Immunologic and Clinical Effects of Repeated Blood Exchange in Familial Erythrophagocytic Lymphohistiocytosis

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Depressed cellular immune function and increased susceptibility to infection characterize familial erythrophagocytic lymphohistiocytosis (FEL), a usually fatal autosomal recessive disease. One component of the immunodeficiency is plasma-mediated inhibition of lymphocyte proliferation. We have tested whether repeated plasma or blood exchange would decrease plasma inhibitory activity and improve cellular immune function in FEL. Following this treatment, reduction in plasma inhibitory activity, reversal of depressed antigen-specific lymphocyte proliferative responses and monocyte antibody-dependent cytotoxic function in vitro, and clinical improvement were complete in two and partial in one of three patients studied. Relapse, which was ultimately fatal, was associated with recurrence of the immune defects. These findings suggest that cellular immunodeficiency in FEL is acquired and possibly related to circulating immunosuppressive activity, the removal of which is associated with transient immunologic and clinical recovery.

Familial erythrophagocytic lymphohistiocytosis (FEL) is a usually fatal disease with an autosomal recessive pattern of inheritance. Neither its direct etiology nor pathogenesis is known. Furthermore, no biochemical or other specific marker of FEL has been identified, although hyperlipidemia has now been found to be a consistent feature of clinically severe FEL.

The clinical presentation and the occurrence of frequently fatal infections led to the suggestion that immunodeficiency could be a component of the syndrome of FEL. A consistent pattern of cellular immune defects has recently been documented and includes depressed lymphocyte proliferative responses and defective monocyte-mediated antibody-dependent cytotoxicity (MMADCC). These defects are accompanied by an inhibitory effect of patient plasma on normal lymphocyte proliferation in vitro, suggesting that presence of an abnormal plasma factor with immunosuppressive activity might be in part responsible for clinical immunodeficiency in FEL. We have therefore assessed the effects of repeated plasma or blood exchange on in vitro immune function and on the clinical course of FEL.

MATERIALS AND METHODS

Patients

Patient 1 was the 5-yr-old son of nonconsanguineous parents. His presenting symptoms and signs included fever, skin rash, irritability, seizures, hepatosplenomegaly, and mild pancytopenia. The diagnosis of FEL was confirmed on liver biopsy, which showed marked infiltration by erythrophagocytic macrophages of benign morphology. The patient's 1.5-yr-old brother died 8 wk after the almost simultaneous onset of similar symptoms. Patient 1 was transferred to UCLA, at which time his plasma triglyceride level was 413 mg/dl and cholesterol 239 mg/dl, with a type IV lipoprotein electrophoretic pattern (elevated pre-beta fraction). Plasmapheresis/exchange therapy was instituted after informed consent for this procedure, approved by the UCLA Human Subject Protection Committee, had been obtained.

Patient 2 (treated at the University of Calgary) presented at 5 mo of age. The parents, unrelated and of Scottish descent, had had another son who died several years earlier at 15 mo of age of an illness diagnosed as malignant histiocytosis. Initial symptoms and findings in patient 2 were irritability, intermittent fevers, and mild pancytopenia. The plasma triglyceride concentration was > 500 mg/dl, cholesterol 268 mg/dl, and lipoprotein electrophoresis also showed a type IV pattern. Treatment with a course of chemotherapy (vinblastine, prednisone, methotrexate, and cyclophosphamide) resulted in a brief clinical response. One month later he developed Pneumocystis carinii pneumonia and required ventilatory assistance during a prolonged hospitalization. Relapse of FEL was diagnosed. Severe gastrointestinal hemorrhage led to administration of a large-volume irradiated blood transfusion, following which marked clinical improvement was noted. With parental consent, exchange therapy was continued.

Patient 3 (treated at San Diego Children's Hospital) was the 4-yr-old sister of patient 1. She developed symptoms similar to those of her brother 9 mo after his death. Plasma lipid levels included a triglyceride of 225 mg/dl, cholesterol of 180 mg/dl, and an elevated pre-beta fraction on lipoprotein electrophoresis. She was treated with multiple plasma exchanges.

Cell Preparation

Whole blood from patients and normal control donors was collected in preservative-free heparin (50 U/ml) and the plasma separated by centrifugation. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation.
gation\textsuperscript{19} and washed 3 times before use in the cytotoxicity and proliferation assays. Both patient and control PBM cell suspensions contained \(<2\%\) granulocytes, \(70\%-80\%\) lymphocytes, and \(20\%-30\%\) monocytes, identified by nonspecific esterase staining of cytocentrifuge preparations.\textsuperscript{11}

**Monocyte-Mediated Antibody-Dependent Cellular Cytotoxicity (MMADCC)**

MMADCC, assessed as previously described,\textsuperscript{12,13} measures the monocyte-mediated lysis of \(^{51}\text{Cr}\)-labeled human type B erythrocytes in the presence of sensitizing human hyperimmune anti-B antiserum (Dade Laboratories, Miami, Fla.). The antiserum was confirmed to be selective, mediating ADCC by monocytes but not by lymphocytes.\textsuperscript{13} Cytotoxic activity of monocytes is expressed as the calculated number of erythrocyte targets lysed per monocyte, in a 20-hr assay, as determined from specific \(^{51}\text{Cr}\) release and the number of monocytes in the effector cell population.\textsuperscript{12} The cytotoxic activity of monocytes from normal donors was \(1.8 \pm 0.6\) targets lysed/monocyte (mean \(\pm\) SD, \(n = 32\)).

**Lymphocyte Proliferative Responses and Plasma Inhibitory Activity**

Proliferative responses of patient PBM cultured in normal control plasma, and the inhibitory effects of patient plasma on the proliferative responses of normal PBM, were quantitated using published methods.\textsuperscript{5,14} All conditions, including cell and stimulant concentrations and culture duration, were selected to result in optimal \(^3\text{H}\)-thymidine uptake by normal PBM. The duration of the cultures was 3 days when the mitogens phytohemagglutinin (PHA), concanavalin-A (Con-A), and pokeweed mitogen (PWM) were used as stimulants, and 6 days when the soluble specific antigens streptokinase-streptodornase (SKSD), Candida antigen, or tetanus or diphtheria toxins were used. The final concentration of plasma in culture was \(7.5\%\) \(\pm\) unless otherwise specified. Mean net \(^3\text{H}\)-thymidine uptake in triplicate stimulated cultures was determined by subtracting the mean cpm of unstimulated cultures. Inhibitory activity of patient plasma was quantitated by determining the mean change in proliferative responses of normal PBM to the four soluble specific antigens listed above, comparing cultures containing patient versus control normal plasma. The mean response of normal PBM cultured in allogeneic normal plasma was \(95\% \pm 35\%\) (mean \(\pm\) SD, \(n = 12\)) of their response when cultured in autologous plasma. Therefore, we defined a patient plasma to be inhibitory if it reduced the proliferative response of normal PBM to less than \(60\%\) of that observed in autologous normal plasma.

**RESULTS**

**Clinical Observations**

**Patient 1**

This child, who never received cytotoxic chemotherapy or steroid therapy, had 6 exchanges over a 5-wk period, including 3 plasma exchanges, 2 plasmaphereses, and 1 exchange transfusion with irradiated whole blood. The exchange transfusion, his second procedure, was undertaken because of unavailability of the plasmapheresis apparatus. Gradual improvement in his clinical condition allowed transfer to his local hospital and subsequent discharge in clinical remission. Two months later he again presented with fever, hepatosplenomegaly, pancytopenia, and hyperlipidemia, all of which had resolved during his previous admission. The diagnosis of relapse of FEL was made, and he had 4 plasma exchanges in a 3-wk period. However, his clinical condition continued to deteriorate and he died 4 wk after his second admission. The immediate cause of death was identified as disseminated \textit{C. albicans} infection. He had survived 8 mo from the time of diagnosis of FEL.

**Patient 2**

After initial chemotherapy and subsequent relapse of FEL, patient 2 was treated with 3 two-volume exchange transfusions in 4 wk, using fresh-frozen plasma and irradiated packed erythrocytes. Plasmapheresis or plasma exchange therapy could not be used because the technology for plasmapheresis of young infants has not yet been developed. He was discharged from the hospital in clinical remission. A second relapse 1 mo later was effectively treated with 2 exchange transfusions. Several weeks later, however, he again relapsed. This time he did not respond to exchange therapy, which was eventually associated with febrile transfusion reactions. Death was caused by \textit{C. albicans} septicemia 7 mo after the diagnosis of FEL.

**Patient 3**

This patient, like her brother (patient 1), did not receive either steroid or cytotoxic chemotherapy. She was treated with 14 plasma exchanges administered

| Table 1. Immunologic Parameters Unaffected in Patients 1–3 |
|---------------------------------|----------------|----------------|----------------|
|                                 | Patient 1     | Patient 2     | Patient 3     |
| Total white blood cell count/cu mm (> 3000)* | 3,400         | 6,000         | 5,200         |
| Absolute lymphocyte count (> 1500) | 1,734         | 4,260         | 2,028         |
| % T cells (55–70) | 57             | 68             | —             |
| % B cells (5–15) | 9              | 6              | —             |
| Immunoglobulin levels (mg/ml)† |                |                |               |
| IgG                 | 11.8           | 3.4            | 9.7           |
| IgA                 | 3.0            | 0.35           | 1.1           |
| IgM                 | 0.8            | 0.65           | 2.0           |
| Mitogen-induced lymphocyte proliferative responses (net cpm \(\times 10^{12}\)) |          |                |               |
| PHA (> 50) | 94             | 79             | 72            |
| Con-A (> 15) | 22             | 35             | 16            |
| PWM (> 15) | 29             | 30             | 26            |

*Normal values.
† All immunoglobulin levels are normal for age.\textsuperscript{16}
twice weekly for 7 wk. Reduced fever, decreased hepatosplenomegaly, clinical improvement, and almost complete normalization of previously elevated liver function tests occurred during the first month of therapy. However, her disease subsequently progressed rapidly, and she died of cardiorespiratory arrest 9 wk after diagnosis of FEL. *C. albicans* was cultured from lung tissue obtained postmortem.

Fig. 1. Sequential study of MMADCC in 3 patients with FEL who received multiple plasma/blood exchanges. Horizontal bars show periods of treatment; vertical bars, patient monocyte antibody-dependent cytotoxicity over the time course indicated on the abscissa.
Immunologic Studies

Studies performed prior to treatment that were found to be normal are summarized in Table 1. All three children had normal numbers of circulating lymphocytes, and both children tested had normal percentages of T and B cells. Immunoglobulin levels were normal for age.15 Proliferative responses of lymphocytes stimulated by the nonspecific mitogens PHA, Con-A, and PWM, when tested in control normal plasma, were intact in all three children at diagnosis (Table 1) and when tested on subsequent occasions (not shown).

Monocyte Effector Function

Monocyte cytotoxic effector function, tested in the MMADCC assay, was depressed prior to treatment in all three patients (Fig. 1). During exchange therapy of patient 1, MMADCC improved rapidly and remained at normal levels (>1.2 targets lysed/monocyte) throughout the first hospitalization and after discharge. MMADCC was again low (0.3 targets lysed/monocyte) at the time of relapse and did not recover significantly prior to the child’s death (Fig. 1, top panel). MMADCC of patient 2, assessed during and after completion of the first treatment with exchange therapy, was also normal (Fig. 1, center panel). When he was seen for a follow-up visit 1 mo after discharge, however, the level of MMADCC had fallen to 1.1 targets lysed/monocyte. The next week recurrence of symptoms indicated relapse of FEL. Three exchange transfusions were administered over a 2-wk period, during which time a twofold increase in MMADCC activity, to 2.3 targets lysed/monocyte, was documented. No blood sample for study was obtained at the time of diagnosis of the patient’s final relapse, but cytotoxic function was documented to be normal during retreatment with exchange transfusions. Cells of patient 3 also showed improved MMADCC during plasma exchange therapy (Fig. 1, lower panel).

These results suggest an association between multiple plasma or blood exchanges and recovery of monocyte cytotoxic function in patients with FEL and show that this defect in cytotoxic function is reversible in vivo.

Patient Antigen-Specific Lymphocyte Proliferative Responses

The proliferative responses of patient PBM to two specific antigens to which all three patients had been previously immunized (diptheria and tetanus toxoids) were tested. These studies were conducted in normal plasma to eliminate possible inhibitory effects of patient plasma in culture as a cause of low proliferative responses of patient PBM. This point is of importance because in comparison to their responses in normal control plasma, the proliferative responses of patient PBM were even further depressed when inhibitory patient plasma was substituted for control plasma (not shown).

Prior to treatment, PBM of all three patients had depressed responses to these two antigens. During clinical remission following the first course of therapy of patients 1 and 2, these lymphoproliferative responses increased 5–10-fold. Although patient 3 did not achieve complete clinical remission, her PBM exhibited an improved response to one of the two antigens, diptheria toxoid (Fig. 2A) on the one occasion during therapy that these proliferative responses were studied. Thus, like monocyte effector function, proliferative responses of lymphocytes to stimulation by specific antigens improved in parallel with the onset of clincial response associated with exchange therapy. The results also demonstrate that the depression of this function is reversible in vivo. Depression of antigen-specific lymphoproliferative responses of patients 1 and 2 was again noted at the time of terminal relapse (Fig. 2) and did not recover significantly during these terminal relapses (not shown).
Plasma Inhibitory Activity

Plasma obtained from all three patients prior to treatment inhibited proliferation of normal PBM stimulated by the four soluble specific antigens listed in Materials and Methods by 80%–99% (Fig. 3). This effect was not due to direct cytotoxicity, since compared with culture in control plasma, culture of unstimulated normal PBM in patient plasma for 6 days did not reduce PBM number or cell viability assessed by trypan blue dye exclusion.

Following completion of the first series of exchanges, plasma of patients 1 and 2 was no longer inhibitory to lymphoproliferation of normal PBM; 76% and 77% of the responses obtained using control normal plasma were seen. A lesser effect was noted during exchange therapy of patient 3, whose plasma never demonstrated complete loss of inhibitory activity. At relapse, plasma of both patients 1 and 2 again exhibited increased inhibitory activity (Fig. 3), which did not significantly diminish during treatment of these ultimately terminal relapses (not shown). These results suggest that exchange therapy had a transient but not definitive effect on plasma immunosuppressive activity in FEL.

To further delineate the effect of exchange therapy on plasma inhibitory activity, four pairs of pre- and post-exchange samples were obtained during the first course of exchange therapy of patient 1 and tested for their effect on proliferative responses of normal PBM (Fig. 4). Early plasma samples were markedly inhibitory, while shortly after the institution of therapy the inhibitory activity of patient plasma began to diminish. Little inhibitory activity was detectable after the fifth exchange, 1 mo later.

Characteristics of Plasma Inhibitory Activity

Experiments were performed to confirm that the observed depression of proliferative responses of normal PBM cultured in patient plasma reflected a truly inhibitory effect rather than lack (in patient plasma) of some plasma constituent that is normally present and is essential for lymphoproliferation. Little change in the proliferative responses of normal PBM cultured in normal plasma was observed when the concentration of plasma was varied between 1.5% and 7.5% v/v (Fig. 5). In contrast, increasing the concentration of patient
Effects of repeated blood exchange in FEL

Fig. 5. Effect of plasma concentration on proliferative response of normal PBM. Each point represents net \(^{3}H\)-thymidine uptake by normal PBM stimulated by Candida antigen (■, □) or tetanus toxoid (●, ○) when cultures contained various concentrations of control normal (closed symbols) or patient 1 (open symbols) plasma.

plasma caused a progressive decrease in proliferation of these same PBM (Fig. 5). In other experiments, the addition of 1.5% normal plasma (alone capable of supporting lymphoproliferation, as seen in Fig. 5) did not reverse the inhibition of proliferation of normal PBM caused by culture in 6% v/v patient plasma (98% inhibition in patient plasma alone versus 95% inhibition when 1.5% normal plasma was also added to the cultures). Together, these data confirmed that an inhibitory activity, not lack of an essential plasma factor, characterized patient plasma.

Reduced \(^{3}H\)-thymidine uptake by stimulated normal PBM cultured in patient plasma was not due to cold thymidine competition. This possibility, which would have suggested that high concentrations of (unlabeled) thymidine were present in FEL plasma or were produced in culture by the exposure of normal PBM to patient plasma, was excluded by showing that (i) net \(^{3}H\)-thymidine uptake by PBM cultured in 7.5% patient plasma and tetanus toxoid for 6 days was not improved by washing the cells (to remove the patient plasma) and resuspending them in medium containing normal plasma just before the addition of \(^{3}H\)-thymidine (2.0 versus 2.8 \(\times\) 10^3 net cpm/culture, washed versus unwashed cells); and (ii) cultures stimulated with tetanus toxoid and incubated in normal plasma that was similarly replaced with patient plasma just before addition of \(^{3}H\)-thymidine exhibited no reduction in \(^{3}H\)-thymidine uptake (53.5 versus 53.7 \(\times\) 10^3 net cpm/culture, washed versus unwashed cells).

Other studies were performed to prove that the inhibition of \(^{3}H\)-thymidine uptake (96% in the above experiment) reflected actual inhibition of lymphoproliferation by FEL plasma. The number of viable cells in cultures exposed to tetanus toxoid was quantitated at the end of the 6-day culture period. When the cultures contained 7.5% normal plasma, the cell count of the stimulated cultures was 219% of that of parallel unstimulated cultures. In contrast, essentially no change in cell number was observed in cultures containing 7.5% patient plasma (cell count of stimulated cultures was 94% of that of unstimulated cultures). These results documented inhibition of lymphocyte proliferation by FEL plasma by the ultimate criterion—lack of increase in cell number.

Immune complexes were not detected in patient plasmas using Clq binding,\(^{16}\) conglutinin,\(^{16}\) and polyethylene glycol assays.\(^{17}\) Likewise, ultracentrifugation of patient plasma, which sediments formed immune complexes, did not eliminate the inhibitory activity. These findings suggest that immune complexes were not responsible for the inhibitory activity. Other experiments showed that the inhibitory activity was not dialyzable using tubing with a molecular weight cutoff of 12,000 daltons, nor was it reduced by heating the plasma to 56°C for 30 min.

Finally, the previously noted correlation between hypertriglyceridemia and plasma inhibitory activity suggested that this activity might reflect an effect of elevated plasma lipid levels. However, when the chylomicrons and very low density lipoproteins, which are rich in triglycerides, were separated from patient plasma by ultracentrifugation,\(^{18}\) the inhibitory activity was retained by the plasma fraction depleted of chylomicrons and VLDL (not shown).

Discussion

Familial erythrophagocytic lymphohistiocytosis is a rare and usually fatal autosomal recessive disease. The prominent pathologic finding in FEL is the widespread accumulation of cells of the monocyte/macrophage series demonstrating striking hemophagocytosis. This accumulation of cells, assumed to represent uncontrolled proliferation, has frequently resulted in the consideration of FEL as a malignancy, even though these cells have normal and not malignant morphological characteristics.\(^{19}\) With few reported exceptions,\(^{6,20,21}\) cytotoxic and steroid therapy have been ineffective in the treatment of FEL.\(^{19}\)
The clinical course of FEL, with wasting, irritability, poor appetite, and frequent infections, resembles that of some primary childhood immunodeficiency syndromes, such as severe combined immunodeficiency disease. Recently, we documented a specific pattern of immunodeficiency in FEL. Pretreatment assessment of immune function in the present three patients gave results consistent with these previous findings. General measures of immunocompetence, including lymphocyte count and percentages of T and B cells, immunoglobulin levels, and the proliferative responses of patient PBM (cultured in normal plasma) to stimulation by non-specific mitogens were normal. Cellular immune defects in the present and previously studied patients included depressed MMADCC and lymphoproliferative responses to specific antigens. Finally, plasma obtained prior to treatment from each of the three patients inhibited the lymphoproliferative responses of normal PBM.

The inhibition of lymphoproliferation by plasma obtained from patients with FEL suggested a therapeutic approach to this disease. Would removal of an abnormal plasma factor with immunosuppressive activity by repeated blood exchange alleviate the immunodeficiency and ameliorate the clinical course of FEL? Such effects might be expected if circulating immunosuppressive activity were in part responsible for the clinical immunodeficiency and if this immunodeficiency significantly contributed to the clinical deterioration seen in children with FEL.

Marked reduction in plasma inhibitory activity was observed following initial exchange therapy in two of the three patients (Fig. 3). In addition, monocyte effector function recovered (Fig. 1) and the proliferative responses of patient lymphocytes to specific antigens improved (Fig. 2). These results suggest that in FEL the cellular immune defects are acquired rather than intrinsic.

Coincident with the improvement in immune function assessed in vitro, transient but complete clinical recovery was observed in two patients, and a partial response occurred in the third patient. The survival of patients 1 and 2 (8 and 7 mo) was prolonged compared with the reported average survival of 6 wk from the onset of symptoms, but their lack of response during the final fatal relapses suggests that exchange therapy cannot be considered a fully effective long-term approach to the treatment of FEL. It is possible, however, that more intensive or longer initial therapy or the continuation of treatment during remission could have prevented or delayed these relapses.

The cause of the inhibitory activity of FEL plasma has not yet been elucidated. We thought very low density lipoproteins (VLDL) might be responsible for this activity for the following reasons. First, VLDL are rich in triglyceride, and hypertriglyceridemia is a characteristic finding in FEL that we and others have noted to resolve during remission. Second, VLDL and plasmas in which they are elevated (as in familial type IV hyperlipoproteinemia) inhibit normal human lymphoproliferation. However, since patient plasma from which VLDL had been removed by ultracentrifugation retained the inhibitory activity, factors other than VLDL must be responsible for the plasma-mediated inhibition.

Why the cellular immunodeficiency in FEL is reversible in vivo but not in vitro is also an unsolved question. One possible explanation is that unresponsiveness of patient cells in vitro may have been caused by prolonged exposure in vivo to an immunosuppressive factor that we quantitated by its inhibitory effect on lymphoproliferation. If this were true, improvement in cellular immune function associated with exchange therapy might reflect the generation of new cells or the functional recovery of previously inhibited immunocytes, once plasma inhibitory activity had been reduced or eliminated. This potential link between cellular immune defects and plasma-mediated inhibition of lymphoproliferation in FEL suggests that further studies to characterize the plasma factor(s) involved should be pursued.

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