Since her tumor cells contain only the deleted 13 chromosome they may provide valuable target cells for probes being developed to identify key genetic differences within 13q14 between normal 13 chromosomes and those which contain small deletions or mutations responsible for the susceptibility to retinoblastoma.

WILLIAM F. BENEDICT
Clayton Ocular Oncology Center and
Division of Hematology-Oncology,
Childrens Hospital of Los Angeles,
Los Angeles, California 90027
A. LINN MURPHREE
Clayton Ocular Oncology Center and
Division of Ophthalmology,
Childrens Hospital of Los Angeles
ASHUTOSH BANERJEE
Division of Hematology-Oncology,
Childrens Hospital of Los Angeles
CELSA A. SPINA
Department of Microbiology and
Immunology, Center for
Health Sciences,
University of California,
Los Angeles 90024
MARYELLEN C. SPARKES
ROBERT S. SPARKES
Division of Medical Genetics,
Departments of Medicine,
Pediatrics, and *Psychiatry,*
Center for Health Sciences,
University of California,
Los Angeles 90024

References and Notes

1. R. S. Sparkes, M. C. Sparkes, M. G. Wilson, J. W. Towner, W. Benedict, A. L. Murphree, J. J. Yunis, *Science* **208**, 1042 (1980).
2. J. J. Yunis and N. Ramsey, *Am J. Dis. Child.* **132**, 161 (1978).
3. J. J. Yunis, *Hum. Pathol.* **12**, 494 (1981).
4. D. N. Comings, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3324 (1973); A. K. Knudsen, Jr., *Semin. Oncol.* **5**, 57 (1978); A. L. Murphree and W. F. Benedict, in preparation.
5. R. S. Sparkes, A. L. Murphree, M. C. Sparkes, L. Field, S. Funderburk, W. F. Benedict, *Science* **219**, 971 (1983).
6. V. M. Riccardi, E. Sujansky, A. C. Smith, U. Francke, *Pediatrics* **61**, 604 (1978); V. M. Riccardi, H. Hittner, U. Francke, J. Yunis, D. Ledbetter, W. Borges, *Cancer Genet. Cytogenet.* **2**, 131 (1980); Y. Kaneko, M. C. Egues, J. D. Rowley, *Cancer Res.* **41**, 4577 (1981); R. M. Slater and J. de Kraker, *Cancer Genet. Cytogenet.* **5**, 237 (1982).
7. G. M. Brodeur, A. A. Green, F. A. Hayes, K. J. Williams, D. L. Williams, A. A. Tsiatis, *Cancer Res.* **41**, 4678 (1981).
8. J. Whang-Peng, C. S. Kao-Shan, E. C. Lee, P. A. Bunn, D. N. Carney, A. F. Gazdar, J. D. Minna, *Science* **215**, 181 (1982).
9. A. J. Cohen, F. Li, S. Berg, D. Marchetto, S. Tsai, S. Jacob, R. S. Brown, *N. Engl. J. Med.* **301**, 592 (1979).
10. P. W. J. Rigby, *Nature (London)* **297**, 451 (1982); G. M. Cooper, *Science* **217**, 801 (1982).
11. R. S. Sparkes, S. Targum, E. Gershon, G. D. Sensabaugh, M. C. Sparkes, M. Crist, *Hum. Genet.* **46**, 319 (1979).
12. D. A. Hopkinson, M. A. Mestriner, J. Cortner, H. Harris, *Ann. Hum. Genet.* **37**, 119 (1973).
13. M. Seabright, *Lancet* **1973-II**, 971 (1973).
14. Supported by NIH grant EY-02715. A portion of this work was done in conjunction with the Clayton Foundation for Research. We thank B. Szirth for photographic assistance.

5 October 1982

Transfusions of Whole Blood Prevent Spontaneous Diabetes Mellitus in the BB/W Rat

Abstract. *Weekly transfusions of whole blood from a nondiabetic subline of BB/W rats reduced the incidence of diabetes in susceptible BB/W rats from 39 to 0 percent and the incidence of pancreatic insulitis from 64 to 6 percent. Responsiveness of lymphocytes to concanavalin A was found to be low in rats with diabetes or insulitis. Transfusion restored concanavalin A responsiveness to levels observed in control rats free of diabetes or insulitis. These data suggest that whole blood alters the course of autoimmune BB/W rat diabetes.*

Polyuria, polydipsia, polyphagia, and weight loss are the principal diagnostic features of human insulin-dependent diabetes mellitus (IDDM). Although the syndrome has been recognized for millennia, the etiology and prevention of diabetes remain enigmatic and elusive goals (*1*). The animal model that most closely mimics the human disease is the BB rat (*2*). As a model of IDDM it exhibits many features that are analogous to its human counterpart. The syndrome develops in 30 to 50 percent of diabetes-prone rats between 60 and 120 days of age. Diabetic rats are nonobese and develop acute ketoacidosis which is lethal within weeks unless treated with insulin.

Several lines of evidence suggest an immune pathogenesis of BB rat diabetes. These include the lymphocytic infiltration of pancreatic islets (insulitis) and prevention of the disease by immunosuppressive agents and neonatal thymectomy (*3*). Transplantation of neonatal bone marrow from another strain of rat also prevents diabetes in susceptible BB rats (*4*). In this last study, mixed lymphocyte cultures from the recipient BB rats demonstrated that they had been "immunologically reconstituted." This suggests that some form of immunodeficiency may be present in susceptible BB rats. The finding of lymphopenia in diabetic and insulitis-bearing BB rats supports this interpretation (*5*). Specifically, helper T lymphocytes alone (*6*), or both helper and suppressor T lymphocytes, may be present in abnormally low numbers in diabetic BB rats (*7*).

To test the hypothesis that deficiency of some circulating blood constituent is permissive to the development of diabetes, we administered whole blood transfusions to diabetes-prone rats. Donor blood from a nondiabetic subline of rats completely prevented the development of the disease in susceptible animals.

Five litters of BB/W diabetes-susceptible animals between 31 and 41 days of age were used (*8*). Rats from each litter were randomly assigned to one of two groups. Group 1 ($N=18$) received weekly transfusions of 1 ml of rat whole blood until the animals were 120 days of age (*9*). Group 2 ($N=18$) received 1 ml of normal saline injected on an identical schedule. Transfused blood was obtained from a line of BB/W rats in which neither diabetes nor insulitis has been observed in over seven generations (*10*). The animals were tested for glycosuria twice weekly from 60 to 140 days of age (*11*). Diabetes was diagnosed on the basis of 2+ glycosuria and plasma glucose in excess of 250 mg/dl (*12*).

When the rats were about 140 days of age, 2 ml of blood was withdrawn into heparinized capillary tubes from the orbital sinus of the transfused and nontransfused rats. The rats were then killed and the pancreata removed and fixed in Bouin's solution. Sections stained with hematoxylin and eosin were examined for the presence of insulitis by a pathologist (A.A.L.) who was not aware of the clinical or experimental status of the animals. The heparinized blood samples were processed to isolate lymphocytes which were then assayed for mitogenic responsiveness to concanavalin A (con A) according to previously described techniques (*13*). Blood from six additional 140-day-old rats from the nondiabetic subline was also used in the con A study.

Table 1 gives the frequency of diabetes in experimental and control animals (*14*). None of the animals that received transfusions became diabetic, whereas 39 percent of the nontransfused animals developed the syndrome. Table 1 also gives the frequency of insulitis among animals that did not become diabetic. Only one of 18 animals (6 percent) that had received the multiple blood transfusions demonstrated a mild focal insulitis. In the nontransfused group, 7 of 11 nondiabetic rats (64 percent) had insulitis.

Figure 1 illustrates the peripheral blood lymphocyte response to con A (*15*). The nontransfused animals with diabetes or insulitis are much less responsive than are nontransfused rats without insulitis or diabetes. The responsiveness of the latter group is comparable to that of rats from the nondiabetic subline. This

Table 1. Occurrence of diabetes and insulitis in transfused and control BB/W rats.

Group	Diabetes*	No diabetes*	Insulitis†	No insulitis†
Transfused	0	18	1	17
Not transfused	7	11	7	4

*$P < .008$. †$P < .002$.

suggests that both insulitis and diabetes are correlated with blunted con A mitogenic stimulation. The con A response of transfused rats is similar to that of both the nondiabetic subline and nontransfused rats without diabetes or insulitis. Transfusion thus appears not only to prevent diabetes but also to return the depressed con A responsiveness associated with insulitis and diabetes to normal.

Since it is possible that immunocompetent lymphoid cells in transfused blood cause a graft versus host reaction (GVHR), the spleens from the control and transfused animals were removed and weighed. Splenomegaly is a sensitive indicator of the presence of GVHR (*16*). Spleen size as well as body weight and hemoglobin concentration measured at the end of the experiment were comparable in the transfused and control groups (data not shown).

One current hypothesis holds that the difference between autoimmunity and self-tolerance depends on a regulatory interaction between suppressor T lymphocytes and self-reactive lymphocytes which holds the latter in check (*17*). The data presented above suggest that we may have transfused a cellular component capable of regulating this interaction which is defective in the diabetes-prone BB/W rat. Production of lymphocyte chimerism in adult animals usually requires preparation of the host by irradiation or treatment with cytotoxic drugs. However, if diabetes-susceptible rats lack a particular subpopulation of regulatory lymphocytes, then chimerism might be produced in that compartment only in an unprepared host. This concept is consistent with a study in which donor-derived B lymphocytes were found to be present after transfusion to previously B cell–deficient CBA/N mice (*18*). Although our findings suggest that we have transferred competent lymphocytes or their precursors from BB rats of the nondiabetic subline into the susceptible animals, we cannot exclude the production of alloantibodies, plasma components, or the introduction of competent macrophages.

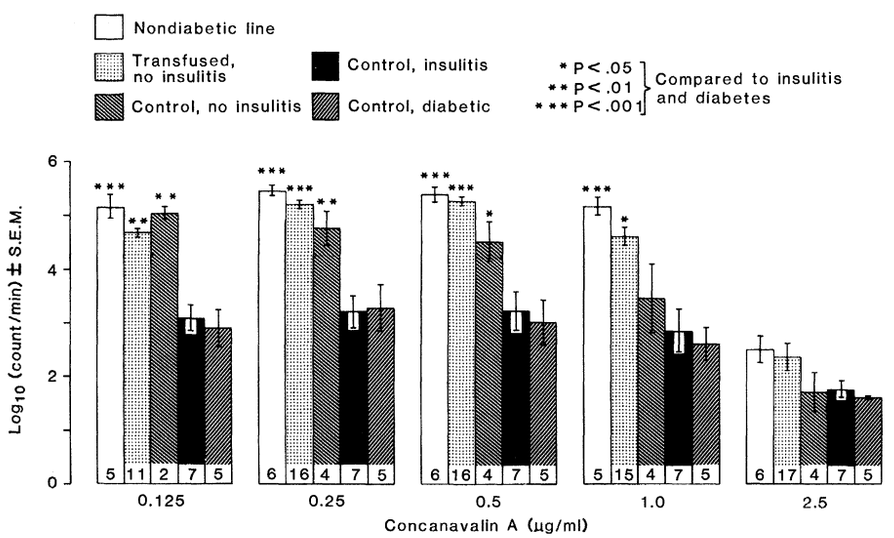

Fig. 1. Response to con A of rats from the nondiabetic subline, diabetes-prone rats which received transfusions of whole blood, and diabetes-prone control rats injected with saline. The last group is subdivided according to whether the animal eventually developed diabetes, insulitis, or neither. The procedure quantitates the incorporation of [^{3}H]thymidine into lymphocytes exposed to varying doses of con A for 18 hours. Data are presented as the mean $\log_{10}$ (counts per minute) ± the standard error of the mean. The number of rats in each group is given at the base of each bar. No transfused rats became diabetic. The single transfused rat with insulitis is omitted. Its results at con A doses 0.25 to 2.5 μg/ml were 5.50, 5.54, 4.25, and 1.95 respectively. Results for cells to which no con A was added are as follows ($\log_{10}$, counts per minute, plus or minus standard error of the mean): nondiabetic line 4.37 ± 0.3; transfused, no insulitis 3.49 ± 0.2; control, no insulitis 2.11 ± 0.4; and control diabetic 3.0 ± 1.0.

It should be noted that depressed con A responsiveness is typical of autoimmunity in animals. This, however, could be either a direct or an indirect consequence of immune regulation (*19*). The restoration of responsiveness by transfusion in this study does not prove the establishment of chimerism.

In our experiments whole blood transfusions from nondiabetic to diabetes-prone BB/W rats protect not only against diabetes, but also against pancreatic insulitis which is the pathological substrate of diabetes. Furthermore, the uniformly good con A response observed in transfused animals suggests that transfusion is able to alter the previously depressed immune response observed in insulitis and diabetes. The transfusion of blood constituents appear to provide some factor which alters the immune system to protect against both diabetes and insulitis. Whether a cellular or humoral component of whole blood produces this response remains to be determined.

ALDO A. ROSSINI*
JOHN P. MORDES
ANNE M. PELLETIER
Department of Medicine, University of Massachusetts Medical School, Worcester 01605
ARTHUR A. LIKE
Department of Pathology, University of Massachusetts Medical School

References and Notes

1. J. E. Craighead, *N. Engl. J. Med.* **299**, 1439 (1978); A. E. Notkins, *Sci. Am.* **241** (No. 5), 62 (1979); G. F. Cahill, Jr., and H. O. McDevitt, *N. Engl. J. Med.* **304**, 1454 (1981).
2. E. B. Marliss, A. F. Nakhooda, P. Poussier, A.A.F. Sima, *Diabetologia* **22**, 225 (1982); A. A. Like, L. Butler, R. M. Williams, M. C. Appel, E. J. Weringer, A. A. Rossini, *Diabetes* **31**, 7 (1982).
3. A. A. Like, A. A. Rossini, D. L. Guberski, M. C. Appel, R. M. Williams, *Science* **206**, 1421 (1979); A. A. Like, E. Kislauskis, R. M. Williams, A. A. Rossini, *ibid.* **216**, 644 (1982).
4. A. Naji, W. K. Silvers, S. A. Plotkin, D. Dafoe, C. F. Barker, *Surgery* **60**, 218 (1979).
5. P. Poussier, A. F. Nakhooda, J. A. Falk, C. Lee, E. B. Marliss, *Endocrinology* **110**, 1825 (1982).
6. R. Jackson, N. Rossi, T. Crump, B. Haynes, G. S. Eisenbarth, *Diabetes* **3**, 887 (1981).
7. A. A. Rossini, J. Stevens, J. P. Mordes, A. A. Like, unpublished observations.
8. Rats from the breeding colony at the University of Massachusetts are designated BB/W rats. The animals are descendants of BB breeders obtained from Bio-Breeding, Ltd. of Ottawa, Canada, in 1977. Approximately 30 to 50 percent of rats in the diabetes-susceptible line used in this study develop the disease (L. Butler, D. Guberski, A. A. Like, *Can. J. Genet. Cytol*, in press).
9. The donor rats from the nondiabetic line of BB/W rats were anesthetized with ether. Blood was obtained by cardiac puncture by means of a heparin-coated glass syringe from which all excess heparin had been expelled. The blood was kept at room temperature. Transfused rats received a 1-ml injection of blood into the tail vein, Deseret catheters being used with 21-gauge needles. Normal saline (0.3 ml) was used to flush the catheter.
10. The nondiabetic subline is descended from diabetic BB/W rats and has been bred specifically for the absence of diabetes. In a test of genetic purity, animals from the nondiabetic line were crossed with diabetic rats. Of 154 offspring of

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-