

## SHORT COMMUNICATION

# Relationship of the Genes for Chediak-Higashi Syndrome (Beige) and the T-Cell Receptor $\gamma$ Chain in Mouse and Man

RANDALL F. HOLCOMBE,\*† WILLIAM STRAUSS,† FRANCES L. OWEN,‡ LAURENCE A. BOXER,§  
 ROBERT W. WARREN|| Mary ELLEN CONLEY,\* JAMES FERRARA,¶ RANDI Y. LEAVITT,\*\*  
 ANTHONY S. FAUCI,\*\* BENJAMIN A. TAYLOR,†† AND J. G. SEIDMANT

\*Division of Hematology, Department of Medicine, Brigham & Women's Hospital, Boston, Massachusetts 02115; †Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115; ‡Department of Pathology, Tufts University School of Medicine, Boston, Massachusetts 02111; §Division of Pediatric Hematology/Oncology, Mott Hospital, University of Michigan, Ann Arbor, Michigan 48109; ||Division of Rheumatology and Immunology, Department of Pediatrics, University of North Carolina, Chapel Hill, North Carolina 27599; #Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104; ¶Division of Hematology/Oncology, Children's Hospital Medical Center, Boston, Massachusetts 02115; \*\*NIAID, National Institutes of Health, Bethesda, Maryland 20892; and ††The Jackson Laboratory, Bar Harbor, Maine 04609

Received September 8, 1987; revised November 24, 1987

The genetic linkage of Chediak-Higashi syndrome and its murine analog, beige (*bg*), to the T-cell receptor (TCR- $\gamma$ )  $\gamma$  chain gene is further defined. Previous studies using recombinant inbred strains of mice demonstrated that the murine *bg* gene is genetically linked to a murine TCR- $\gamma$  gene. We report that in the mouse the frequency of recombination between these two markers is 0.025. Further, we tested the hypothesis that these two genes are linked in the human genome by analyzing restriction fragment length polymorphisms (RFLPs) in five families with children afflicted with Chediak-Higashi syndrome. In three families, RFLPs in TCR- $\gamma$  genes were inherited discordantly from Chediak-Higashi syndrome, demonstrating nonlinkage. We postulate that there is an evolutionary chromosomal breakpoint between the *bg* gene and the TCR- $\gamma$  gene. © 1987 Academic Press, Inc.

Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder characterized by oculocutaneous albinism, a predisposition to pyogenic infections, and large abnormal cytoplasmic masses in all granule-containing cells (Blume and Wolff, 1972). Death may occur at an early age due to infection or a lymphoma-like accelerated phase which may be of T-cell origin (Argyle *et al.*, 1982) or rather a lymphohistiocytic proliferation related to EBV infection (Rubin *et al.*, 1985). Other cellular processes that have been shown to be abnormal include absent or decreased natural killer (NK) activity (Haliotis *et al.*, 1980; Klein *et al.*, 1980; Merino *et al.*, 1983; Roder and Duwe, 1979), altered responsiveness to EBV infection

(Merino *et al.*, 1986), decreased antibody-dependent cell-mediated cytotoxicity (ADCC) (Klein *et al.*, 1980), and altered neutrophil digestive capabilities (Quie and Cates, 1977). Beige (*bg*) mice have many of these features, and several groups have proposed that the murine *bg* mutation is analogous to the mutation in patients with CHS (Brandt *et al.*, 1975; Lutzner *et al.*, 1966; Windhorst and Padgett, 1973). Here we further define the map distance between the murine *bg* gene and the murine T-cell receptor (TCR)  $\gamma$  chain gene and demonstrate that the human CHS gene is not closely linked to the human  $\gamma$  chain gene.

We have previously shown that a murine TCR- $\gamma$  chain gene is closely linked to the *bg* gene in the crinkled (*cr*), *bg*, and extra-toes (*Xt*) cluster by mapping this locus using recombinant inbred lines and taking advantage of a restriction fragment length polymorphism (RFLP) that exists at this locus (Owen *et al.*, 1986). The TCR- $\gamma$  chain is an immunoglobulin-like polypeptide that comprises one chain of the T-cell  $\gamma$ - $\delta$  receptor. Murine  $\gamma$  chains are encoded by three closely linked immunoglobulin-like genes located on murine chromosome 13 (Kranz *et al.*, 1985). Because there is diminished NK activity in beige mice (Roder and Duwe, 1979), and because it has been shown that T-cells with a  $\gamma$ - $\delta$  T-cell receptor are capable of non-MHC restricted (or NK-like) killing (Ang *et al.*, 1987; Borst *et al.*, 1987; Brenner *et al.*, 1987), the possibility existed that the gene for the TCR- $\gamma$  chain was involved in the pathogenesis of CHS/beige. In this study we present data from breeding studies in mice demonstrating that the *bg* mutation can be genetically sepa-

**TABLE 1**  
**Results of Backcross of F<sub>1</sub> (C3H/HeJ × C57BL/6J-*bg*) to C57BL/6J-*bg* to Examine the Linkage of TCR- $\gamma$  to *bg***

	F <sub>1</sub> (C3H/HeJ × C57BL/6- <i>bg</i> )			
	Nonrecombinant gametes		Recombinant gametes	
	<i>Tcrg</i> <sup>a</sup> +	<i>Tcrg</i> <sup>b</sup> <i>bg</i>	<i>Tcrg</i> <sup>b</sup> +	<i>Tcrg</i> <sup>a</sup> <i>bg</i>
C57BL/6J- <i>bg</i>				
<i>Tcrg</i> <sup>b</sup> <i>bg</i>	59	60	0	3
(gamete)	(+)	( <i>bg</i> )	(+)	( <i>bg</i> )

*Note.* *Bg* mice were obtained from Jackson Laboratories, Bar Harbor, Maine, and maintained according to institutional guidelines for animal care with institutional approval. C3H/HeJ were mated with C57BL/6J-*bg* to generate the F<sub>1</sub> animals. 122 F<sub>2</sub> mice were scored for coat color initially. Analysis of RFLP patterns at the TCR- $\gamma$  locus using tail DNA was performed as previously described (18) by hybridization with a murine  $\gamma$  chain probe. Phenotypic coat color is indicated in parentheses. Wild-type (+) phenotype is agouti for C3H/HeJ and black for C57BL/6J. Agouti is dominant to black in heterozygotes. The beige phenotype is easily discernible in either the agouti or black background. *Tcrg*<sup>a</sup> and *Tcrg*<sup>b</sup> indicate the restriction fragment patterns of C3H/HeJ and C57BL/6J, respectively.

rated from a murine TCR- $\gamma$  locus and is thus probably not altered in beige mice.

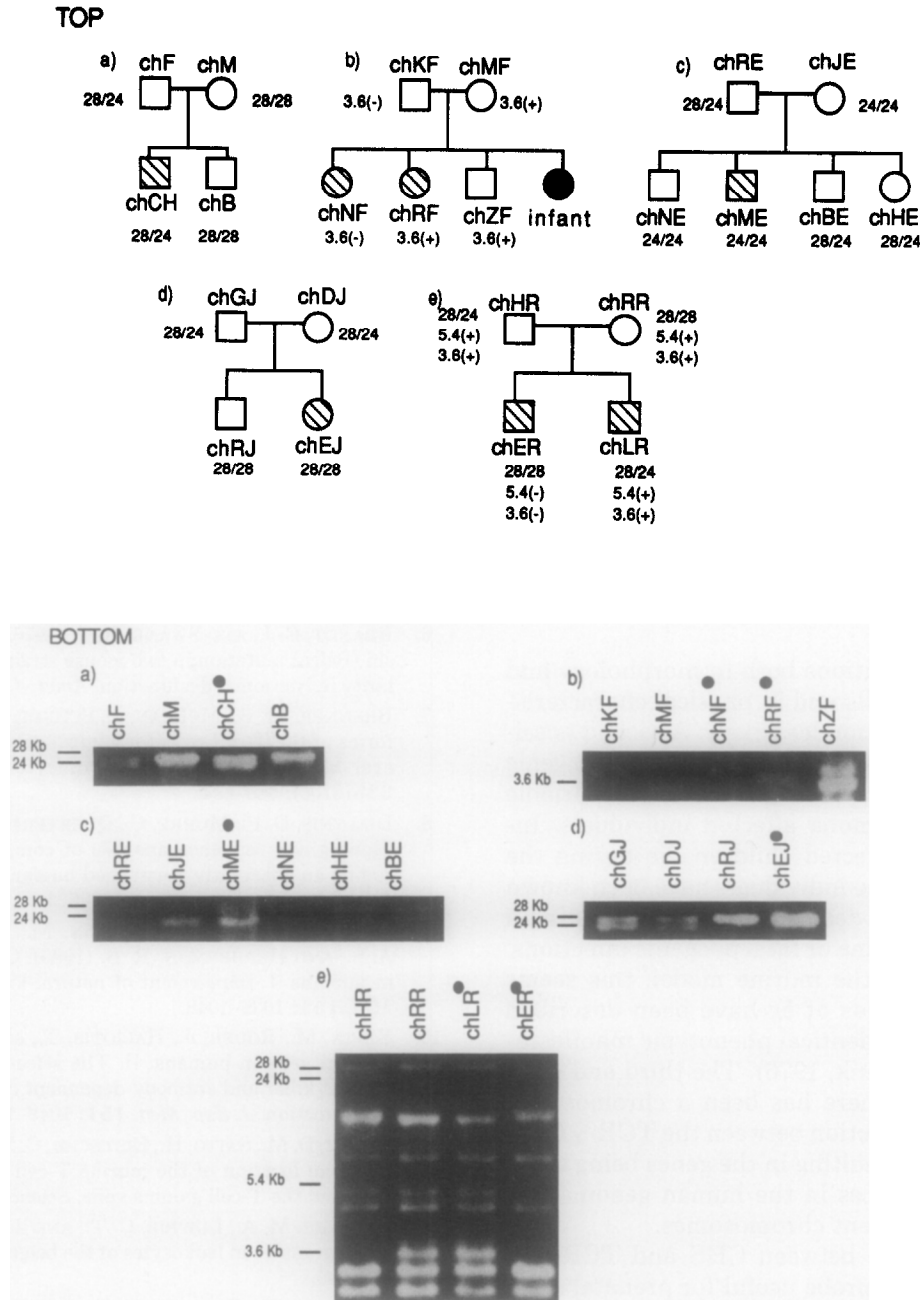
To determine the genetic linkage between the *bg* mutation and the murine TCR- $\gamma$  genes, a backcross between F<sub>1</sub> heterozygotes (C3H/HeJ × C57BL/6J-*bg*) and C57BL/6J-*bg* homozygotes was performed (Table 1). Progeny were analyzed for the presence of *bg* homozygosity by observing coat color. The beige phenotype in a C3H/HeJ background and the beige phenotype in a C57BL/6J mouse are easily distinguished from the wild-type coat colors. Previous studies have demonstrated that the TCR- $\gamma$  genes in C3H/HeJ mice can be distinguished from the TCR- $\gamma$  genes in C57BL/6J mice by RFLPs. Southern blot analysis was performed on DNA derived from progeny of the cross to ascertain which of the TCR- $\gamma$  polymorphism patterns was present. There were three recombinants out of 122 backcross mice, appearing in two separate litters. All three recombinants were heterozygous at the TCR- $\gamma$  locus and homozygous for *bg*. The frequency of recombination between these two markers is estimated to be 0.025 with 95% confidence limits of 0.005 and 0.07.

Linkage of homologous loci in mammals is often conserved (Nadeau and Taylor, 1984). To determine whether human CHS is linked to the TCR- $\gamma$  chain genes, we have examined the inheritance of the TCR- $\gamma$  chain genes in families affected by CHS. Fam-

ily pedigrees are illustrated in Fig. 1, top. The human  $\gamma$  chain genes are encoded by an immunoglobulin-like gene cluster that is about 200 kb long and is encoded on chromosome 7 (Murre *et al.*, 1985; Strauss *et al.*, 1987). Very few RFLPs have been previously identified at this locus. Because only five relatively small families were available for this analysis, more RFLPs of the TCR- $\gamma$  gene locus were required to make all of these families informative. Thus, DNAs from 20 normal individuals were screened for the presence of RFLPs with a number of restriction enzymes (data not shown) using standard techniques (Maniatis *et al.*, 1982). *Xba*I identifies a number of useful RFLPs. Figure 1, bottom, shows the autoradiographs of the Southern blots following digestion of DNA from the five families with *Xba*I and hybridization with a  $\gamma$  chain cDNA probe, pT $\gamma$ 1 (Dialynis *et al.*, 1986). Polymorphic bands are present at 28, 24, 5.4, and 3.6 kb. In family 1 (a), analysis of the 24 and 28-kb RFLPs, which are inherited in a Mendelian fashion, demonstrates concordance of inheritance because only the father (chF) and the affected child (chCH) show the 24-kb band. The pattern of these alleles in family 3 (c) is also consistent with linkage of Chediak-Higashi syndrome and TCR- $\gamma$ . Other RFLPs of this locus were also inherited, in these families, in a fashion consistent with linkage (data not shown).

Clear discordance of allelic inheritance is demonstrated in families 2, 4, and 5 (Figs. 1b, d, and e, bottom). In family 2, the 3.6-kb polymorphism is present in one parent (chMF) and one affected child (chRF), indicating that this band is representative of the allele bearing the gene for the disease. However, the other affected child in this family, chNF, has not received this allele from the parent as expected if linkage were present. The 24- and 28-kb bands are again informative with respect to family 4. Both parents in this family are heterozygous for this polymorphism yet both children have inherited only the allele producing the 28-kb band on *Xba*I digestion. As one child has the disease and the other is unaffected, nonlinkage is shown. The final family demonstrates discordance of allelic inheritance between siblings, both of which have CHS, for RFLPs at 24, 5.4, and 3.6 kb. In this case, none of the RFLPs present upon digestion with *Xba*I, and subsequent hybridization with pT $\gamma$ 1, has segregated with the disease. Negative LOD scores for recombination frequencies of 0.010–0.410 for the five families are shown in Table 2, demonstrating the absence of linkage at a frequency of 0.200 and below.

Three models can be proposed to explain the divergence of chromosomal linkage between these loci in mouse and man. One possibility is that the beige mouse may not be a true animal analog of Chediak-Higashi syndrome. However, the numerous similar-



**FIG. 1.** Evidence of nonlinkage between human CHS and the TCR- $\gamma$  gene locus. **Top:** Family pedigrees for the five families with offspring afflicted with Chediak-Higashi syndrome. Squares represent males, and circles represent females. One infant was not tested for RFLPs and is indicated by a solid circle. Cross-hatching indicates the presence of Chediak-Higashi syndrome. The presence or absence of relevant RFLPs is indicated for each family member. **Bottom:** Autoradiograms of Southern blots after digestion with *Xba*I and hybridization with the cDNA probe pT $\gamma$ 1 (8) for families 1-5 (a-e, respectively). Blood samples (5-20 cc) were obtained from index cases with CHS and all immediate family members after informed consent. Mononuclear cells were separated by Ficoll/Hypaque and, after T-cells were eliminated with cyclosporin A, B-cells were transformed by EBV (Epstein-Barr virus), establishing cell lines for each family member. DNA was obtained and 10  $\mu$ g was digested with restriction enzymes. Bars indicate the relevant RFLPs for analysis and are labeled with the appropriate size. For simplicity, only the informative bands are shown in a through d. All fragments detected by hybridization with pT $\gamma$ 1 after *Xba*I digestion are displayed in e. Superscript solid circles identify patients with Chediak-Higashi syndrome.  $\lambda$  DNA cut with *Hind*III was used as a size marker during electrophoresis through a 1% agarose gel. See text for discussion.

TABLE 2

Degree of Nonlinkage between the Genes for Human Chediak-Higashi Syndrome and the TCR- $\gamma$  Chain

$\theta$	LOD score
0.010	-21.79
0.060	-8.47
0.110	-4.64
0.160	-2.68
0.210	-1.54
0.260	-0.85
0.310	-0.43
0.360	-0.20
0.410	-0.07

Note. A computer program entitled Linkage 3.5, written by Jurg Ott, was used for calculation of LOD scores. Because of peculiarities within the program, "affection status" was converted to a binary phenotype with internally consistent estimation of heterozygosity of unaffected siblings.  $\theta$  represents recombination frequency.

ities between the conditions both in morphology and function of affected cells and in physical characteristics make this explanation less likely. A second possibility may be that the human CHS is a multigenic disease. This might explain the somewhat variable clinical course seen among affected individuals. Indeed, though most affected children die during the first decade of life, some individuals have been known to live into the fourth decade without manifesting a lymphoma-like syndrome or fatal pyogenic infections. Again, by analogy to the murine model, this seems unlikely. Several alleles of *bg* have been described previously, each with identical phenotypic manifestations (Brandt and Swank, 1976). The third and most likely model is that there has been a chromosomal breakpoint during evolution between the TCR- $\gamma$  locus and the beige locus, resulting in the genes being separated by larger distances in the human genome and possibly even on different chromosomes.

Because the linkage between CHS and TCR- $\gamma$  is not present in man, a probe useful for prenatal diagnosis is not currently available. However, we imagine that segments of DNA linked to the murine *bg* locus may be linked to the human CHS defect and may offer an approach to identifying a DNA probe that will be a useful diagnostic tool for this disease.

## ACKNOWLEDGMENTS

R.F.H. is supported by National Heart Lung and Blood Institute Training Grant HL-07446. This work was supported by NIH Grants AI19148 and AI18436 to J.G.S., AI20065 to L.A.B., and GM18684 to B.A.T. F.L.O. is supported by Grant IM394 from the American Cancer Society. M.E.C. has received a grant from the Immune Deficiency Foundation. Thanks to Dr. Margaret Johnson who assisted in locating patients, Dr. Thomas Quertermous for his

advice, and Eric Hoffman and Dr. Samuel Latt for their statistical guidance.

## REFERENCES

- ANG, S. L., SEIDMAN, J. G., PETERMAN, G. M., *et al.* (1987) Functional gamma chain-associated T cell receptors on cerebrospinal fluid-derived natural killer-like T cell clones. *J. Exp. Med.* **165**(5): 1453-1458.
- ARGYLE, J. C., KJELDSBERG, C. R., MARTY, J., SHIGEOKA, A. O., AND HILL, H. R. (1982). T-cell lymphoma and the Chediak-Higashi syndrome. *Blood* **60**: 672-676.
- BLUME, R. S., AND WOLFF, S. M. (1972). The Chediak-Higashi syndrome: Studies in four patients and a review of the literature. *Medicine (Baltimore)* **51**: 247-280.
- BORST, J., VAN DE GRIEND, R. J., VAN OOSTVEEN, J. W., *et al.* (1987). A T-cell receptor gamma/CD3 complex found on cloned functional lymphocytes. *Nature (London)* **325**(6106): 683-688.
- BRANDT, E. J., ELLIOTT, R. W., AND SWANK, R. T. (1975). Defective lysosomal enzyme secretion in kidneys of Chediak-Higashi (Beige) mice. *J. Cell Biol.* **67**: 774-788.
- BRANDT, E. J., AND SWANK, R. T. (1976). The Chediak-Higashi (Beige) mutation in two mouse strains: Allelism and similarity in lysosomal dysfunction. *Amer. J. Pathol.* **82**: 573-588.
- BRENNER, M. B., MCLEAN, J., SCHEFT, H., *et al.* (1987). Two forms of the T-cell receptor gamma protein found on peripheral blood cytotoxic T lymphocytes. *Nature (London)* **325**(6106): 689-694.
- DIALYNSIS, D. P., MURRE, C., QUERTERMOUS, T., *et al.* (1986). Cloning and sequence analysis of complementary DNA encoding an aberrantly rearranged human T-cell  $\gamma$  chain. *Proc. Natl. Acad. Sci. USA* **83**: 2619-2623.
- HALIOTIS, T., RODER, J., KLEIN, M., ORTALDO, J., FAUCI, A. S., AND HERBERMAN, R. B. (1980). Chediak-Higashi gene in humans. I. Impairment of natural-killer function. *J. Exp. Med.* **151**: 1039-1048.
- KLEIN, M., RODER, J., HALIOTIS, T., *et al.* (1980). Chediak-Higashi gene in humans. II. The selectivity of the defect in natural-killer and antibody-dependent cell-mediated cytotoxicity function. *J. Exp. Med.* **151**: 1049-1058.
- KRANZ, D. M., SAITO, H., DISTECHE, C. M., *et al.* (1985). Chromosomal location of the murine T-cell receptor alpha-chain gene and the T-cell gamma gene. *Science* **227**: 941-945.
- LUTZNER, M. A., LOWRIE, C. T., AND JORDAN, H. W. (1966). Giant granules in leukocytes of the beige mouse. *J. Hered.* **58**: 299-300.
- MANIATIS, T., FRITSCH, E. F., AND SAMBROOK, J. (1982). "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- MERINO, F., AMESTY, C., HENLE, W., L'AYRISSE, Z., BIANCO, N., AND RAMIREZ-DUQUE, P. (1986). Chediak-Higashi syndrome: Immunological responses to Epstein-Barr virus studies in gene heterozygotes. *J. Clin. Immunol.* **6**: 242-248.
- MERINO, F., KLEIN, G. O., HENLE, W., RAMIREZ-DUQUE, P., FORSGREN, M., AND AMESTY, C. (1983). Low NK activity and elevated anti-EBV antibodies in Chediak-Higashi patients. *Clin. Immunol. Immunopathol.* **27**: 326-339.
- MURRE, C., WALDMAN, R. C., MORTON, C. C., *et al.* (1985). Human gamma-chain genes are rearranged in leukemic T cells and map to the short arm of chromosome 7. *Nature (London)* **316**: 549-552.
- NADEAU, J. H., AND TAYLOR, B. A. (1984). Lengths of chromo-

- somal segments conserved since divergence of man and mouse. *Proc. Natl. Acad. Sci. USA* **81**: 814-818.
18. OWEN, F. L., TAYLOR, B. A., ZWEIDLER, A., AND SEIDMAN, J. G. (1986). The murine  $\gamma$ -chain of the T cell receptor is closely linked to a spermatocyte specific histone gene and the beige coat color locus on chromosome 13. *J. Immunol.* **137**: 1044-1046.
  19. QUIE, P. G., AND CATES, K. L. (1977). Clinical conditions associated with defective polymorphonuclear leukocyte chemotaxis. *Amer. J. Pathol.* **88**: 711-725.
  20. RODER, J., AND DUWE, A. (1979). The beige mutation in the mouse selectively impairs natural killer cell function. *Nature (London)* **278**: 451-453.
  21. RODER, J. C., HALIOTIS, T., KLEIN, M., *et al.* (1979). A new immunodeficiency disorder in humans involving NK cells. *Nature (London)* **284**: 553-555.
  22. RUBIN, C. M., BURKE, B. A., MCKENNA, R. W., *et al.* (1985). The accelerated phase of Chediak-Higashi syndrome: An expression of the virus-associated hemophagocytic syndrome? *Cancer* **56**: 524-530.
  23. STRAUSS, W. M., QUERTERMOUS, T., AND SEIDMAN, J. G. (1987). Measuring the human T cell receptor  $\gamma$ -chain locus. *Science* **237**: 1217-1219.
  24. WINDHORST, D. B., AND PADGETT, B. (1973). The Chediak-Higashi syndrome and the homologous trait in animals. *J. Invest. Dermatol.* **60**: 529-537.