Localization of a Gene for Familial Hemophagocytic Lymphohistiocytosis at Chromosome 9q21.3-22 by Homozygosity Mapping

Mina Ohadi,[1] Michel R. A. Lalloz,[1] Pak Sham,[2] Jinghua Zhao,[2] Andrew M. Dearlove,[3] Caroline Shiach,[4] Sally Kinsey,[4] Michael Rhodes,[3] and D. Mark Layton[1]

[1]Department of Haematological Medicine, King's College School of Medicine and Dentistry, and [2]Department of Psychological Medicine, Institute of Psychiatry, Denmark Hill, London; [3]UK HGMP Resource Centre, Hinxton, Cambridge; and [4]Department of Pediatric Haematology-Oncology, St. James University Hospital, Leeds, England

Summary

Familial hemophagocytic lymphohistiocytosis (FHL), also known as familial erythrophagocytic lymphohistiocytosis and familial histiocytic reticulosis, is a rare autosomal recessive disorder of early childhood characterized by excessive immune activation. Linkage of the disease gene to an ~7.8-cM region between markers D9S1867 and D9S1790 at 9q21.3-22 was identified by homozygosity mapping in four inbred FHL families of Pakistani descent with a combined maximum multipoint LOD score of 6.05. This is the first genetic locus to be described in FHL. However, homozygosity by descent across this interval could not be demonstrated in an additional affected kindred of Arab origin, whose maximum multipoint LOD score was −0.12. The combined sample revealed significant evidence for linkage to 9q markers (LOD score with heterogeneity, 5.00). Identification of the gene(s) involved in the pathogenesis of FHL will contribute to an understanding of the control of T-lymphocyte and macrophage activation, which is central to homeostasis in the immune system.

Introduction

Familial hemophagocytic lymphohistiocytosis (FHL; MIM 267700), first described by Farquhar and Claireaux (1952), is a lethal disorder of immune regulation, characterized by uncontrolled T-lymphocyte and macrophage activation, with an estimated incidence of 1/50,000 live births (Henter et al. 1991*c;* Hirst et al. 1994; Arico et al. 1996). Inheritance is autosomal recessive. Infiltration of the liver, spleen, bone marrow, and central nervous system by activated T cells and macrophages results in a multisystem disorder with onset in early infancy, which—in the absence of treatment with epipodophyllotoxins, immunosuppressive agents, or bone marrow transplantation—progresses rapidly, with a median survival of 2 mo (Janka 1983; Fischer et al. 1985; Blanche et al. 1991).

Cardinal clinical features include fever, hepatosplenomegaly, cytopenia, and neurological abnormalities frequently accompanied by hypofibrinogenemia, hypertriglyceridemia, and hyperferritinemia (Henter 1991*a*). The characteristic histological finding is a diffuse infiltration by lymphocytes and non–Langerhans cell histiocytes, with the latter showing phagocytosis of blood cells (hemophagocytosis). FHL is included among the histiocytoses, a heterogeneous group of disorders characterized by abnormal proliferation of antigen-presenting or antigen-processing cells (Favara et al. 1997). Recently, the locus for a novel autosomal recessive histiocytic disorder associated with sensorineural deafness and joint contractures in a single family was mapped to chromosome 11q25 (Moynihan et al. 1998). Thus far, this syndrome, termed "Faisalabad histiocytosis" (MIM 602782), has not been identified in other kindreds.

The mechanism that underlies failure to abrogate T-lymphocyte and macrophage activation in FHL is unknown. Elevation of the concentrations of circulating cytokines, including γ-interferon, interleukin-1 (IL-1), IL-6, and tumor necrosis factor α, is accompanied by high serum levels of soluble IL-2 receptor and CD8 (Komp et al. 1989; Henter et al. 1991*b;* Osugi et al. 1997). These immunological features suggest a failure to down-regulate proinflammatory cellular immune responses mediated by T-helper 1 (Th1) lymphocytes, which leads to sustained macrophage activation. This pathogenetic model is consistent with the efficacy of T-lymphocyte–targeted therapy in the form of antithymocyte globulin or cyclosporin A (Stephan et al. 1993; Abella et al. 1996). Further support for a transregulatory

Received June 17, 1998; accepted October 15, 1998; electronically published January 6, 1999.

Address for correspondence and reprints: Dr. D. Mark Layton, Department of Haematological Medicine, King's College School of Medicine and Dentistry, Denmark Hill, London SE5 9PJ, United Kingdom. E-mail: mlayton@hgmp.mrc.ac.uk

0002-9297/99/6401-0022$02.00

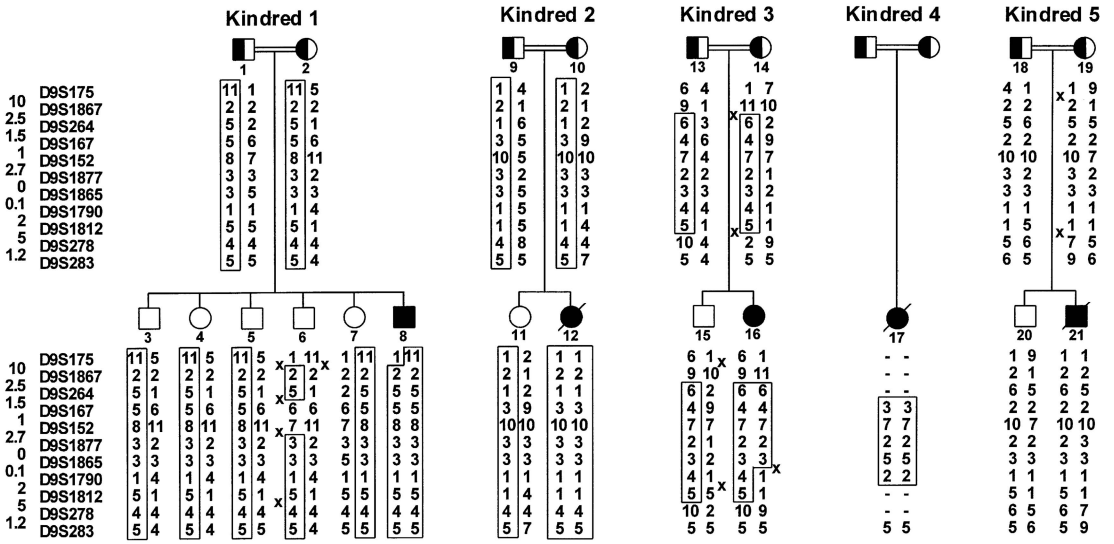

Figure 1 Allele segregation of linked and flanking markers in FHL families. Only key subjects are shown. Markers are shown telomeric to centromeric (*top to bottom*). Disease-linked haplotypes are boxed. Since limited material was available from the affected individual in kindred 4, we could genotype only markers D9S167, D9S152, D9S1877, and D9S1865 within the critical region in addition to markers D9S1790 and D9S283.

immune defect in FHL is provided by evidence that allogeneic bone marrow transplantation may be curative in the presence of mixed chimerism, despite limited donor engraftment (Landman-Parker et al. 1993) and the occurrence of hemophagocytic disorders secondary to T-lymphoproliferative disorders (Falini et al. 1990). Selective deficiency in natural killer cell activity (Ladisch et al. 1982; Perez et al. 1984) and T-cell cytotoxicity (Arico et al. 1988; Egeler et al. 1996) is frequently observed, although its relation to the pathogenesis of FHL remains uncertain.

The genes for Chediak-Higashi and Griscelli syndromes (MIM 214500 and MIM 214450)—autosomal recessive disorders associated with pigmentary dilution, cellular immunodeficiency, and evolution in the latter stages to an "accelerated phase" associated with uncontrolled T-cell and macrophage activation—have been cloned and mapped to chromosomes 1q43 (Barbosa et al. 1996; Nagle et al. 1996) and 15q21 (Pastural et al. 1997), respectively. Proliferation of activated T cells is also a feature of the autosomal recessive disorder Omenn syndrome (MIM 267700), shown recently to result from impaired genomic rearrangement of antigen receptor loci due to a mutation of the *RAG1* or *RAG2* genes at chromosome 11p13 (Villa et al. 1998). Omenn syndrome is demarcated from FHL and other lymphocyte/macrophage activation disorders by a distinct phenotype in which severe combined immunodeficiency with erythroderma, alopecia, lymphadenopathy, liver and spleen enlargement, protracted diarrhea, and failure to thrive is accompanied by elevated serum IgE concentrations and eosinophilia due to oligoclonal expansion of T-helper 2 lymphocytes, which secrete IL-4 and IL-5. In contrast to FHL, Th1 lymphocyte activity is suppressed.

The genetic basis of FHL is currently unknown. Human (Le Deist et al. 1996) and mouse (Schultz et al. 1993; Waterhouse et al. 1995) mutants that resemble FHL phenotypically have provided a rationale for candidate gene approaches. Genetic defects of SHP-1 (a protein tyrosine phosphatase involved in the down-regulation of signaling in hematopoietic cells), CTLA-4 (which negatively regulates activated T cells), and Fas (CD95)/Fas ligand (mediators of lymphocyte and macrophage apoptosis) have been excluded in FHL by mutation or linkage analysis (Fischer et al. 1997; Tabrizi et al. 1998; M. Ohadi, unpublished data). In the present study, homozygosity mapping localized the gene responsible for FHL in four affected kindreds of Pakistani origin to an ~7.8-cM interval on the long arm of chromosome 9 (bands q21.3-22). An affected kindred originating from Saudi Arabia exhibited negative multipoint LOD scores for markers at this interval. This study represents a first step toward identification of the gene defect(s) in FHL, which promises to illuminate the molecular and cellular basis for regulation of T-lymphocyte and macrophage activation.

Subjects and Methods

Families

Five consanguineous families originating from Pakistan (kindreds 1–4) or Saudi Arabia (kindred 5) were

included in the study (fig. 1). The diagnosis of FHL fulfilled the criteria proposed by the Histiocyte Society (Henter et al. 1991*a*). The parents were first cousins in kindreds 1, 3, 4, and 5 and second cousins in kindred 2. In four of the five families (kindreds 1–4), the proband presented between 1 and 2 mo of age. In kindred 5, onset of disease in the proband was delayed until 10 mo. All affected children exhibited typical features of FHL, which included fever, liver and spleen enlargement, bi- or pan-cytopenia, hypertriglyceridemia, and hypofibrinogenemia. We identified hemophagocytosis on examination of bone marrow and, in two cases (individuals 12 and 16), cerebrospinal fluid. All patients received treatment with etoposide and corticosteroids alone or in combination with cyclosporin A. Individual 8 subsequently underwent successful allogeneic bone marrow transplantation from an HLA-identical sibling donor (individual 6) and remains disease free 54 mo after diagnosis. One patient (individual 16) has survived in partial remission with continuing therapy for 15 mo. The remaining three patients died 9–17 mo after diagnosis because of disease progression despite treatment. Healthy siblings were aged 5–18 years at the time of the study. Blood samples for genetic analysis were collected after we obtained informed consent. Genotyping was performed on DNA extracted from paraffin-embedded tissue obtained from the deceased proband in family 4.

Genotype Analysis

We performed a genomewide screen by means of the ABI PRISM Linkage Mapping Set version 1 (Perkin-Elmer Applied Biosystems), consisting of 358 fluorescent-labeled PCR primer pairs, spaced at intervals of ~10 cM and including reverse primer-tailing chemistry (Brownstein et al. 1996), selected to amplify highly informative microsatellite loci from the Généthon human linkage map (Weissenbach et al. 1992; Gyapay et al. 1994; Dib et al. 1996). The methods we employed have been detailed by Diehl et al. (1990) and Reed et al. (1996). In brief, we extracted genomic DNA from whole blood in accordance with a standard protocol (Sambrook et al. 1988), and we extracted DNA from paraffin-embedded tissue according to the manufacturer's (Qiagen) protocol by means of xylene extraction modification.

We performed PCR reactions in 10-μl reactions with a Perkin-Elmer 9600 thermal cycler. Pooled PCR products were separated on a 4.5% polyacrylamide gel by electrophoresis on an ABI 377 XL sequencer. The PCR products were sized by GENESCAN 2.1 and scored by GENOTYPER 2.0 programs (Applied Biosystems). Once allele sizes for each microsatellite marker had been determined for DNA samples from all the families studied, allele numbers were assigned, and Mendelian inheritance was verified.

Statistical Analysis

Individual markers were examined for homozygosity in the affected members of the FHL families. We performed linkage analysis of the disease locus and markers at 9q with GENEHUNTER software (Kruglyak et al. 1996). We assumed an autosomal recessive model with complete penetrance in both sexes and a frequency of 0.004 for the disease allele. Since no information regarding the frequency of individual microsatellite alleles in the Pakistani population was available, we estimated allele frequencies of the 9q markers by genotyping 50 unrelated, randomly selected, healthy Pakistani subjects. For the families described, LOD scores were estimated at 4.61 and 3.67 for markers (assuming five equally frequent alleles) 5 and 10 cM from the disease gene, respectively (Terwilliger and Ott 1994).

Results

Primary Mapping of a Locus for the FHL Gene in Pakistani Kindreds

The initial genomewide screen of kindreds 1–3 identified markers D9S167 and D9S283, both located on chromosome 9q, to be the only markers with consistent homozygosity in the affected members (fig. 1). The marker D9S167 showed homozygosity in each of the affected family members, yielding a combined two-point LOD score of 3.1. In addition, all affected individuals were homozygous at marker D9S283, 12 cM telomeric to D9S167 (although the LOD score was reduced because of parental homozygosity for this marker), whereas the affected individuals of families 1 and 3 were heterozygous at D9S175, 14 cM centromeric to D9S167. These preliminary findings established linkage of the disease gene in these families to marker D9S167 and limited the centromeric boundary of the disease gene interval to marker D9S175.

Confirmation of Linkage and Delineation of the Disease Gene Interval by Fine Mapping

Eight microsatellite markers between D9S175 and D9S283 were selected from the Généthon microsatellite panel (Dib et al. 1996) to confirm and potentially narrow the disease gene interval. Extended haplotypes constructed by use of these markers, the order of which is consistent with recent genetic linkage maps available from the National Center for Biotechnology Information and Center for Medical Genetics web sites, are shown in figure 1. Regions of homozygosity by descent were observed in all affected individuals between and including D9S264 and D9S1865. Subject 16 in family 3 was heterozygous at D9S1867 and D9S1790 because of two recombination events on the maternal side. In this fam-

ily, an inferred double recombination in the maternal grandfather narrowed the centromeric limit of the FHL disease gene interval. A second recombination event in the mother of the proband (subject 14) between markers D9S1865 and D9S1790 further resolved the telomeric boundary, limiting the disease gene interval to ~7.8 cM between and excluding markers D9S1867 and D9S1790, with a combined maximum multipoint LOD score of 4.9.

Inclusion of a fourth affected Pakistani patient in the study (family 4) revealed homozygosity for markers D9S167, D9S152, D9S1877, D9S1865, D9S1790, and D9S283 in the affected individual (fig. 1), which increased the maximum multipoint LOD score to 6.05 in the interval defined. The limited quantity of archival necropsy material available precluded further marker analysis in this case.

Consideration of Locus Heterogeneity in FHL

In the affected member (individual 21) of family 5 (fig. 1) included in the fine mapping, negative multipoint LOD scores were obtained in the interval D9S1867–D9S1790 (a maximum of −0.12). Extended haplotype analysis in the five families resulted in a maximum multipoint LOD of 4.40 and a maximum LOD score with heterogeneity (HLOD) of 5.00 at an estimated proportion of 0.81 of families linked to chromosome 9q markers at the interval D9S1867–D9S1790 (fig. 2). The admixture χ^2 test of heterogeneity (Smith 1963) gave a value of 2.76 ($P = .1$), providing tentative evidence in favor of locus heterogeneity.

Discussion

This study represents the first report of linkage in FHL and underscores the power of homozygosity mapping to detect linkage in rare recessive disorders on the basis of a small number of affected families from a homogeneous background (Lander and Botstein 1987).

The maximum multipoint HLOD score of 5.00 in the five families studied identifies a locus for FHL at 9q21.3-22 in an ~7.8-cM interval between markers D9S1867 and D9S1790. Among the four kindreds (1–4) that show linkage (a maximum multipoint LOD score of 6.05) to this locus, disease-linked haplotypes are shared among several families (fig. 1). Kindreds 1 and 2 share the haplotype 3–3–1 at D9S1877, D9S1865, and D9S1790, and kindreds 1–3 share allele 3 at D9S1865. This, however, does not contribute further to the localization of the FHL gene at 9q21.3-22, since D9S1877 (3), D9S1865 (3), and D9S1790 (1) alleles are the ones most frequently encountered in the Pakistani population (allele frequencies of 0.42, 0.61, and 0.50, respectively).

Interestingly, a constitutional pericentric inversion of chromosome 9 has recently been described in a sporadic case of hemophagocytic lymphohistiocytosis (Hasle et al. 1996). It is unlikely that disruption of the FHL gene

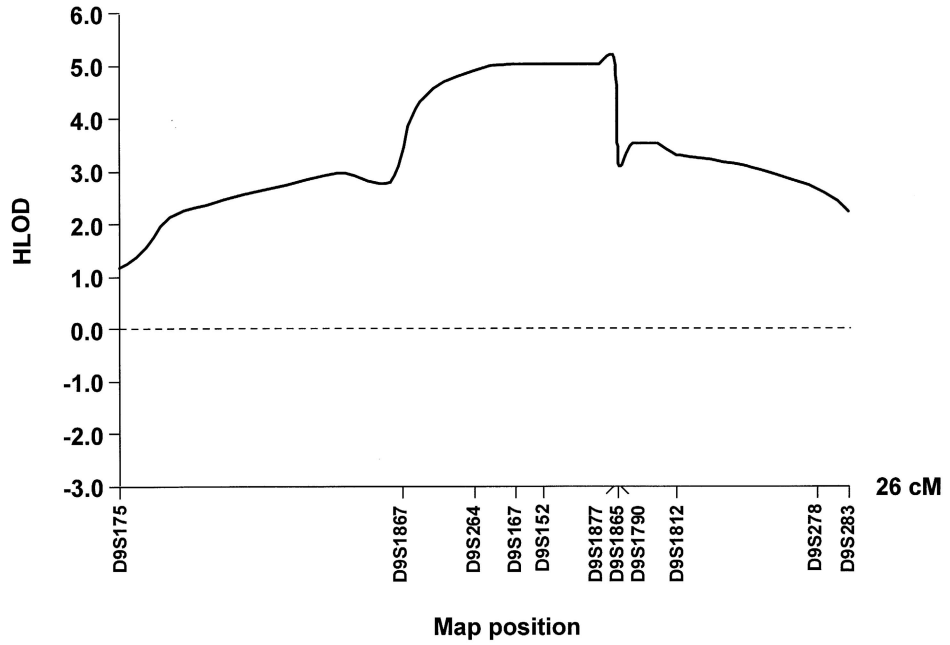

Figure 2 HLOD curve for the five FHL families, maximized over the proportion of families linked

at 9q21.3-22 contributed to pathogenesis in this case, since the breakpoints of the inversion at 9p23 and 9q31 lie a considerable distance from this locus.

Several possibilities could explain the failure to detect linkage to chromosome 9q21.3-22 in kindred 5. Lack of parental informativity within the critical interval (D9S167, D9S1865, and D9S1790) renders it impossible to differentiate between homozygosity by state and by descent at these loci. The occurrence in a consanguineous kindred of a recessive phenotype, although suggestive, does not signify homozygosity by descent, per se, and the possibility, albeit a remote one, that distinct mutant alleles at the 9q locus segregate in family 5 cannot be excluded. Alternatively, FHL may exhibit locus heterogeneity, as suggested by the result of admixture testing. The χ^2 test value obtained (2.76) is compatible, although not conclusive, in this respect. Heterogeneity with respect to cellular immune dysfunction in FHL (Ladisch et al. 1982; Stark et al. 1987) is consistent with the possibility that multiple defects underlie the disease phenotype. Since the ethnic background of family 5 varied from that of the other families included in the study, it is conceivable that within individual populations defects of different genes predominate among FHL kindreds. Further studies of affected families from diverse ethnic backgrounds will clarify this.

Two genes involved in negative regulation of the cell cycle—*CKS2* (Demetrick et al. 1996) and *GAS1* (Del Sal et al. 1992; Evdokiou et al. 1993)—are located at 9q21.3-22 within the D9S1867–D9S1790 interval. Although they are not specifically implicated in T-cell and macrophage regulation, a defect in one of these genes could result in failure to check cellular proliferation, with persistence of an activated state. Such a mechanism, although speculative, would be consistent with the diffuse lymphohistiocytosis that characterizes FHL. The observation that deletion or rearrangement of chromosome band 9q22 occurs in myeloid leukemias and non-Hodgkin's lymphoma, including malignant histiocytosis (Mitelman et al. 1997), suggests the presence within this region of one or several genes that play an important role in the regulation of hematopoietic cell proliferation or differentiation.

In conclusion, a gene for FHL has been localized by homozygosity mapping to chromosome 9q21.3-22 between D9S1867 and D9S1790. Identification of the causative gene within this interval will facilitate carrier and prenatal diagnosis of FHL, for which no approach is currently available, and delineate the mechanisms underlying physiological control of T-lymphocyte and macrophage activation, an essential pathway in regulation of the immune response.

Acknowledgments

Genotyping was undertaken at the Linkage Hotel, United Kingdom–Human Genome Mapping Project Resource Centre. We are grateful to Amanda J. Thompson for technical assistance and to Professor Robert Mueller and Dr. Philip Mason, who provided samples for allele frequency estimation in the Pakistani population.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, http://www.marshmed.org/genetics (for genetic linkage maps used for homozygosity mapping)
Généthon, http://www.genethon.fr (for microsatellite markers)
National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov (for genetic linkage maps used for homozygosity mapping)
Online Mendelian Inheritance in Man (OMIM), http://www3.ncbi.nlm.nih.gov/Omim (for the autosomal recessive disorders FHL [MIM 267700]; Chediak-Higashi syndrome [MIM 214500], linked to 1q43; and Griscelli syndrome [MIM 214450], linked to 15q21)

References

Abella EM, Artrip J, Schultz K, Ravindranath Y (1997) Treatment of familial erythrophagocytic lymphohistiocytosis with cyclosporin. Am J Pediatr 130:467–470

Arico M, Nespoli L, Maccario R, Montagna D, Bonetti F, Caselli D, Burgio GR (1988) Natural cytotoxicity impairment in familial haemophagocytic lymphohistiocytosis. Arch Dis Child 63:292–296

Barbosa MDFS, Mguyen QA, Tchernev T, Ashley JA, Detter JC, Blaydes SM, Brandt SJ, et al (1996) Identification of the homologous beige and Chediak-Higashi syndrome genes. Nature 382:262–265

Blanche S, Caniglia M, Girault D, Landman J, Griscelli C, Fischer A (1991) Treatment of hemophagocytic lymphohistiocytosis with chemotherapy and bone marrow transplantation. Blood 78:51–54

Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: primer modifications that facilitate genotyping. Biotechniques 20:1004–1009

Del Sal G, Ruaro ME, Philipson L, Scheider C (1992) The growth arrest–specific gene, *gas1,* is involved in growth suppression. Cell 70:595–607

Demetrick DJ, Zhang H, Beach DH (1996) Chromosomal mapping of the human genes *CKS1* to 8q21 and *CKS2* to 9q22. Cytogenet Cell Genet 73:250–254

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of

the human genome based on 5,264 microsatellites. Nature 380:152–154

Diehl SR, Siegle J, Buck GA, Reynolds TR, Weber JL (1990) Automated genotyping of human DNA polymorphisms. Am J Hum Genet 47:A177

Egeler RM, Shapiro R, Loechelt B, Filipovich A (1996) Characteristic immune abnormalities in hemophagocytic lymphohistiocytosis. Pediatr Hematol Oncol 18:340–345

Evdokiou A, Webb GC, Peters GB, Dobrovic A, O'Keefe DS, Forbes IJ, Cowled PA (1993) Localization of the human growth arrest–specific gene (*GAS1*) to chromosome bands 9q21.3-q22, a region frequently deleted in myeloid malignancies. Genomics 18:731–733

Falini B, Pileri S, De Solas I, Martelli MF, Mason DY, Delsol G, Gatter KC, et al (1990) Peripheral T-cell lymphoma associated with hemophagocytic syndrome. Blood 75: 434–444

Farquhar JW, Claireaux AE (1952) Familial hemophagocytic reticulosis. Arch Dis Child 27:519–525

Favara BE, Feller AC, with members of the WHO Committee on Histiocytic/Reticulum Cell Proliferations (1997) Contemporary classification of histiocytic disorders. Med Pediatr Oncol 29:157–166

Fischer A, Cavazzana-Calvo M, De Saint Basile G, De Villartay JP, Di Santo JP, Hivraz C, Rieux-Laucat F, et al (1997) Naturally occurring primary deficiencies of the immune system. Annu Rev Immunol 15:93–124

Gyapay G, Morisette J, Vignal A, Dib C, Fizames C, Millaseau P, Marc S (1994) The 1993–1994 Généthon human genetic linkage map. Nat Genet 7:246–339

Hasle H, Brandt C, Kerndrup G, Kjeldsen E, Sorensen AG (1996) Haemophagocytic lymphohistiocytosis associated with constitutional inversion of chromosome 9. Br J Haematol 93:808–809

Henter JI, Elinder G, Ost A, and the HLH Study Group of the Histiocyte Society (1991*a*) Diagnostic guidelines for hemophagocytic lymphohistiocytosis. Semin Oncol 18:29–33

Henter JI, Elinder G, Soder O, Hansson M, Anderson B,-Anderson U (1991*b*) Hypercytokinemia in familial hemophagocytic lymphohistiocytosis. Blood 78:2918–2922

Henter JI, Elinder G, Soder O, Ost A (1991*c*) Incidence in Sweden and clinical features of familial hemophagocytic lymphohistiocytosis. Acta Pediatr Scand 80: 428–435

Hirst WJR, Layton DM, Singh S, Mieli-Vergani G, Chessells M, Strobel S, Pritchard J (1994) Haemophagocytic lymphohistiocytosis: experience at two UK centres. Br J Haematol 88:731–739

Janka GE (1983) Familial hemophagocytic lymphohistiocytosis. Eur J Pediatr 140:221–230

Komp DM, McNamara J, Buckley P (1989) Elevated soluble interleukin-2 receptor in childhood hemophagocytic histiocytic syndromes. Blood 73:2128–2132

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

Ladisch S, Ho W, Matheson D, Pilkington R, Hartman G (1982) Immunologic and clinical effects of repeated blood exchange in familial erythrophagocytic lymphohistiocytosis. Blood 60:814–821

Lander ES, Botstein D (1987) Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. Science 236:1567–1570

Landman-Parker J, Le Deist F, Blaise A, Brison O, Fischer A (1993) Partial engraftment of donor bone-marrow cells associated with long-term remission of haemophagocytic lymphohistiocytosis. Br J Haematol 85:37–41

Le Deist F, Emile JF, Rieux-Laucat F, Benderou M, Roberts I, Brousse N, Fischer A (1996) Clinical, immunological, and pathological consequences of Fas-deficient conditions. Lancet 348:719–723

Mitelman F, Mertens F, Johansson B (1997) A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. Nat Genet 15 (special issue):417–474

Moynihan LM, Bundey SE, Health D, Jones EL, McHale DP, Mueller RF, Markham AF, et al (1998) Autozygosity mapping to chromosome 11q25, of a rare autosomal recessive syndrome causing histiocytosis, joint contractures, and sensorineural deafness. Am J Hum Genet 62:1123–1128

Nagle DL, Karim MA, Woolf EA, Holmgren L, Bork P, Misumi DJ, McGrail SH, et al (1996) Identification and mutation analysis of the complete gene for Chediak-Higashi syndrome. Nat Genet 14:307–311

Osugi Y, Hara J, Tagawa S, Takai K, Hosoi G, Matsuda Y, Ohta H (1997) Cytokine production regulating Th1 and Th2 cytokines in hemophagocytic lymphohistiocytosis. Blood 89:4100–4103

Pastural E, Barrat FJ, Dufourcq-Lagelouse R, Certain S, Sanal O, Jabado N, Seger R, et al (1997) Griscelli disease maps to chromosome 15q21 and is associated with mutations in the *myosin-Va* gene. Nat Genet 16:289–292

Perez N, Virelizier JL, Arenzana-Seisdedos F, Fischer A, Griscelli C (1984) Impaired natural killer activity in lymphohistiocytosis syndrome. J Pediatr 104:569–573

Reed PW, Davies JL, Copeman JB, Bennett ST, Palmer SM, Pritchard LE, Gough CL, et al (1994) Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. Nat Genet 7:390–395

Sambrook J, Fritsch E, Maniatis T (eds) (1989) Molecular cloning: a laboratory manual. Vol 103. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Schultz LD, Schweitzer PA, Rajan TV, Yi T, Ihle JN, Matthews RJ, Thomas ML, et al (1993) Mutations at the murine moth-eaten locus are within the hematopoetic cell protein–tyrosine phosphatase (*Hcph*) gene. Cell 73:1445–1454

Smith CAB (1963) Testing for heterogeneity of recombination fraction values in human genetics. Ann Hum Genet 27: 175–182

Stark B, Cohen IJ, Pecht M, Umiel T, Apte RN, Friedman E, Levin S (1987) Immunologic dysregulation in a patient with familial hemophagocytic lymphohistiocytosis. Cancer 60: 2629–2636

Stephan JL, Donadieu J, Le Deist F, Blanche C, Griscelli C, Fischer A (1993) Treatment of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins, steroids and cyclosporin A. Blood 82:2319–2323

Tabrizi M, Yang W, Jiao H, DeVries EMG, Platanias LC, Arico M, Yi T (1998) Reduced Tyk2/SHP-1 interaction and lack of SHP-1 mutation in a kindred of familial hemophagocytic lymphohistiocytosis. Leukemia 12:200–206

Terwilliger JD, Ott J (1994) Handbook of human genetic linkage. Johns Hopkins University Press, Baltimore, pp 243–260

Villa A, Santagata S, Bozzi F, Giliani S, Frattini A, Imberti L, Gatta LB (1998) Partial V(D)J recombination activity leads to Omenn syndrome. Cell 93:885–896

Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, et al (1995) Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. Science 270:985–988

Weissenbach J, Gyapay G, Dib C, Vignal A, Morrissette J, Millasseau P, Vaysseiz G (1992) A second-generation linkage map of the human genome. Nature 359:794–801