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Natural cytotoxicity impairment in familial haemophagocytic lymphohistiocytosis

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SUMMARY Ten children with the characteristic clinical and haematological features of haemophagocytic lymphohistiocytosis are reported. Four patients treated with a combination of drugs comprising etoposide, methotrexate, and steroids were in complete remission after 10 to 30 months.

Natural cytotoxic mechanisms including natural killer cell activity, antibody dependent cell mediated cytotoxicity, lymphokine activated killer cell activity, and natural killer cell like activity were persistently absent or severely impaired in these four patients despite their clinical remission. Their parents and one healthy sibling also had impaired natural cytotoxic mechanisms. Constitutional impairment of natural cytotoxic mechanisms could be important in the pathogenesis of haemophagocytic lymphohistiocytosis.

Familial haemophagocytic reticulosis was first described by Farquhar and Claireaux in 1952.[1] Now known as familial haemophagocytic lymphohistiocytosis (FHL), it is a rare disease that is probably often wrongly diagnosed. It usually presents with fever, hepatosplenomegaly, and pancytopenia in infants.[2–4] The central nervous system is often affected. Hypertriglyceridaemia and hypofibrinogenaemia are common, and often reversible with treatment.[5] Genetic analysis of most of the sibships reported elsewhere indicates that there is an autosomal recessive inheritance in about 75% of cases.[6] The disease has been rapidly fatal in most patients due to visceral infiltration of lymphocytes and histiocytes.[2] Initial attempts at treatment with plasma and blood exchange led to some improvement in the clinical course.[7] Recently long term remission has been achieved by treatment with etoposide[8 9] and teniposide.[10] Combined treatment with etoposide, steroids, and methotrexate given intrathecally, seems to result in long term clinical remission, and preliminary results of prevention of fatal relapse from symptoms in the central nervous system by cranial irradiation have been reported.[11]

Evaluation of the immunological state of some patients has shown that in some cases impairment of the immune system is an essential feature of FHL.[12–14] Impairment of natural killer cell activity has recently been reported.[15]

We report the clinical and immunological features of 10 new cases of FHL. In particular we investigated various natural cytotoxic mechanisms including natural killer cell activity, antibody dependent cellular cytotoxicity, lymphokine activated killer cell activity, and natural killer cell like activity generated in allogeneic mixed lymphocyte cultures in four affected children who achieved continuous complete remission, and in their families.

Patients and methods

Nine boys and one girl aged between 1 and 44 months from six Italian families were studied between 1983 and 1986. They all had FHL according to current diagnostic criteria.[2 4] Cases 3 and 4 and cases 5 and 6 were brothers, and cases 1, 9, and 10 (the latter being male monozygous twins) were members of the same family. The families of cases 7 and 8, and of 1, 9, and 10, were also studied; they comprised six healthy parents aged from 25 to 35, and the 3 year old healthy brother of cases 1, 9, and 10. The control group (from whom the normal values were derived) comprised healthy adult subjects who underwent immunological evaluation at the same time as the patients.

CELL PREPARATION

Lymphocytes were isolated from heparinised peripheral blood as previously described.[16]

ASSAY OF NATURAL KILLER CELL ACTIVITY

The assay was performed as previously described.[17] K562 cells were used as the target line, and they were labelled with 100 μCi of sodium ^{51}chromate (New England Nuclear) for 60 minutes at 37°C. The cells were plated in duplicate in concentrations of 5×10^3/μl, and varying numbers of effector cells were then added to give effector:target cell ratios of 100:1, 30:1, and 10:1. The plates were incubated in a humidified atmosphere containing 5% carbon dioxide for four hours at 37°C. After the incubation period the amount of radioactivity in the supernatant was measured by a gamma counter. The results were expressed as percentage cytotoxicity measured by specific release of ^{51}chromate and calculated as follows: % release=experimental release minus spontaneous release/total release minus spontaneous release×100.

ASSAY OF ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY

P815 cells, which are resistant to the activity of natural killer cells, were sensitised with rabbit specific IgG before being used as target cells. These cells, in concentrations of $1–2\times10^6$, were labelled with ^{51}chromate and then incubated with antiP815 antiserum in a dilution of $1:10^5$ for 30 minutes at room temperature. The assay was performed under the same conditions as that of natural killer cell activity.

ASSAY OF LYMPHOKINE ACTIVATED KILLER CELL ACTIVITY

Lymphocytes were cultured in 24 well plates at a concentration of 2×10^6/ml in 2 ml of Roswell Park Memorial Institute (RPMI) 1640 medium, which was supplemented with 10% human AB serum, 50 μg/ml gentamicin, and 2 mM glutamine (complete medium) in the presence of 500 U/ml human recombinant interleukin 2 (R IL-2, Roche). After being incubated for seven days in a humidified atmosphere with 5% carbon dioxide the cells were recovered and their cytotoxicity measured against Daudi target cells, which are resistant to the activity of natural killer cells, under the same conditions as described above.

ASSAY OF ALLOGENEIC MIXED LYMPHOCYTE RESPONSE AND NATURAL KILLER CELL LIKE ACTIVITY

Responder lymphocytes were cultured in 24 well plates at a concentration of 10^6/ml in complete medium. Allogeneic human mononuclear cells were used as the stimulators after they had been irradiated with 2000 rads, and were added at the same concentration in a total volume of 2 ml. Cultures were incubated at 37°C in a humidified atmosphere with 5% carbon dioxide. After seven days cells were recovered and natural killer cell like cytotoxicity was measured against HL-60 target cells, which are resistant to natural killer cell activity, under the same conditions as described above.

Table 1 *Clinical and laboratory data of 10 children with haemophagocytic lymphohistiocytosis*

	Case nos									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Sex	M	M	M	M	M	M	F	M	M	M
Familial occurrence	Yes	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Age at onset (months)	2	17	1	2	25	44	13	5	2	2
Fever (>38·5°C)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hepatosplenomegaly	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lymphadenopathy	No	No	No	No	Yes	Yes	Yes	Yes	No	No
Jaundice	No	No	Yes	No	Yes	No	No	Yes	No	No
Skin rash	No	No	Yes	No	Yes	No	No	No	No	No
Polymorphonuclear leucocytes $<1\times10^9$/l	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Platelet count $<1000\times10^9$/l	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Serum fibrinogen concentration <1g/l	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Serum triglyceride concentration >1·7 mmol/l	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
White cell count in cerebrospinal fluid $>5\times10^6$/l	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No
Erythrophagocytosis in bone marrow, and liver or spleen	Yes	Yes	Yes	Yes	*	Yes	Yes	Yes	Yes	Yes
Cranial radioprophylaxis (18 Gy in 10 doses)	No	No	No	No	No	No	Yes	Yes	No	No
Outcome	Died	Died	Died	Died	Died	Died	Well	Well	Well	Well
Age at death, or at time of writing (months)	4	23	12	20	26	46	43	24	12	12

*Observation not made by us.

Results

These 10 children showed the clinical and laboratory features that are typical of FHL (table 1). Seven of the 10 had the familial disease, the other three being isolated cases.

Case 3 ran a fulminant course with a graft versus host reaction after a blood transfusion, so the diagnosis of congenital severe combined immune deficiency had to be excluded. Later his brother (case 4) developed typical FHL. The complete clinical record of case 5 was available from another hospital where the child had died of what had been assumed to be a lymphoproliferative disorder. Cases 1–6 all died of severe FHL, cases 5 and 6 within 26 months of age. The last four patients (cases 7–10) were still alive, and in continuous complete remission 10 to 30 months after diagnosis.

Immunological investigation of the 10 patients showed an inconsistent pattern of abnormalities, as reported elsewhere.[18] In the four surviving children (cases 7–10) natural killer cell activity, lymphokine activated killer cell activity, antibody dependent cellular cytotoxicity, and natural killer cell like activity were profoundly impaired despite continuous clinical remission; fibrinogen and triglyceride concentrations had returned to normal, and haemophagocytosis was no longer detectable (table 2).

Table 3 shows that the parents of the surviving patients and the healthy 3 year old brother of patients 1, 9, and 10 all showed impairment in their natural cytotoxic mechanisms. In particular natural killer cell activity was extremely low or absent in the mothers of cases 7 and 8, the father of 8, and in the healthy brother; it was at the lower limit of normal in the father of case 7. Antibody dependent cellular cytotoxicity was low in four of the six parents tested;

Table 2 *Natural cytotoxicity evaluation in four surviving patients*

	Effector: target cell ratio	*Surviving case nos*				*Normal values mean (SD)*
		7	*8*	*9*	*10*	
Natural killer cell activity	100:1	2	6	34	17	61 (13)
	30:1	0	2	21	23	40 (12)
	10:1	0	0	10	17	28 (9)
Antibody dependent cellular cytotoxicity	100:1	0	28	7	10	68 (17)
	30:1	0	0	2	8	52 (15)
	10:1	0	0	2	2	27 (10)
Lymphokine activated killer cell activity	100:1	0	22	35	27	74 (14)
	30:1	0	15	26	9	53 (11)
	10:1	0	13	16	9	31 (9)
Natural killer cell like activity	100:1	0	2	22	12	55 (21)
	30:1	0	0	18	7	47 (13)
	10:1	0	0	7	4	22 (13)

Activities are expressed as % of specific lysis.

Table 3 *Cellular cytotoxicity and natural killer cell phenotype in six parents and one healthy brother of cases 7–10*

	Effector: target cell ratio	*Case 7*		*Case 8*		*Cases 1, 9, and 10*			*Normal values mean (SD)*
		Father	*Mother*	*Father*	*Mother*	*Father*	*Mother*	*Brother*	
Natural killer cell activity	100:1	32	8	21	17	56	46	25	61 (13)
	30:1	18	4	15	8	46	45	28	40 (12)
	10:1	5	4	7	6	39	46	24	28 (9)
Antibody dependent cellular cytotoxicity	100:1	27	24	33	27	69	46	19	68 (17)
	30:1	21	12	30	18	76	33	12	52 (15)
	10:1	12	7	7	9	56	25	11	27 (10)
Lymphokine activated killer cell activity	100:1	52	52	19	10	16	71	54	74 (14)
	30:1	41	33	12	3	15	62	55	53 (11)
	10:1	23	21	4	2	7	59	46	31 (9)
Natural killer cell like activity	100:1	67	38	28	27	46	59	33	55 (21)
	30:1	64	26	15	22	19	48	21	47 (13)
	10:1	42	6	6	5	20	40	11	22 (13)
With monoclonal antibody Leu7		12	9	8	12	ND	ND	ND	18 (7)
With monoclonal antibody Leu11		18	7	15	14	ND	ND	ND	23 (6)

Activities are expressed as % of specific lysis. Leu7 and Leu11 are expressed as % of positive cells.

lymphokine activated killer cell activity was low in both parents of case 7 and in the father of cases 9 and 10, and natural killer cell like activity was low in the parents of case 8 and at the lower limit of normal in the mother of case 7 and in the brother of cases 1, 9, and 10.

Discussion

We have studied 10 cases of FHL from six Italian families. Three patients came from families with no other affected children, but were included as they showed all the clinical and laboratory features of FHL with the exception of a positive family history. It is reported that there is only a slight preponderance of males affected,[6] whereas we found a male:female ratio of 9:1. The clinical course was severe and all but the last four children died, most within 2 years of age. The four surviving patients were treated with a combination of drugs (etoposide, steroids, and methotrexate given intrathecally). Cases 7 and 8 received cranial irradiation in a dose of 18 Gy, as recently suggested.[11] They were all in continuous remission at the time of writing after 10 to 30 months. How long chemotherapy should be continued in patients in remission is still debatable. We decided not to suspend treatment because of the severe prognosis and the evidence of early relapse after stopping treatment found by others (C Griscelli, personal communication).

The pathogenesis of FHL is unknown. A primary disorder of lymphocyte response with secondary impairment of the monocytic response has been suggested. A defect in natural killer cell activity that is reversible by cytotoxic treatment, has also been suggested.[15] In the four surviving children in our report (cases 7–10), natural cytotoxic mechanisms including natural killer cell activity, antibody dependent cellular cytotoxicity, lymphokine activated killer cell activity and natural killer cell like activity were persistently absent or severely impaired even during the long lasting complete remission. Impairment of cytotoxicity is not due to treatment with drugs,[15] because we detected it in the healthy, untreated subjects as well as the patients. Moreover, this study provides evidence that the parents and the only healthy brother of our patients with FHL have impairment of one or more of their natural cytotoxic mechanisms. These data suggest that the defect may be genetically transmitted from the healthy heterozygous parents to the offspring. In our patients impairment of natural cytotoxicity appeared to be a characteristic feature of FHL, independent of clinical state, typical biochemical aberrations, and treatment.

The present data are not in keeping with a previous report of an apparently reversible defect in natural killer cell activity in FHL.[11] On the contrary, evidence of restoration of natural killer cell activity in a patient with FHL who received an HLA matched bone marrow transplant[19] suggests that the successful graft led to clinical remission, and to biological replacement of the impaired lymphocyte clone. It can be hypothesised that in FHL functional imbalance of different natural killer cell subsets is responsible for the persistent severe impairment in natural cytotoxic mechanisms. Nevertheless, our results confirm previous reports[20, 21] that showed that the percentage of circulating natural killer cells (calculated using Leu 7 and Leu 11 monoclonal antibodies) is low only in some patients, and does not correlate with low functional activity. Moreover, incubation with interferon (the production of which also seems to be impaired) has been reported as being ineffective in restoring natural killer cell activity in patients with FHL.[20]

Natural killer cell activity develops in humans during the prenatal period, is still depressed in the newborn, and becomes complete—that is, the same as normal adult activity—within a few months.[22–25] Nevertheless, newborn infants display normal or even high antibody dependent cellular cytotoxicity, natural killer cell like and lymphokine activated killer cell activities when compared with adult controls.[26]

It was recently reported that homozygosity in the genes controlling the major histocompatibility complex influences natural killer cell activity in man.[27] Thus a link between the gene or genes controlling the development of FHL and those controlling the level of natural killer cell activity, which are all located close to the genes coding for luman leucocyte antigen, could be possible. The various mechanisms of natural cytotoxicity are currently thought to play a principal part in the defence against proliferating aberrant cells or cells infected with viruses, as well as having a regulatory role in the modulation of hemopoiesis.[28 29] A persistent constitutional impairment of these mechanisms seems to be important in the pathogenesis of FHL. Moreover, the identification of this biological marker is also important as it may have a practical application if prenatal diagnosis is attempted.

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