Transfusion-associated GVHD after fludarabine therapy in a patient with systemic lupus erythematosus

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BACKGROUND: Fludarabine, a purine antimetabolite with potent immunosuppressive properties, has previously been associated with the development of transfusion-associated GVHD (TA-GVHD) in patients with hematologic malignancies. Its role as a risk factor for TA-GVHD in patients without underlying leukemia or lymphoma is uncertain.

STUDY DESIGN AND METHODS: A 42-year-old female with refractory lupus nephritis received three monthly cycles of fludarabine (30 mg/m^2/day on Days 1-3) and cyclophosphamide (500 mg/m^2 on Day 1). Three months after the last dose of fludarabine, she received 2 units of packed RBCs and 6 units of pooled random platelets, none of which were irradiated. Two weeks later, fever, rash, aminotransferase elevations, hyperbilirubinemia, and pancytopenia developed.

RESULTS: Marrow biopsy showed severe aplasia and skin biopsy was consistent with GVHD. Allele-level HLA typing on circulating lymphocytes revealed extra HLA alleles not present in her pretreatment sample, but identical to the HLA haplotypes of an unrelated platelet donor. Treatment with antithymocyte globulin, cyclosporine, and prednisone was followed by preparatory conditioning for a PBPC transplant from an HLA-identical sibling, but the patient died of disseminated candidiasis before transplant.

CONCLUSIONS: Fludarabine and other purine analogs are increasingly used in the treatment of disorders other than hematologic malignancy, such as autoimmune disease. The occurence of TA-GVHD after fludarabine therapy in a patient with lupus strongly suggests that this drug is sufficiently immunoablative to be an independent risk factor for TA-GVHD. Irradiation of blood components should be considered in all patients who receive fludarabine therapy.

Transfusion-associated GVHD (TA-GVHD) is a rare but devastating complication of transfusion. It is mediated by immunocompetent allogeneic lymphocytes present in cellular blood products, which can engraft, proliferate, and mount a severe immunologic reaction against the host.[1] Risk factors for the development of TA-GVHD are well defined and include a state of profound immunodeficiency in the host, due either to leukemia, lymphoma, congenital immunodeficiency disorders, the neonatal state, or to the effects of potent cytotoxic and immunosuppressive drugs given as part of hematopoietic transplant conditioning regimens.[2] Transfusion of HLA antigen haplotype-homozygous blood to an immunocompetent recipient who is heterozygous for that haplotype, such as a family member, may also incite the reaction.[3] TA-GVH reactions are almost invariably fatal, and thus attention has focused on prospective recognition of patients at risk. Irradiation of blood components eliminates the mitotic potential of passenger lymphocytes and prevents TA-GVHD.[1] Thus, identification of new risk factors for TA-GVHD is critically important to allow the irradiation of blood components in the indicated setting. Universal irradiation of blood components has not been recommended due to a small but significant

ABBREVIATIONS: CLL = chronic lymphocytic leukemia; SLE = systemic lupus erythematosus; TA-GVHD = transfusion-associated GVHD.

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TABLE 1. Prior reports of TA-GVHD in association with fludarabine therapy

Reference	Unique patient number	Diagnosis*	Age (years)	Prior therapy	Cycles of fludarabine	Implicated component	Interval between flu and GVHD	Outcome
Maung et al., 1994	1	B-CLL	61	chlorambucil	1	RBCs	NS	Death
	2	B-CLL	47	chlorambucil	4	NS	1 month	Death
	3	B-CLL	59	chlorambucil	4	NS	NS	Death
Briz et al., 1995	4	B-CLL	61	chlorambucil COP, CHOP†	6	6 U RBCs 6 U PC‡	Several months	Death
Williamson et al., 1996	5	B-CLL	62	COP, CHOP	4	PC	11 months	Death
	6	B-CLL	59	chlorambucil	5	RBCs	10 days	Death
	7	B-CLL	64	chlorambucil	2	PC	1 month	Death
Deane et al., 1997	8	AML	20	daunarubicin AraC, TG§	3	NS	NS	Death
Zelig et al., 1999	9	B-NHL	67	CHOP, XRT¶	4	2 U RBCs	Several months	Death

* B-CLL = B-cell chronic lymphocytic leukemia; AML = acute myelogenous leukemia; B-NHL = B-cell non-Hodgkin's lymphoma.
† COP = cyclophosphamide, vincristine, prednisone; CHOP = cyclophosphamide, vincristine, doxorubicin, prednisone.
‡ PCs = platelet concentrates.
§ AraC = cytosine arabinoside; TG = thioguanine.
¶ XRT = radiation therapy.

decrease in in vivo RBC recovery after RBC storage in the irradiated state.[4]

Fludarabine is a synthetic purine antimetabolite, a fluorinated analog of adenine that is relatively resistant to deamination by adenosine deaminase.[5] It blocks DNA synthesis in dividing cells through inhibition of ribonucleotide reductase and hinders DNA repair mechanisms in resting cells through inhibition of DNA polymerase alpha.[5] Fludarabine causes profound lymphopenia, depleting T cells more markedly than B cells. Sustained reductions are seen in all T cell subsets, particularly in CD4 helper cells.[6] Fludarabine has potent activity against lymphoid malignancies, particularly chronic lymphocytic leukemia (CLL), where it is considered the drug of choice for salvage and, in many centers, initial therapy.[7,8] The potent immunosuppressive properties of fludarabine have recently led to its use as an investigational agent in patients with refractory autoimmune disease.[9,10]

Fludarabine has been previously associated with TA-GVHD in nine patients, all of whom had hematologic malignancies and seven of whom had CLL (Table 1).[11-15] No prior cases of TA-GVHD have been reported in CLL in the absence of fludarabine therapy, suggesting that the drug confers a risk for TA-GVHD in this setting. The role of fludarabine as a possible risk factor for TA-GVHD in patients without underlying leukemia or lymphoma remains unknown. We report a case of fatal TA-GVHD in a patient with systemic lupus erythematosus (SLE) receiving fludarabine and low-dose cyclophosphamide.

MATERIALS AND METHODS

Patients

Patients with moderate to severe lupus nephritis, refractory to other therapies, were enrolled in an approved investigational protocol. Written informed consent was obtained from all patients. Therapy consisted of monthly cycles of fludarabine 30 mg per m^2 given subcutaneously on Days 1 through 3, cyclophosphamide 500 mg per m^2 orally on Day 1, and prednisone orally on a tapering schedule. Three patients had completed five or six cycles of therapy and had experienced only the expected toxicities of this regimen, including severe but reversible neutropenia. None had required blood transfusions. The fourth patient to be treated on this protocol is described below.

CASE REPORT

A 42-year-old female was diagnosed with SLE in 1991. She received 16 g of pulse cyclophosphamide from 1996 to 1998 for diffuse proliferative nephritis but failed to achieve a sustained remission. She was referred to NIH and enrolled in the current study. Two weeks after completing the third monthly cycle of fludarabine and cyclophosphamide, Grade IV myelotoxicity occurred and further treatment was discontinued. Her WBC count improved after G-CSF administration, but moderate anemia and thrombocytopenia persisted. Plateletpheresis concentrates were given on Days 20 and 27, 2 units of RBCs on Day 88, and 6 units of pooled random platelets on Day 90 after the last dose of fludarabine. The RBCs, but not the platelets, were leukoreduced by filtration; none of the components was irradiated. Ten days after the last transfusions, fever, diffuse rash, aminotransferase elevations, elevated bilirubin, and pancytopenia developed. Marrow biopsy showed severe aplasia, and skin biopsy was consistent with GVHD. Treatment with G-CSF, antithymocyte globulin, and cyclosporine was followed by high-dose cyclophosphamide (60 mg/kg/day for 2 days) and fludarabine (25 mg/m^2/day for 5 days) in preparation for a PBPC transplant from an HLA-matched sibling. However, she

TABLE 2. Clinical course after fludarabine administration

	Cycle 1		Cycle 2		Cycle 3							
	D1	D7	D1	D7	D1	D7	D14	D20-7	D33	D90	D102	D116
WBC/μL	8010	3300	6630	1800	1910	300	2100	5900	2005	2650	781	25
Neut/μL	5520	2700	5090	1044	1290	42	1911	na	700	1960	565	10
Lymphocytes/μL	1520	363	560	400	350	210	105	na	620	477	31	10
Hb (g/dL)	10.9	10.2	10.8	9.9	12.2	11.1	8.5	8.4	8.2	9.2	10.8	7
Platelets (10^3/μL)	378	290	318	248	159	125	25	6	44	20	13 930	11 878
ALT (IU/L)	31							△△		↑↑		
Fludarabine (mg/m^2)	30 × 3		30 × 3		30 × 3			platelets		RBCs ▲▲▲ ▲▲▲	25 × 5	
CY (g/m^2)	0.5		0.5		0.5					platelets	120 mg/kg	
Symptoms									Fatigue, dyspnea, diarrhea			Fever, rash Death

△ = plateletpheresis concentrate; ↑ = RBC unit; ▲ = random-donor platelet unit; neut = neutrophil; CY = cyclophosphamide.

TABLE 3. HLA typing of patient and 10 blood donors

Day**	Component† or sample	LR‡	HLA-A		HLA-B		HLA DRβ1		DQβ1		DRβ	
–60	Patient before fludarabine	–	*24	*33	*5001	*78	*0701	*1301	*02	*06	3*01	4*01
+120	Patient during GVHD	–	*24, ***02**	*33	*5001, ***13**	*78, ***44**	*0701, ***04**	*1301	*02	*06, ***03**	3*01	4*01
+20	PC	No	02	02	51	60	–	–	–	–	–	–
+27	**PC**	No	02	32	44	60	–	–	–	–	–	–
+88	**PRBC**	Yes	*02	*2301	*15	*4501	*04	*0901	*02	*03	–	4*01
+88	**PRBC**	Yes	*02	*02	*08	*15	*03	*11	*02	*03	3*01	3*02
+90	PLT	**No**	***02**	***02**	***13**	***44**	***04**	***07**	***02**	***03**	**–**	**4*01**
+90	PLT	No	*01	*2301	*08	*14	*01	*13	*05	*06	3*02	–
+90	PLT	No	*01	*24	*55	*57	*07	*11	*03	*03	3*02	4*01
+90	PLT	No	*01	*26	*18	*55	*11	*11	*03	–	3*02	–
+90	PLT	No	*02	*3101	*15	*40	*04	*04	*03	–		4*01
+90	PLT	No	*02	*24	*27	*40	*04	*15	*03	*06	4*01	5*01

** Day = day relative to third cycle of fludarabine.
† PC = single-donor plateletpheresis concentrate; PLT = random-donor platelet unit.
‡ LR = leukoreduced by filtration.

died of disseminated candidiasis with sepsis and multiorgan system failure before receiving a PBPC infusion. Her course is shown in Table 2.

Measurements

Allele-level HLA typing using sequence-specific primers was performed on circulating lymphocytes obtained from the patient at study entry (before fludarabine) and at the onset of the GVHD reaction, on a sample from her sibling, and on samples from the 10 donors whose blood she had received in the period between fludarabine treatment and the onset of the GVHD reaction.

RESULTS

HLA typing

HLA typing of the patient's circulating lymphocytes, performed early in the course of the GVHD reaction, revealed additional HLA alleles not present in her prefludarabine sample. These alleles were identical to those of an unrelated platelet donor (Table 3). A nonleukoreduced, single-unit platelet concentrate from this donor had been transfused 90 days after the last dose of fludarabine and 10 days before the onset of GVHD symptoms. The patient shared an HLA DRβ1 and a DQβ1 epitope with this donor, but she also shared DRβ1 and DQβ1 epitopes with 5 other donors, whose cells were not circulating in her blood at the time of the GVHD reaction. None of the 10 involved donors was HLA homozygous and none shared an HLA haplotype with the patient.

Autopsy findings

Autopsy demonstrated invasive infection with yeast forms and pseudohyphae in the brain, lungs, kidneys, and throughout the small intestine, which were identified as *candida albicans*. The skin, lung, liver, and kidneys

showed fibrin microthrombi consistent with disseminated intravascular coagulation. The lungs revealed diffuse consolidation, hyaline membrane formation, and alveolar hemorrhage, with viral inclusions identified as respiratory syncytial virus. The marrow was aplastic and marked lymphoid depletion was seen in the lymph nodes, liver, and spleen.

DISCUSSION

Fludarabine and other purine analogs constitute a class of potent immunosuppressive medications that are increasingly used in the treatment of malignant and nonmalignant disorders. Profound, sustained lymphopenia is a predictable effect of these agents, affecting both T and B cells.[16] A greater than 90-percent decrease in circulating B cells and a 70-percent reduction in CD4+ T cells were observed after fludarabine therapy in patients with rheumatoid arthritis.[17] The number of CD20+ B cells returned to 73 percent of baseline at 1 year, whereas the recovery of CD4+ T cells was more prolonged, with less than 50 percent recovery at 1 year.[17]

Patients at risk for TA-GVHD are characterized by profound T-cell immunosuppression, due either to their underlying disease or to the effects of immunoablative drug regimens. Before the current case report, TA-GVHD had been described in association with fludarabine therapy in nine patients: seven with CLL, one with non-Hodgkin's lymphoma, and one with acute myelogenous leukemia.[11-15] Patients with lymphoma and acute leukemia are known to be at risk of TA-GVHD, but the reaction had not been previously described in patients with CLL. Immune dysfunction in B-cell CLL is due to hypogammaglobulinemia rather than T-cell suppression, and CLL in itself had not been considered a risk factor for TA-GVHD.

The occurrence of TA-GVHD in CLL subjects has aroused substantial controversy. In one institution, three of the first eight B-cell CLL patients receiving fludarabine developed lethal TA-GVHD reactions.[11] However, in a large multicenter study of fludarabine in CLL, none of 100 patients, half of whom had received prior therapy, was noted to develop this complication. In the latter study, the number of patients receiving transfusions was not given, but 14 percent of all subjects developed Grade III/IV thrombocytopenia and were likely to have needed platelet support.[7] In another center, 20 of 42 patients treated with fludarabine for CLL were transfused with a mean of 23 nonirradiated blood products, and none developed TA-GVHD after a mean of 10 months of follow-up.[18] However, the clinical manifestations of TA-GVHD may mimic other conditions, and the diagnosis is likely to be overlooked in the absence of a high index of suspicion. Concomitant or prior therapy with alkylating agents may confer additional GVHD risk. A synergistic cytotoxic effect of cyclophosphamide and fludarabine has been suggested in laboratory as well as in clinical studies.[19] It is therefore of particular interest that all CLL patients reported to develop TA-GVHD, as well as the patient described in this report, received alkylating agents (chlorambucil or cyclophosphamide) in addition to fludarabine. Nonetheless, the number of cases of fludarabine-associated TA-GVHD reported in Britain was of sufficient concern for the Blood Transfusion Task Force of the British Committee on Standards in Hematology to recommend prophylactic irradiation of blood components given to all patients who have received fludarabine.[20]

The current case may serve to resolve some of this controversy. The occurence of TA-GVHD after fludarabine therapy in a patient with SLE strongly suggests that fludarabine is sufficiently immunoablative to be an independent risk factor for this reaction. Prior and concomitant cyclophosphamide therapy may have compounded the risk in our patient. This is the first report of TA-GVHD after use of a nonmyeloablative agent in an adult patient without hematologic malignancy (other than patients receiving HLA-haplotype homozygous blood components).

It is not clear why one particular donor rather than another was implicated in the reaction. Haplotype homozygosity was not present in any donor, and several donors shared Class II HLA alleles with the patient. It is possible that the WBC content, freshness, or storage conditions of the implicated component contributed to the occurence of the GVHD reaction. The platelet unit had not been leukoreduced and would thus be expected to contain 2 to 3×10^8 total lymphocytes; it was relatively fresh as well, having been stored at room temperature for 3 days before transfusion. The fact that the implicated component was administered 3 months after the last dose of fludarabine suggests that the immunosuppressive effects of the drug can be prolonged. In prior reports, cases of TA-GVHD occurred after transfusions given 11 months after the last dose of fludarabine.[15]

Heightened surveillance for cases of TA-GVHD is recommended in all patients receiving fludarabine and other purine analogs, such as deoxycoformycin (pentostatin) and chlorodeoxyadenosine (cladribine), so that a better understanding can be gained of the degree of TA-GVHD risk associated with such therapy. In support of this recommendation, a case of TA-GVHD after cladribine therapy was recently reported in a patient with non-Hodgkin's lymphoma.[21] Until such time as risk can be better quantitated, it seems reasonable to recommend that all cellular blood components given to patients who have received fludarabine be irradiated, regardless of the underlying diagnosis. This precaution should be maintained for at least 1 year, and consideration should be given to continuing this restriction for the patient's lifetime.

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