this mating none were diabetic (L. Butler, D. Guberski and A. A. Like, *Can. J. Genetics Cytol.*, in press).
11. For this we used testape, Eli Lilly and Co., Indianapolis, Ind.
12. Samples of tail blood for plasma glucose were collected in heparin-treated pipettes and assayed as described previously [A. A. Rossini, M. Berger, J. Shadden, G. F. Cahill, Jr., *Science* **183**, 424 (1974)].
13. J. T. Oppenheim and B. Schecter, in *Manual of Clinical Immunology*, N. R. Rose and H. Friedman, Eds. (American Society for Microbiology, Washington, D.C., 1980). Blood was diluted in RPMI-1640 medium (Microbiological Associates). This mixture was then layered on Lympholyte M (Cedarline Laboratories). The cells were counted with a hemocytometer and viability was ascertained with trypan blue. Viability was greater than 90 percent in all experiments. Cell number was adjusted to 1×10^6 cells per well with RPMI-1640 medium supplemented with 5 percent pooled, heat-inactivated rat serum, 5×10^{-5} *M* 2-mercaptoethanol, and, per milliliter, 3 mg of glutamine, 100 U of penicillin, and 100 μg of streptomycin. Concanavalin A (Miles-Yeda, Rehovot, Israel) was prepared to provide a stock solution of 1 mg/ml in buffered saline from which dilutions were then made. Limbro 96-well flat bottom microtiter plates were used. Appropriate amounts of con A were added to lymphocytes to achieve doses of 0.125, 0.25, 0.5, 1.0, or 2.5 μg/ml in a final volume of 200 μl per well. Measurements at each dose were performed in triplicate and subsequently averaged. The cells were cultured for 72 hours in an incubator with CO_2 plus air at 37°C. After incubation, methyl [^{3}H]thymidine (1 μCi per well, specific activity 6.7 Ci/*m*M, New England Nuclear) was added, and the cells were incubated for an additional 18 hours. The cells were collected on glass fiber filters with a Mash II harvester, and [^{3}H]thymidine incorporation was measured with a Hewlett-Packard liquid scintillation spectrophotometer. Two diabetic rats died before con A testing could be performed. Not all rats were tested at the con A dose of 0.125 μg/ml. At other con A doses, a total of five data points were discarded because of extreme discordance among the triplicate values. The decision to omit these points was made without knowledge of the treatment or outcome status of the animal.
14. Statistical analysis of 2 by 2 tables was performed with the use of Fisher's exact probability test [S. Siegal, *Nonparametric Statistics* (McGraw-Hill, New York, 1956), pp. 96–104].
15. Significance at each dose level was determined by using a one-way analysis of variance and the Scheffe procedure for *a posteriori* contrasts [N. H. Nie, C. H. Hull, J. G. Jenkins, K. Steinbrenner, D. H. Bant, *Statistical Package for the Social Sciences* (McGraw-Hill, New York, 1975)].
16. E. S. Golub, *The Cellular Basis of the Immune Response* (Sinauer, Sunderland, Mass., 1981), pp. 42–43.
17. B. Benacerraf and E. R. Unanue, *Textbook of Immunology* (Williams & Wilkins, Baltimore, 1979), pp 253–255.
18. C. J. Paige, P. W. Kincade, M. A. S. Moore, G. Lee, *J. Exp. Med.* **150**, 548 (1979).
19. A. Altman *et al.*, *ibid.* **154**, 791 (1981).
20. We thank L. Caouette, E. Ennis, M. Geisberg, and L. Carreaux for technical assistance and D. Parker for critical reading of the manuscript. Supported in part by PHS grants AM25306 and AM19155 and a grant from the Kroc Foundation, Inc.

* Send correspondence to Department of Medicine, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester 01605.

13 September 1982

Resistance to a Malignant Lymphoma in Chickens Is Mapped to Subregion of Major Histocompatibility (*B*) Complex

Abstract. *A genetic recombinant within the major histocompatibility* (B) *complex of the chicken has revealed the chromosomal subregion effecting resistance to Marek's disease—a malignant lymphoma induced by a herpesvirus. The recombinant,* $B^{F21\text{-}G19}$, *occurred spontaneously among the progeny of a male heterozygous for resistant* $B^{F21\text{-}G21}$ *and susceptible* $B^{F19\text{-}G19}$ *haplotypes. Exposure to Marek's disease of families segregating for the recombinant showed that this new F-G arrangement conferred a level of resistance equivalent to that of the resistant parental haplotype. Thus, a gene, or genes, within or closely linked to the* B-F *region of the* B *complex appears to be responsible for the observed resistance to Marek's disease.*

Haplotype B^{21} (*1*) of the major histocompatibility complex (*2, 3*) of the chicken imparts a strong resistance to Marek's disease (MD), a herpesvirus-induced, malignant lymphoma (*4*), whereas B^{19} is associated with a high degree of susceptibility (*5, 6*). Two of the three regions constituting the *B* complex (*7*) code for erythrocyte alloantigens which serve as the basis for differentiating *B* haplotypes by hemagglutination. The *B-F* region determines the specificity of histocompatibility antigens present on lymphocytes and erythrocytes, whereas *B-G* codes for a series of nonhistocompatibility antigens present only on erythrocytes. The third region, *B-L*, determines Ia-like antigens (I-region associated) found primarily on immunoglobulin-bearing lymphocytes and on cells of the monocyte-macrophage series (*8*). In this report we describe a recombinant resulting from crossing over between the *B-F* and *B-G* regions as found in haplotypes B^{21} and B^{19} and the use of the recombinant in determining the portion of the *B* complex that is active in resistance to MD.

The fortuitous recombinant between highly resistant B^{21} and very susceptible B^{19} haplotypes resulted from the mating of two White Leghorn chickens, a male of the genotype B^{19}/B^{21} to a female of the genotype B^6/B^{19}. With the use of alloantisera B6(422–496), B19(339–97), and B21(305–517), each specific for one of the three alloantigens transmitted collectively by the parents, the phenotypes of 10 of 11 progeny were clearly explicable by the four genotypic combinations expected: B^6/B^{19}, B^6/B^{21}, B^{19}/B^{21}, and B^{19}/B^{19}. The cells of one chick, a male, showed distinct reactivity with each of the three antisera. This aberrant chick was grown to maturity and mated to a female of the genotype B^3/B^{15}. The normal B^6 haplotype was transmitted to 14 of his 23 progeny and a recombinant haplotype, resulting in hemagglutination with both B19(339–97) and B21(305–517), was transmitted to the remaining nine progeny.

The origin of the segments in the recombinant was first evaluated by using the graft-versus-host (GVH) splenomegaly reaction (*3, 9*), which is especially suited to determining *B-F* homologies between recombinant and parental haplotypes. Testing for this reaction consists of injecting intravenously whole blood from an adult chicken (0.1 ml of peripheral blood diluted 33 percent with physiologically balanced citrate solution) into 14-day embryos and continuing incubation for an additional 5 days, when the embryos are killed and their spleens weighed. If the immunocompetent lymphocytes from the adult blood recognize a foreign *B* complex histocompatibility antigen in the embryo, the weight of the spleen will markedly increase, usually four- to tenfold, beyond that of embryos that have been treated with compatible donor cells.

The GVH results obtained by injecting the cells of the original male possessing the recombinant into B^{21}/B^{21} and B^{19}/B^{19} embryos clearly showed that the recombinant haplotype was compatible with B^{21} but was incompatible with B^{19} (Table 1). Similarly, donor cells from chickens of genotype B^6/B^{21} were injected into B^{21}/B^{21} and B^{19}/B^{19} embryos and donor cells from B^2/B^{19} were injected into B^2/B^{19} and B^{21}/B^{21} embryos to confirm the natural incompatibility between B^{19} and B^{21}. These data show that the *B-F* segment of the recombinant haplotype was derived from B^{21} and, if we assume that the recombination resulted from normal reciprocal crossing over, the *B-G* segment was derived from B^{19}. To indicate the segment derived from each of the parental haplotypes, we symbolize the recombinant as $B^{F21\text{-}G19}$; the symbols for the normal haplotypes B^{21} and B^{19} represented in this extended nomenclature are $B^{F21\text{-}G21}$ and $B^{F19\text{-}G19}$, respectively.

A second method used to determine the haplotype origin of the *B-F* and *B-G* segments in the recombinant was the GVH inhibition test (*10*), in which exposure of donor lymphocytes to antibodies against T-cell antigens prevents splenomegaly. A recipient of the genotype B^{15}/B^{19} was immunized with blood from a donor of genotype $B^{F21\text{-}G19}/B^{19}$, producing antiserum B-F21(468/267). A recipient of the genotype B^6/B^{21} was immu-

Table 1. Graft-versus-host splenomegaly reactions characterizing recombinant $B^{F21\text{-}G19}$ relative to parental haplotypes B^{21} and B^{19}. Donor blood of genotype $B^{6}/B^{F21\text{-}G19}$ was from the original recombinant male. Injecting B^{21}/B^{21} embryos with $B^{19}/B^{F21\text{-}G19}$ blood from adult progeny of the recombinant male also failed to produce positive GVH reaction.

Genotypes		Number of embryos	Spleen weight (mg)	P*
Adult donor	Embryo recipient			
$B^{6}/B^{F21\text{-}G19}$	B^{21}/B^{21}	11	21.9	< .002
$B^{6}/B^{F21\text{-}G19}$	B^{19}/B^{19}	6	105.1	
B^{6}/B^{21}	B^{21}/B^{21}	5	14.1	< .001
B^{6}/B^{21}	B^{19}/B^{19}	5	166.4	
B^{2}/B^{19}	B^{2}/B^{19}	3	27.5	< .05
B^{2}/B^{19}	B^{21}/B^{21}	2	178.8	

*Probability based on Student's t-test.

Table 2. Incidence of Marek's disease among White Leghorn families segregating for recombinant $B^{F21\text{-}G19}$.

Mating code	Genotype of parents		Genotype of progeny			P*
	Sire	Dam	B^{21}/B^{19}	$B^{F21\text{-}G19}/B^{19}$	B^{19}/B^{19}	
A	B^{19}/B^{19}	$B^{F21\text{-}G19}/B^{21}$	6/81† (7.4%)	5/81 (6.2%)		> .7
B	B^{19}/B^{19}	$B^{F21\text{-}G19}/B^{19}$		7/60 (11.7%)	35/57 (61.4%)	< .001

*Tests of independence were based on χ^2 tests in 2 by 2 contingency tables. †Number with MD compared to number exposed (includes six deaths for nonspecific reasons between 91 and 112 days: three B^{21}/B^{19}, one $B^{F21\text{-}G19}/B^{19}$, and two B^{19}/B^{19}).

nized with blood from a $B^{F21\text{-}G19}/B^{21}$ donor, producing antiserum B-G19(486/569). When B^{21}/B^{21} donor lymphocytes were exposed to the B-F21 antiserum and then injected into incompatible embryos, no GVH reaction resulted; in contrast, when B^{19}/B^{19} donor lymphocytes were exposed to the B-G19 antiserum and injected into incompatible embryos, a full GVH reaction was obtained. This suppression of donor T cells carrying B^{21} and lack of suppression of donor T cells carrying B^{19} confirm that the recombinant consists of a *B-F* segment from B^{21} and a *B-G* segment from B^{19}.

This recombinant haplotype, $B^{F21\text{-}G19}$, provided an opportunity to determine the extent to which the *B-F* segment derived from the B^{21} haplotype might be as effective as normal B^{21} in expression of *B* complex resistance to MD. Parents for an appropriate experimental mating were produced by mating individuals carrying the $B^{F21\text{-}G19}$ haplotype to birds of the genotype B^{19}/B^{21}; progeny possessing the $B^{F21\text{-}G19}$ haplotype were then used in matings A and B of Table 2. In mating A, six White Leghorn males of the genotype B^{19}/B^{19} were mated individually by artificial insemination to 15 White Leghorn females of the genotype $B^{F21\text{-}G19}/B^{21}$. In mating B, five of the six males were simultaneously mated to 10 $B^{F21\text{-}G19}/B^{19}$ females. Chicks were produced in a single hatch and transported the same day to experimental facilities in Haslett, Michigan, to be challenged with MD virus. Each chick was inoculated intraperitoneally at 14 days of age with 2000 plaque-forming units of the JM strain of the virus (clone 102W, propagated on duck embryo fibroblasts and maintained as standard stock at the Regional Poultry Research Laboratory, East Lansing, Michigan). Blood samples for alloantigen typing were taken when the chicks were 16 days of age. The chicks were observed for the presence of MD through 112 days of age. Necropsies were made on all dead birds and on any individuals showing clinical signs of MD at the end of the test period. Of the birds dying from MD, 79 percent had enlarged and edematous nerves, 15 percent had tumors in one or more visceral organs, and 6 percent had lesions in both nerves and viscera.

In evaluating the relative influence of particular genotypes, it is essential that MD resistance be compared for genotypes segregating within family groups, for only by such procedures can one randomize other genetic effects on the trait under consideration. From matings A and B (Table 2) there resulted 15 and 10 full-sib family groups, respectively, with a minimum of three progeny in any genotypic category. The progeny of mating A were assigned to the genotypic classes as shown in Table 2 from alloantigen phenotypes resulting from typing with a reagent specific for B-G21, produced in a $B^{19}/B^{F21\text{-}G19}$ recipient injected with cells from a $B^{21}/B^{F21\text{-}G19}$ donor. In the progeny of this mating, the detection of antigen B-G21 proves the presence of B^{21}. The progeny from mating B were typed with a reagent specific for B-F21, present only in individuals receiving the recombinant haplotype. Before the family data were pooled as shown in Table 2, analysis by heterogeneity χ^2 had shown that the distribution of MD mortality among genotypic classes from both A and B matings were homogeneous across dam and sire families. The MD incidence among the B^{21}/B^{19} and $B^{F21\text{-}G19}/B^{19}$ sibs of mating A was 6 out of 81 (7.4 percent) and 5 out of 81 (6.2 percent), respectively, indicating that the recombinant $B^{F21\text{-}G19}$ is equivalent to B^{21} in effecting MD resistance. Furthermore, from mating B the relative MD incidence among genotypes $B^{F21\text{-}G19}/B^{19}$ and B^{19}/B^{19} was 7 out of 60 (11.7 percent) and 35 out of 57 (61.4 percent), respectively, resembling in magnitude the comparative resistance of full sibs having genotypes B^{21}/B^{19} and B^{19}/B^{19} (8.6 and 69.7 percent, respectively) previously reported (*6*).

The gene, or genes, encoding the B-F21 antigen, and other genes of the *B* complex that may be located on the near side of the crossover break, appear to fully account for the MD resistance associated with the B^{21} haplotype. Considering these data in light of the prominent role of cell-mediated immunity in genetic resistance to MD (*6*) suggests that a gene, or genes, within or closely linked to the *B-F* region plays a significant role in T-cell response—possibly through selective sensitization of T cells to altered self-antigens (*11*) resulting from tumorigenesis.

W. Elwood Briles
Ruth W. Briles
Rolf E. Taffs
Department of Biological Sciences, Northern Illinois University, DeKalb 60115

Howard A. Stone
Omega Chicks, 1827 Lyndhurst Street, Haslett, Michigan 48840

References and Notes

1. W. E. Briles, W. H. McGibbon, M. R. Irwin, *Genetics* **35**, 633 (1950); W. E. Briles, C. P. Allen, T. W. Millen, *ibid.* **42**, 631 (1957). The haplotype designations in the present report, B^{19} and B^{21}, are presumed, on the basis of current reaction patterns with alloantisera, to be identical with corresponding haplotypes in the latter reference.
2. L. W. Schierman and A. W. Nordskog, *Science* **134**, 1008 (1961); D. G. Gilmour, *Heredity* **18**, 123 (1963); R. E. Gleason and R. C. Fanguy, *Transplantation* **2**, 509 (1964); V. C. Miggiano, I. Birgen, J. R. L. Pink, *Eur. J. Immunol.* **4**, 397 (1974).
3. W. P. Jaffe and E. M. McDermid, *Science* **137**, 984 (1962).
4. H. G. Purchase, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **31**, 1634 (1972).

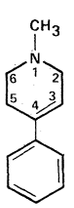
5. M. P. Hansen, J. N. Van Zandt, G. R. J. Law, *Poult. Sci.* **46**, 1268 (1967); W. E. Briles and H. A. Stone, *ibid.* **54**, 1738 (1975); B. M. Longenecker, F. Pazderka, J. S. Gavora, J. L. Spencer, R. F. Ruth, *Immunogenetics* **3**, 401 (1976).
6. W. E. Briles, H. A. Stone, R. K. Cole, *Science* **195**, 193 (1977).
7. K. Hála, M. Vilhelmová, J. Hartmanová, *Immunogenetics* **3**, 97 (1976); J. R. L. Pink, W. Droege, K. Hála, V. C. Miggiano, A. Ziegler, *ibid.* **5**, 203 (1977).
8. D. L. Ewert and M. D. Cooper, *ibid.* **7**, 521 (1978); D. L. Ewert, D. G. Gilmour, W. E. Briles, M. D. Cooper, *ibid.* **10**, 169 (1980).
9. M. Vilhelmová, V. C. Maggiano, J. R. L. Pink, K. Hála, J. Hartmanová, *Eur. J. Immunol.* **7**, 674 (1977).
10. M. Simonsen, K. Hála, M. Vilhelmová, in *Avian Immunology*, A. A. Benedict, Ed. (Plenum, New York, 1977), p. 267.
11. P. C. Doherty and R. M. Zinkernagle, *J. Exp. Med.* **141**, 502 (1975).
12. We thank B. Blondin for assistance in preparation of the manuscript. This work was supported by PHS grant CA12796 awarded by the National Cancer Institute.

13 August 1982; revised 8 September 1982

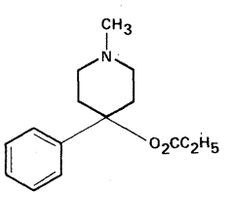
Chronic Parkinsonism in Humans Due to a Product of Meperidine-Analog Synthesis

Abstract. *Four persons developed marked parkinsonism after using an illicit drug intravenously. Analysis of the substance injected by two of these patients revealed primarily 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) with trace amounts of 1-methyl-4-phenyl-4-propionoxy-piperidine (MPPP). On the basis of the striking parkinsonian features observed in our patients, and additional pathological data from one previously reported case, it is proposed that this chemical selectively damages cells in the substantia nigra.*

1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) is a commercially available compound that is sold as a chemical intermediate (*1*). The biological effects of this substance seem not to have been systematically investigated in animals or in man. MPTP is also a by-product in the synthesis of 1-methyl-4-phenyl-4-propionoxy-piperidine (MPPP), a meperidine analog (*2*). Admixed with varying amounts of MPPP, MPTP was recently sold as a new "synthetic heroin" in a limited region of northern California and used intravenously by a number of addicted individuals who subsequently developed severe parkinsonism. In this report we describe four of these patients, each of whom has been examined and followed by one or more of us.



MPTP



MPPP

The patient group consisted of one female and three males, whose ages ranged from 26 to 42 years. Each patient had a history of previous heroin abuse (3 months to 14 years). Patients 1 and 2 obtained a "new heroin" sample in San Jose, California, on 1 July 1982; patients 3 and 4 (brothers) received theirs in Watsonville, California, about 3 weeks earlier. Dosages ranged from 5 g over 4 days (cases 1 and 2) to 20 g over 5 to 8 days (cases 3 and 4). All patients became symptomatic within a week after starting to use the new drug. First symptoms included almost immediate visual hallucinations (one patient), jerking of limbs (two patients), and stiffness (one patient). Generalized slowing and difficulty in moving occurred within 4 to 14 days after the initial use of the substance. In at least one patient, symptoms continued to evolve over a period of 3 to 5 days after the drug was stopped.

Three of the four patients were hospitalized within 14 days to 6 weeks of first use of the drug. Examination in each revealed near total immobility, marked generalized increase in tone, a complete inability to speak intelligibly, a fixed stare, marked diminution of blinking, facial seborrhea, constant drooling, a positive glabellar tap test, and cogwheel rigidity in the upper extremities. One patient exhibited a "pill-rolling" tremor (5 to 6 cycles per second) at rest in the right hand. All patients exhibited a flexed posture typical of fully developed Parkinson's disease. The fourth patient (case 4) was seen as an outpatient 4 weeks after onset of symptoms. He was able to walk but exhibited a short-stepped, slow, shuffling gait, "en bloc" turning, and generalized bradykinesia. In our examination of him, he was otherwise similar to the other three patients.

Additional findings in these patients included apraxia of eyelid opening in three patients (*3*) and limitation of vertical gaze in two patients. Electroencephalograms were normal in three patients; a fourth exhibited intermittent bitemporal slowing. Results of cerebral computerized tomography were normal in the three patients who underwent the procedure. Lumbar puncture revealed an elevated protein in three of the four patients (range 47 to 120 mg/dl). Tests for Wilson's disease were negative in all four patients. Complete toxic screens for acidic, neutral, and basic drugs in plasma and urine including phenothiazines were negative in the two patients tested (*4*). Heavy metal screens in these two patients were also negative.

All patients responded to therapy with a combination of L-dopa and carbidopa (Sinemet). Addition of dopamine agonist therapy (bromocriptine or lisuride) provided additional therapeutic response in two patients. Five months after onset, none of these patients have shown signs of remission. All continue to require substantial doses of these medications, and one patient is now experiencing dose-limiting side effects. One patient (case 3) had all medications stopped 2 months after onset of symptoms for 5 days. During this period the patient completely reverted to his original state of complete immobility and rigidity, being able to move only his eyes. Swallowing was impaired to the point that he required intravenous hydration until his medications could be effectively resumed.

Analyses of all drug samples were performed by using thin-layer chromatography (TLC), gas chromatography (GC), and combined gas chromatography and mass spectrometry (GC/MS). Both MPTP and MPPP were identified by means of GC/MS (Fig. 1). Confirmation and quantitation was performed with reference material obtained from Aldrich Chemical Company (*1*) and the National Institute of Mental Health (*5*). A sample of the powder which was injected by patients 1 and 2 was obtained from their residences. This white crystalline powder contained 3.2 percent MPTP and 0.3 percent MPPP (by weight).

Three additional samples were obtained from the individual who supplied drugs to patients 3 and 4. These samples were said to contain the same substance our patients had been taking when symptoms developed and additional amounts of "good heroin" (presumably the target compound, MPPP) which had been added later to make it more salable. These brown granular samples also contained MPTP at concentrations ranging between 2.5 and 2.9 percent (by weight). They also contained large amounts of MPPP (between 22 and 27 percent by weight). No other drugs were detected in any of the samples analyzed.

A case reported by Davis *et al.* (*6*) has great bearing on the patient material re-