activity, six have developed lymphomas and four have common varied immunodeficiency. Of the two affected individuals with normal NK activity, one has developed a lymphoma and the other has common varied immunodeficiency. Surface marker studies on peripheral blood mononuclear cells showed no differences among affected males, carrier females, and normal individuals with regard to T lymphocyte (sheep red blood cell rosettes) or B lymphocyte (surface membrane immunoglobulin) markers.

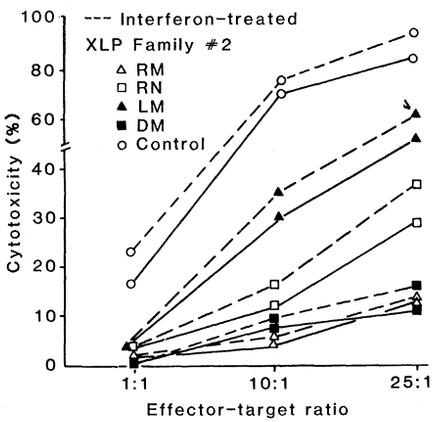

Fig. 2. Spontaneous NK cell activity and interferon augmentation against cell line K562 in four XLP-affected males. Experiments were performed as described in the legend to Fig. 1. Interferon augmentation was carried out by incubating peripheral blood leukocytes (5×10^6), with or without 100 to 1000 units of type I leukocyte or fibroblast interferon, in 1 ml of RPMI medium 1640 for 1 hour at 37°C. The peripheral blood leukocytes were then placed in the standard chromium release assay with K562 target cells. The ratio of effector cells to target cells varied from 1:1 to 25:1.

To ascertain whether deficient NK cell activity in affected males was due to obstruction of the maturation of NK cells (*7, 8*) secondary to deficient production of interferon, effector cells were incubated in interferon before the spontaneous cytotoxic activity for the K562 target cells was measured. Figure 2 shows the interferon-augmented activity of NK cells from four affected males in one XLP family. Activity of cells from three of the four was deficient at each effector-to-target ratio studied. In each case, NK activity could be augmented by preincubation with interferon. No differences in augmentation were observed with fibroblast or leukocyte interferon. Augmentation with interferon did not result in increases in NK activity to levels observed in controls. Similar experiments with nine affected males from six XLP-affected kindreds demonstrated that the activity of effector cells from seven of the males could be augmented by preincubation in type I fibroblast or leukocyte interferon. Effector cells from two affected males showed no increase in activity following incubation with interferon. Effector cells from affected males with the most deficient NK activity levels frequently showed the most marked increase in activity following interferon augmentation. However, they were markedly deficient compared to NK cells from carrier females and controls. When dose-response curves were established, with NK activity expressed as lytic units per 10^6 lymphocytes (*11*), eight of the nine samples tested showed severe deficiency. Activity in NK cells from five of nine female carriers also decreased in terms of lytic units. However, all showed an increase in activity following treatment in vitro with type I interferon.

The human myeloid line K562 is a sen-

sitive target cell for NK activity, and only small increments in lysis are observed after preincubation of effector cells with interferon (Fig. 2). Saksela *et al.* (*8*) demonstrated that K562 is a potent stimulator of interferon when cultured with human lymphocytes. Interferon causes inactive NK cells to become active in vitro. Dramatic augmentation of NK activity following incubation with interferon has been observed with Daudi cells used as targets. The Daudi line, unlike K562, does not induce interferon production when cultured with lymphocytes in vitro (*7*, *8*) and is more sensitive to augmentation by exogenous interferon. When Daudi cells are used as targets, the activity of NK cells from the peripheral blood of affected males with XLP is augmented even more strikingly. However, as with K562 target cells, this activity never reaches the levels seen in NK cells from normal controls.

Recent studies suggest that the NK cell may be an important factor in recovery from human cytomegalovirus and herpes simplex (*12*). The presence of circulating NK cells in the peripheral blood of individuals with infectious mononucleosis is well documented (*13*), and our data suggest a role for the NK cell during EBV infection as well. Viral infection of target cells increases their sensitivity to NK cells (*14*), and Blazar *et al.* (*15*) showed that when Burkitt's lymphoma-derived Raji cells enter the EBV cycle, induced by superinfection with the P3HR-1 virus or by treatment of cells with *n*-butyric acid, their susceptibility to killing by human peripheral blood NK cells is markedly increased. Roder *et al.* (*16*) demonstrated that NK cells from patients with Chédiak-Higashi syndrome have a defect in their ability to spontaneously lyse tumor cell targets in vitro. Chédiak-Higashi syndrome and severe combined immunodeficiency are the only immunodeficiency disorders with a high frequency of defective NK cell activity (*17*).

Our study of males with XLP syndrome supports the hypothesis that NK cells represent an important aspect of the immune response during viral infection in man and play a role in immune surveillance against lymphoproliferation. Patients with XLP syndrome do not show a normal immune response to EBV (*18*). Defective NK cell activity in affected males may reflect a primary defect or may be a consequence of the virus-host relationship. Chronic EBV infection in males with XLP frequently results in a lymphoproliferative disorder in which malignant cells contain the EBV genome (*10*). An important role for interferon in host responses to EBV is suggested by the report of defective interferon production in a girl who succumbed to overwhelming EBV-induced lymphoproliferation (*19*). Our demonstration that interferon augments NK cell activity in patients with XLP syndrome suggests that careful clinical investigation of the effects of interferon on uncontrolled EBV infections in vivo is warranted.

JOHN L. SULLIVAN
KEVIN S. BYRON
FRANK E. BREWSTER
DAVID T. PURTILO

Departments of Pediatrics and *Pathology, University of Massachusetts Medical School, Worcester 01605*

References and Notes

1. R. Kiessling, E. Klein, H. Pross, H. Wigzell, *Eur. J. Immunol.* **5**, 117 (1975); R. B. Herberman, M. E. Nunn, H. T. Holden, D. H. Lavrin, *Int. J. Cancer* **16**, 320, (1975); S. Gupta, G. Fernandes, M. Nair, R. A. Good, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5137 (1978); R. B. Herberman *et al.*, *Immunol. Rev.* **14**, 43 (1979).
2. M. Minato, B. R. Bloom, C. Jones, J. Holland, L. M. Reid, *J. Exp. Med.* **149**, 1117 (1979).
3. J. Roder and A. Duwe, *Nature (London)* **278**, 451 (1979).
4. J. E. Talmadge, K. M. Meyers, D. J. Prieus, J. R. Starkey, *ibid.* **284**, 622 (1980).
5. R. M. Welsh, R. M. Zinkernagel, L. A. Halenbeck, *J. Immunol.* **122**, 475 (1979).
6. G. V. Quinnan and J. E. Manischewitz, *J. Exp. Med.* **150**, 1549 (1979).
7. G. Trinchieri, D. Santoli, R. R. Dee, B. B. Knowles, *ibid.* **147**, 1299 (1978).
8. E. Saksela, T. Timonen, K. Cantell, *Scand. J. Immunol.* **10**, 257 (1979).
9. P. K. Pattengale, R. W. Smith, P. Gerber, *Lancet* **1973-II**, 93 (1973); M. Jondal, G. Klein, *J. Exp. Med.* **138**, 1365 (1973).
10. D. T. Purtilo, L. A. Paquin, D. DeFlorio, F. Virzi, R. Sahkuja, *Semin. Hematol.* **16**, 309 (1979); J. K. Hamilton *et al.*, *J. Pediatr.* **96**, 669 (1980).
11. H. D. Kay, G. D. Bonnard, W. H. West, R. B. Herberman, *J. Immunol.* **118**, 2058 (1977).
12. C. Ching and C. Lopez, *Infect. Immun.* **26**, 49 (1979); G. V. Quinnan, N. Kirmani, W. H. Burns, E. Esber, R. Saral, J. Manischewitz, R. Roger, in *Human Herpes Viruses: An Interdisciplinary Perspective*, A. Nahmias, W. Dowdle, R. Schinazi, Eds. (Elsevier/North-Holland, New York, in press); S. Shore, in *ibid.*; C. Lopez, in *ibid.*
13. E. Svedmyr and M. Jondal, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 401 (1975).
14. D. Santoli, G. Trinchieri, F. S. Leif, *J. Immunol.* **121**, 526 (1978).
15. B. Blazar, M. Patarroyo, E. Klein, G. Klein, *J. Exp. Med.* **151**, 614 (1980).
16. J. C. Roder *et al.*, *Nature (London)* **284**, 553 (1980).
17. H. S. Koren, D. B. Amos, R. H. Buckley, *J. Immunol.* **120**, 796 (1978); H. F. Pross, S. Gupta, R. A. Good, M. G. Baines, *Cell Immunol.* **43**, 160 (1979).
18. K. Sakamoto, H. Freed, D. Purtilo, *J. Immunol.* **125**, 921 (1980).
19. J. L. Virelizier, G. Lenoir, C. Griscelli, *Lancet* **1978-II**, 231 (1978).
20. We thank the many physicians who referred their patients for study. We also thank C. W. Upper for preparing the manuscript. Supported by NIH grant CA 23561-01 and by a grant from the Burroughs Wellcome Company.

11 July 1980; revised 26 August 1980

Genetic Diversity and Structure in *Escherichia coli* Populations

Abstract. *A survey of electrophoretic variation in 20 enzymes from 109 clones of* Escherichia coli *from natural populations yielded an estimate of mean genetic diversity approximately twice that reported in an earlier study and four to five times larger than estimates for most eukaryotic species. Despite this extensive variability, the number of distinctive genotypes apparently is rather limited. Identical clones were obtained from unassociated hosts, and a clone that is electrophoretically indistinguishable from the laboratory strain* Escherichia coli *K-12 was isolated from a human infant. The results suggest that rates of genetic recombination in natural populations of* Escherichia coli *are low. These findings have implications for our understanding of the genetic structure of* Escherichia coli *populations and the factors determining the amount of neutral gene variability in this bacterial species.*

Levels of genetic polymorphism and heterozygosity have been estimated for hundreds of species of plants and animals from electrophoretic surveys of protein variation (*1*). However, Milkman's (*2*) survey of 829 clones of *Escherichia coli* from humans and other mammals is the only attempt to estimate genetic diversity in natural populations of bacteria (*3*). Four of the five enzymes that he studied were polymorphic, and the mean genetic diversity was 0.23, a value not much greater than those reported for some species of higher organisms (*1*). These results were interpreted by Milkman as evidence against the neutral theory of structural gene variation and molecular evolution (*4*) since the observed level of genetic diversity was much smaller than that predicted for populations with a genetically effective size as large as he assumed for *E. coli* (*5*).

We present here the results of a survey of variation in 20 enzymes, as assayed by starch-gel electrophoresis, in 109 clones (*6*) of *E. coli* from the following sources: (i) 17 clones from 16 human infants in a Massachusetts hospital nursery (*7*); (ii) 34 clones from 30 adults and children in Iowa and two adults in Massachusetts (*8*); (iii) 55 clones from 41 mammals, of 28 species, in North America (*9*); (iv) one clone from a lizard; and (v) two clones from water in a well. The clones from Massachusetts were isolated by us, and the others were obtained from Milkman's collection and had been used in his earlier studies (*10*). We also examined one clone each of the laboratory

0036-8075/80/1031-0545$00.50/0