BONE MARROW TRANSPLANTATION REVISITED

MEDICAL GRAND ROUNDS
PARKLAND MEMORIAL HOSPITAL
AUGUST 25, 1977

CASE PRESENTATION

APLASTIC ANEMIA

- Bone Marrow Transplantation in Identical Twins
- Allogeneic Bone Marrow Transplantation
- Summary of Clinical Results
- Analysis of Factors Associated with Graft Rejection

ACUTE LEUKEMIA

- Identical Twin Bone Marrow Transplantation for Hematologic Neoplasia
- Allogeneic Bone Marrow Transplantation for Acute Leukemia

SUMMARY

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Bone Marrow Transplantation Revisited

Case Summary P.M.H. #54-66-38

JL was a 19 year old white female who was admitted to Parkland Memorial Hospital on 5/25/77 with the chief complaint of easy bruisability. Two weeks prior to admission she had noticed a petechial rash over her lower extremeties. Shortly thereafter purpura, intermittent gingival bleeding, blood streaked sputum and hematochezia appeared. She noted premature initiation of her menstrual period with a very heavy flow. Since the birth of her second child 5 months prior, she had experienced a 40 pound weight loss. She described a recent exposure to parathon and had an episode of flu 3 months prior to admission. Her medication included norinyl, aspirin and occasional acetaminophen. Family history was noncontributory. The patient denied a history of hepatitis or mononucleosis.

On physical examination she appeared as a well developed, well nourished white female in no apparent distress. Numerous petechiae and ecchymosis were noted over the extremeties, back, and trunk. Her vital signs were normal. She had scattered petechiae over her buccal mucosa and under her tongue. Her liver was not enlarged to palpation and there was no splenomegaly. Her stools were guaiac negative.

Laboratory studies revealed a white blood cell count of 5500, with 46 PMNs, one band, 51 lymphocytes, 3 eosinophils. Hemoglobin was 11.1 grams and hematocrit was 31.9. Platelet count was 6000 per cubic millimeter. VDRL was nonreactive as was hepatitis B surface antigen. Serum protein electrophoresis was within normal limits, monospot test was negative as was the sugar water test. Serum iron and iron binding were within normal limits as were other laboratory studies.

Immediately upon admission she was placed on prednisolone therapy and a bone marrow biopsy was obtained. This revealed a hypocellular marrow with absent megakaryocytes, absent erythroid precursors, abundant granulocytes and their mature precursors, but without evidence of promyelocytes or blast forms. Her platelet count fell to 1500 and her hemoglobin, hematocrit and white blood cell count fell progressively over the next several days. The patient received 6 units of platelets 6 days after admission and required another 6 units one week later. During this two week interval her absolute neutrophil count plummeted, reaching a nadir of 700. At this time she spiked a fever to 100.4°F. She was placed on carbenicillin, gentamycin and cephalothin. Within 24 hours she was afebrile; chest x rays and microbiological studies failed to reveal specific evidence of infection. The house staff in consultation with the hematology service considered the possibility of a bone marrow transplantation. The patient and her family were then subjected to HLA tissue typing. The patient had 9 siblings; both parents were available for testing. One sibling, MG, had serologically identical HLA antigens as the patient and was mixed leukocyte culture nonreactive suggesting that the two were HLA haplo-identical. With the identification of a suitable donor, arrangements for bone marrow transplantation were made and the patient was transferred to City of Hope Hospital, California, on June 10, 1977, by air ambulance.

The subject of bone marrow transplantation as a therapeutic measure in clinical medicine was last reviewed at Medical Grand Rounds more than 5 years ago. Since then, considerable change has taken place. This attitudinal transformation is probably best exemplified by the fact that within a few short weeks of the diagnosis of aplastic

anemia in this case, the house staff and consultants had arranged to have the patient prepared for bone marrow transplantation even though the more traditional modes of therapy had not been instituted. In a comparable clinical setting 6 years ago, allogeneic bone marrow transplantation would have been considered only when all else had been tried and failed. The purpose of this seminar is to attempt to evaluate the current status of clinical bone marrow transplantation, to identify the relevant experiences of the past 5 years that have led to this turn around in clinical thinking, to identify the major problems that continue to bedevil the clinician and his patient for whom he proposes to carry out this therapeutic maneuver. For these purposes, I have chosen to consider the experiences and results in only two disorders for which bone marrow transplantation has been employed: aplastic anemia and acute leukemia.

Aplastic anemia

Aplastic anemia is a primary disorder of the hematopoietic bone marrow in which a relative to absolute failure of formation of blood cells occurs. A review of the etiology, pathogenesis, clinical course, prognosis and conventional modes of therapy for this disease is well beyond the scope of this presentation. A relatively recent series of 24 consecutive patients with acquired aplastic anemia were reported. Only 6 patients survived beyond 4-6 months, 5 of whom received anabolic-androgenic steroids. The remainder died of bleedings and/or infection. This exceedingly grim prognosis prompted these authors to state that "an alternative form of therapy must be considered for this group" (1). Since the pathogenesis of this disorder would appear to be a deficiency of cells capable of generating new blood cells, it would seem logical that appropriate treatment would result from taking healthy hematopoietic stem cells from a normal individual and grafting them into the marrow cavity of afflicted persons. However, with the exception of identical twin pairs, bone marrow transplantation between human beings elicits a state of transplantation immunity which, if untreated, overshadows any possible benefits the grafted cells might provide. The problem that donor/host histoincompatibility creates will be discussed more fully later in this presentation, but its consideration should not blot out consideration of other factors, of a nonimmunologic nature, which complicate bone marrow transplantation even between identical twins. It would be appropriate to deal with these matters before going on to the more complicated allogeneic reactivity.

Bone marrow transplantation in identical twins

The factors which influence the outcome of bone marrow transplantation in situations where donor and host are of identical genotype can be categorized as etiologic, mechanical, and clinical. (See Table 1.)

TABLE 1

PROBLEMS ASSOCIATED WITH ISOGENEIC BONE MARROW TRANSPLANTATION FOR APLASTIC ANEMIA

ETIOLOGIC:	STEM CELL VS. HIM DEFICIENCY, DRUG
MECHANICAL:	NO. OF CELLS, METHOD, SUPPORT
CLINICAL:	INFECTION, BLEEDING DIATHESIS, PREVIOUS THERAPY

Precise identification of the etiology of aplastic anemia is impossible for these patients as a group, and usually not possible even for any individual patient. There are of course only two major possibilities: either the deficiency results from a failure of the pluripotent hematopoietic stem cell to continue to proliferate and differentiate into the cellular lines which make up the blood, or it can be ascribed to a deficiency in the hematopoietic inductive microenvironment (H.I.M.) which provides an appropriate milieu for hematopoiesis to occur. In mice, genetic disorders prototypic for each of these possibilities have been described (2,3). A third possibility, less clearly documented or understood, suggests that the aplasia results from an auto-antibody which inhibits hematopoietic precursor cells (4). One might imagine that bone marrow transplantation would offer potential therapeutic benefit only in the situation where the defect resides within the stem cell rather than the H.I.M. Insufficient data exists in man to select between these possible etiologies. Thomas et al. have recently claimed that the relative success with bone marrow transplantation in aplastic anemia suggests that the preponderant type of defect in this disorder resides in the stem cell rather than the environment (5). In most series, only a minority of patients are thought to develop aplastic anemia because of drug or toxin exposure. Obviously, if the exposure is unsuspected and continues, it would limit the possibility of success for an attempted bone marrow transplantation.

Even when donor and patient share the identical genotypes, it remains essential that a sufficient number (sic) of hematopoietic stem cells be administered in order for successful engraftment to take place. The mechanical procedures for bone marrow transplantation have been described before (6,7) and need be only briefly summarized here. The donor is lightly anesthetized or tranquilized; multiple bone marrow aspirations are made from the sternum, and from the anterior and posterior iliac crests. The aim is to transplant 10 to 50 billion nucleated cells of donor marrow. The marrow cells are collected in a sterile plastic bag and when sufficient numbers of cells have been obtained, they are administered to the patient intravenously as though a transfusion of blood. The hematopoietic stem cells [small numbers of which normally circulate in the peripheral blood-(8)] are capable of finding their way through the circulatory system to take up residence in the inductive environment of the bone marrow. Until the graft has established tenure and can produce adequate numbers of erythrocytes, leukocytes and platelets, the patient requires extensive clinical support to tide him over.

The clinical condition of the patient at the time of bone marrow transplantation is a critical determinant of whether or not the procedure will succeed. The case which initiated this Grand Rounds is representative of the problems which must be faced. Severe thrombocytopenia signals a high risk of a fatal bleeding episode, a risk that continues until the grafted marrow can produce sufficient platelets to reconstitute the deficiency. Similarly, the patient with profound granulocytopenia is exceedingly vulnerable to overwhelming sepsis. Successful bone marrow transplantation between identical twins depends heavily upon the clinical state of the patient, especially with regard to serious infection and bleeding propensity either or both of which can be lethal before the graft has taken.

Allogeneic bone marrow transplantation

Besides the problems just enumerated when bone marrow transplantation is attempted between identical twins, a new set of difficulties emerges when donor and host are genetically nonidentical. The tissues of each express unique alloantigens which can elicit a strong state of transplantation immunity threatening the viability of the graft and the integrity of the host. As outlined in Table 2, the grafted tissue

TABLE 2

SPECIAL PROBLEMS WITH ALLOGENEIC BONE MARROW TRANSPLANTATION FOR APLASTIC ANEMIA

HOST-VS-GRAFT RESPONSE: REJECTION

GRAFT-VS-HOST RESPONSE: GVH DISEASE

PRE-EXISTING HOST IMMUNITY (REFRACTORY TO RANDOM PLATELETS)

NEED FOR IMMUNOSUPPRESSION

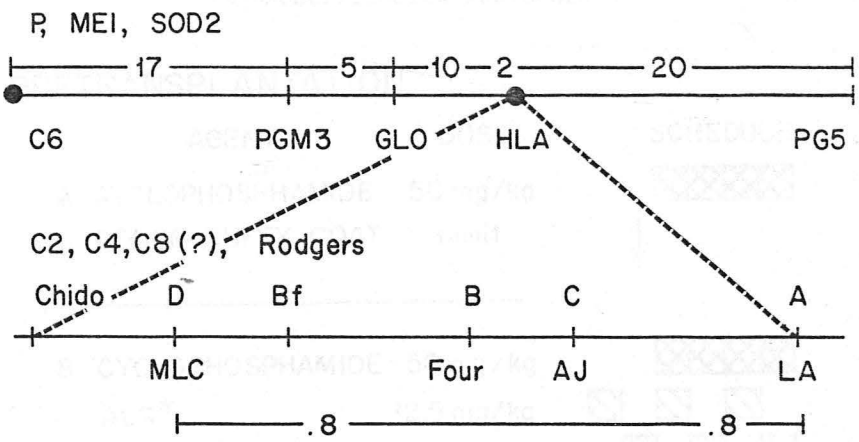
confronts the host with a variety of antigens which stimulate within the host a primary immunologic response, the net result of which is an aggressive destructive reaction aimed at destroying the graft. But, in bone marrow transplantation, the graft is unique in that it possesses immunologic competence in its own right; cells within the graft are able to recognize alloantigenic determinants on host tissues and mount an equally destructive reaction (9).

In an attempt to avoid the serious sequellae of host-versus-graft and graft-versus-host reactions in this situation, two approaches have been utilized: first, to minimize the histoantigenic disparity between donor and host by appropriate tissue typing and second, to utilize immunosuppressive drugs to prevent or blunt the developing immune response. A further complication results from the repeated transfusions of whole blood, leukocytes and platelets which are usually required to maintain the patient in reasonable clinical status. Whether from unrelated donors or genetically related members of the family, these blood products undoubtedly sensitize the patient to antigens which may be present in donor marrow cells. This pre-existent immunity erects a formidable barrier to subsequent bone marrow engraftment (10). Refractoriness to random platelets wherein greater and greater numbers of platelets are required to effect hemostasis in patients with profound thrombocytopenia may be an expression of this sensitization process and auger poorly for a successful marrow transplant.

The absolute number of histocompatibility loci governing transplant immunity in man is unknown. At present, only the major histocompatibility locus, HLA, and the ABO isohemagglutinin system have been definitively implicated in the allotransplant rejection process. After considerable experience with mismatched ABO combinations, Storb and his colleagues (11) and Gale et al. (12) have independently concluded that this important isohemagglutin locus plays little or no role in bone marrow transplant related immunity. This is a rather surprising finding, but the data seems unimpeachable.

All agree that disparity at the HLA complex is of overriding importance, however. Since both donor and host immune potential must be considered in allogeneic bone marrow transplantation, it is essential that HLA identity be established for donor and host. Using alloantisera and mixed lymphocyte cultures, the HLA haplotypes of patient and family must be determined. The only acceptable donor is an HLA identical sibling since partial identity is as hazardous as total nonidentity. Unfortunately even when donor and host share identical HLA markers, the incidence and

FIGURE 1
HLA COMPLEX



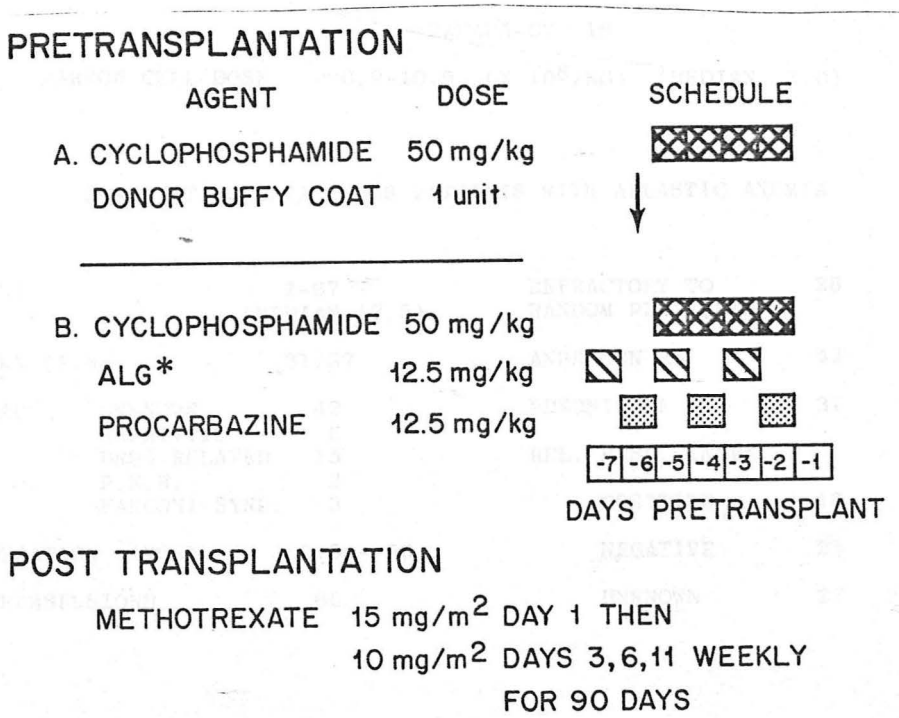
severity of graft-versus-host disease is very great (13), indicating that disparity at loci unlinked to HLA are important in the pathogenesis of this grave complication of marrow transplantation. At the present time, we are unable to type for these "minor" antigens. As will be discussed later, the strikingly high incidence of moderate to severe graft-versus-host disease in patients receiving HLA identical sibling marrow reveals that description of non-HLA antigen systems and their incorporation in tissue typing remain important goals especially in bone marrow transplantation.

Because of the mutual allogeneic reactivity of both host and donor lymphoid cells (14), it is necessary to use immunosuppressive agents to ameliorate the destructive immune response. Figure 2 outlines the types of protocols that are currently employed (15). In an attempt to blunt host-versus-graft reactivity which would ultimately be responsible for rejection of the bone marrow graft, the patient preparation begins with an inoculation of donor buffy coat cells which express donor antigens. Over the next four days, cyclophosphamide, 50 mg. per kilogram, is given in order to effect antigen-specific immunosuppression (16). Immediately following transplantation, the patient is placed on methotrexate therapy with two goals in mind: the suppression of any host-versus-graft reactivity that might emerge, but more importantly, suppression of the more sinister graft-versus-host reaction (17). The schedule B regimen is employed when the patient is known or suspected of having been preimmunized to non-HLA donor antigens.

Immunosuppression in the face of pre-existing immunity is always much more demanding. Consequently, schedule B attempts to overcome extant immunity by adding alternating courses of procarbazine and antilymphocyte globulin to cyclophosphamide conditioning.

FIGURE 2

IMMUNOSUPPRESSION PROTOCOLS



Clinical experience with allogeneic bone marrow transplantation

There are several medical centers in the United States and Europe that perform bone marrow transplantation on a regular basis (17). However, the experience of one group of clinical investigators outstrips that of all others and has set a standard for the field. Accordingly, much of the material to be presented in this Grand Rounds is drawn from published reports from the laboratory of Dr. E. Donnell Thomas at the University of Washington, Seattle, Washington. He and his group have recently reported on more than 70 patients with aplastic anemia who were treated with allogeneic bone marrow transplantation since 1970 (18). The descriptive data which characterize these patients is listed in Table 3. Points worth noting are that in the vast majority of patients, their disease was idiopathic, virtually all had required transfusions and almost 40% had become refractory to random platelets. It should also be noted that all patients and their donors were HLA identical and that 59 of the 68 were ABO matched.

TABLE 3

DESCRIPTIVE DATA ON 68 DONORS FOR BMT IN APLASTIC ANEMIA

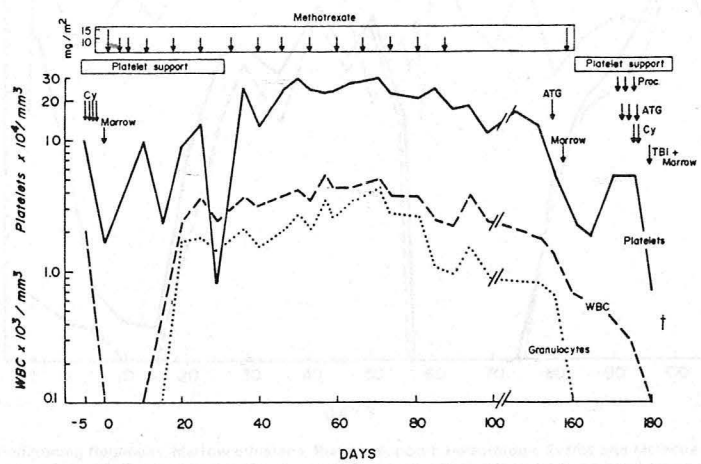
AGE	4-66	(MEDIAN - 18.5)
SEX (F/M)	49/19	
HLA-A,B,D IDENTITY	68	
SEX MATCHES	29	
ABO MATCHES	59	
PATIENT CONDITIONING:	CY	44
	X-RAY	8
	PAPAPA-CY	16
MARROW CELL DOSE	0.9-10.9	(X 10^8/KG) (MEDIAN 3.0)

DESCRIPTIVE DATA ON 68 PATIENTS WITH APLASTIC ANEMIA

AGE	2-67 (MEDIAN-17.5)		REFRACTORY TO RANDOM PLATELETS	28
SEX (F/M)	31/37		ANDROGEN RX	43
CAUSE:	UNKNOWN	42	PREDNISONE RX	37
	HEPATITIS	6		
	DRUG-RELATED	15	REL. RESP. INDEX:	
	P.N.H.	2		
	FANCONI SYND.	3	POSITIVE	16
DURATION (MOS)	0.5 - 96		NEGATIVE	25
TRANSFUSIONS	65		UNKNOWN	27

The clinical course of two patients are illustrative of the spectrum of patient response to bone marrow transplantation. In Figure 3, the patient was prepared according to schedule A above with donor cells and cyclophosphamide. Posttransplantation, methotrexate was administered primarily to alleviate graft-versus-host reactivity. Successful engraftment appears to have taken place with the first sign of granulocytes in the blood approximately 10 days postgrafting. The patient did fairly well until about 150 days when there was a gradual fall in granulocyte and platelet numbers while donor cells persisted in residence.

FIGURE 3

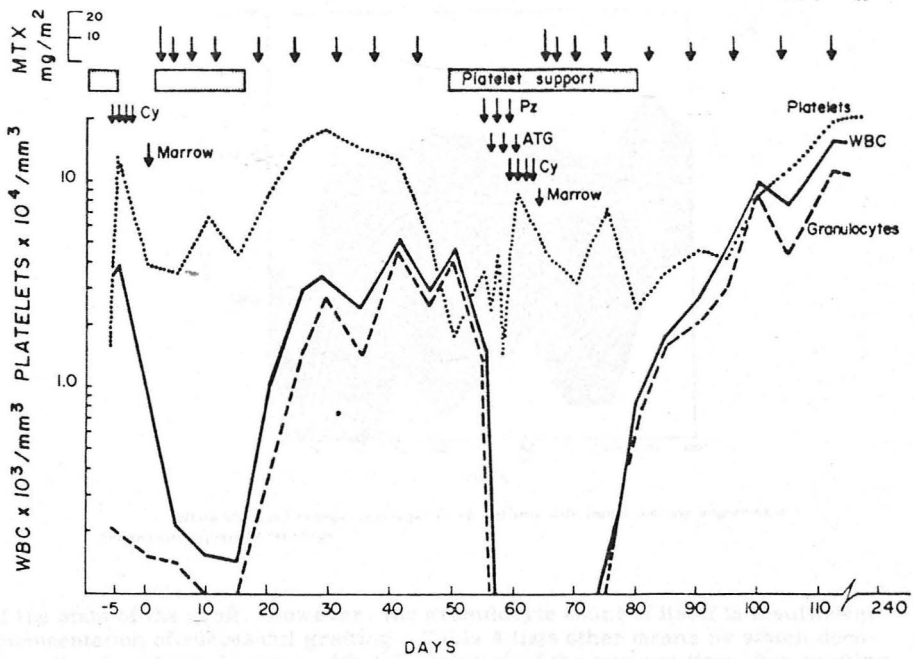


Conditioning regimens, marrow infusion, platelet support, hematologic events, and methotrexate administration in case 48.

One dose of ATG and another infusion of donor marrow were attempted on the belief that marrow "exhaustion" not rejection was occurring. This proved to be of no avail and death intervened before heroic immunosuppression and a third marrow graft could be evaluated. In this instance, graft-versus-host disease played little role in the clinical picture. Similarly, host-versus-graft reactivity also appeared to be minimal. Instead, attrition of the graft of unknown cause seemed to be the basis of the clinical failure.

Figure 4 demonstrates the clinical course of another patient prepared once again in the conventional way. Engraftment is evidenced by increasing numbers of granulocytes in the peripheral blood and dramatic reduction in requirements for platelet support. However, between the 40th and 45th days, the patient experienced precipitous drop in platelets and granulocytes, a much more rapid drop than in the previous case; it was the clinicians' judgment that this situation was probably the result of rejection rather than hypoplasia. As a consequence the patient was treated intensively with procarbazine, antithymocyte globulin, additional cytoxan and a second marrow infusion. Within 15 days granulocytes and platelets began to reappear within the peripheral blood as evidence that the second graft had established itself.

FIGURE 4

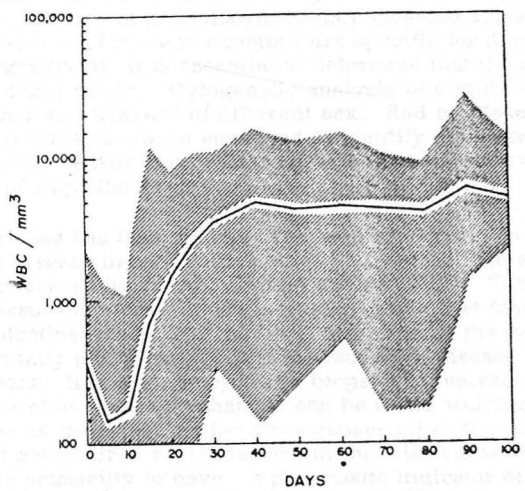


Conditioning Regimens, Marrow Infusions, Platelet Support, Hematologic Events and Methotrexate Administration in a Patient (Case 43) Showing Initial Marrow-Graft Rejection and Subsequent Successful Marrow Engraftment. MTX denotes methotrexate, Cy cyclophosphamide, 50 mg per kilogram, Pz procarbazine, 12.5 mg per kilogram, ATG antithymocyte globulin, 12 mg of IgG per kilogram, and WBC white blood cells.

Three major problems complicate allogeneic bone marrow transplantation as it is currently performed: failure to "take" and/or rejection, graft-versus-host disease, and infections, especially interstitial pneumonia. These themes will recur throughout this presentation, and are introduced here to establish the basis of analysis.

Examination of these two clinical courses reveals that pretransplant conditioning produces (if it does not already exist) a profound granulocytopenia which continues following administration of donor marrow. The first evidence that engraftment has taken place is usually given by the appearance of increasing numbers of granulocytes in the peripheral blood. Figure 5 graphically depicts the mean course of granulocyte counts during the posttransplant interval. Even more than the platelet count, the granulocyte count provides the most accessible indicator

FIGURE 5



White blood cell changes (average) in 46 patients with initial marrow engraftment. Shaded area represents the range.

of the state of the graft. However, the granulocyte count of itself is insufficient documentation of successful grafting. Table 4 lists other means by which documentation is achieved, along with a description of the average time after grafting

TABLE 4

DOCUMENTATION OF ENGRAFTMENT

METHOD	DAY POSTGRAFTING MEDIAN	RANGE
MARROW HISTOLOGY	14	6-46
RISE IN GRANULOCYTE COUNT	16	8-47
DONOR TYPE CELLS BY		
CYTOGENETICS	18	6-66
ISOENZYMES	368	96-767
RBC ANTIGENS	368	87-767
GVHD (AUTOPSY)	17	

when the particular indicator becomes positive. The earliest indication that engraftment has taken place is revealed by bone marrow biopsy since histologic evaluation can confirm the presence of hematopoietic stem cell precursors . Shortly thereafter, the rise in granulocyte count expresses successful engraftment. However, neither of these parameters are specific for donor cells; in order to verify engraftment, it is essential to determine that the newly emergent blood cells are of donor origin. Cytogenetic analysis of circulating lymphocytes may be used if donor and host are of different sex. Red cell isoenzymes and red cell isoantigens have also been employed to identify donor cells. Histologic demonstration of graft-versus-host disease within host tissues is also taken as positive evidence of engraftment.

Much experience has been gained over the past 6 years in the area of clinical graft-versus-host disease in man. Attempts have been made to stage graft-versus-host disease and to assess its overall clinical severity (19). Tables 5 and 6 indicate that while many tissues may be affected in graft-versus-host disease, in man the skin, the gastrointestinal tract, and the liver appear to be the major organs of involvement. Virtually every case of graft-versus-host disease has a prominent cutaneous component. It is mandatory that a biopsy of involved skin be taken; virtually pathognomonic histologic changes can be found which corroborate the diagnosis. This is an important matter since causes other than GVHD can give rise to rashes and generalized erythroderma in this clinical setting. The purpose of staging GVHD is primarilty to have a prognostic indicator of the patient's ultimate course. Patients with no evidence of GVHD or only Grade I disease fair far better than do patients with Grades II to IV; in these individuals, survival approximates only 15%.

TABLE 5

CLINICAL STAGE OF GRAFT-VERSUS-HOST DISEASE

ACCORDING TO ORGAN SYSTEM

STAGE	SKIN	LIVER	INTESTINAL TRACT
+	MACULOPAPULAR RASH <25% OF BODY SURFACE	BILIRUBIN 2-3 mg/100 ml	>500 ml DIARRHEA/DAY
++	MACULOPAPULAR RASH 25-50% BODY SURFACE	BILIRUBIN 3-6 mg/100 ml	>1000 ml DIARRHEA/DAY
+++	GENERALIZED ERYTHRO-DERMA	BILIRUBIN 6-15 mg/100 ml	>1500 ml DIARRHEA/DAY
++++	GENERALIZED ERYTHRO-DERMA WITH BULLOUS FORMATION AND DESQUAMATION	BILIRUBIN >15 mg/100 ml	SEVERE ABDOMINAL PAIN, WITH OR WITHOUT ILEUS

TABLE 6

CLINICAL GRADING OF SEVERITY OF GRAFT-VERSUS-HOST DISEASE

GRADE	DEGREE OF ORGAN INVOLVEMENT
I	+ TO ++ SKIN RASH; NO GUT INVOLVEMENT; NO LIVER INVOLVEMENT; NO DECREASE IN CLINICAL PERFORMANCE.
II	+ TO +++ SKIN RASH; + GUT INVOLVEMENT OR + LIVER INVOLVEMENT (OR BOTH); MILD DECREASE IN CLINICAL PERFORMANCE.
III	++ TO +++ SKIN RASH; ++ TO +++ GUT INVOLVEMENT OR ++ TO ++++ LIVER INVOLVEMENT (OR BOTH); MARKED DECREASE IN CLINICAL PERFORMANCE.
IV	SIMILAR TO GRADE III WITH ++ TO ++++ ORGAN INVOLVEMENT AND EXTREME DECREASE IN CLINICAL PERFORMANCE.

Either as a consequence of the long term effects of graft-versus-host disease or because of the intensive, protracted immunosuppressive therapy, these patients develop severe infections, the third major clinical problem. In particular interstitial pneumonia often due to cytomegalovirus and pneumocystis carinii has dominated the scene.

Summary of Clinical Results

Storb et al. (18) in the most recent Seattle summary of 49 consecutive cases of aplastic anemia reported that 20 patients survived 186 to 990 days after allogeneic marrow grafting. Nineteen of these patients have normal marrow histology and function, while the 20th patient has a mild thrombocytopenia. Fifteen are entirely well and have returned to normal activities. Five patients, however, suffer from chronic graft-versus-host disease either of the skin and/or the liver. Three of these 5 have returned to near normal activity and none requires hospitalization.

Twenty-six patients whose bone marrow grafts established residence have gone on to die, 12 apparently because a primary marrow graft rejection process intervened, although the terminal event was usually an infection. In 8 patients, graft-versus-host disease appeared to be the primary cause of death. In the remaining patients, interstitial pneumonia which followed resolution of graft-versus-host disease or occurred spontaneously was the terminal event. Thus, approximately 40% of patients that present with aplastic anemia to the Seattle group can expect to achieve outstanding therapeutic success with reversal of their disease following bone marrow transplantation.

As another measure of the relative efficacy of bone marrow transplantation in aplastic anemia, the Seattle group has also evaluated the follow-up of long term survivors (20). They were able to examine 11 patients out of an initial group of 24 that were grafted between 1970 and 1973 and who were still surviving 3 to 5 years later. The current hemtologic data on these patients is listed in Table 7.

TABLE 7

CURRENT HEMATOLOGY DATA

PATIENT	AGE (YR)/ SEX	PREPARATION FOR GRAFTING	WBC ($x10^9$)	GRANULOCYTES ($x10^9$)	PLATELETS ($x10^9$)	HCT (%)
2	16/M	CY	9.5	4.4	222	49.0
4	13/M	CY	4.9	3.4	150	44.0
5	23/M	CY	5.4	Normal	133	49.8
10	17/M	TBI	5.4	2.7	300	45.0
15	3/F	CY	6.9	3.0	197	42.6
16	31/F	CY	5.0	2.0	181	44.0
19	5/F	PAPAPA-CY	6.8	3.1	270	38.6
20	10/F	CY	3.5	2.3	220	37.4
21	6/F	CY	10.3	5.2	194	31.0
23	34/M	CY	4.6	2.8	283	42.0
24	10/M	CY	7.8	3.4	178	40.0

Ten of 11 patients lead perfectly normal lives with no immunosuppressive or other drug therapy. One patient has chronic graft-versus-host disease of the skin which is gradually improving and requires no therapy. As the table indicates, the white blood cell count in all patients is within the range of normal as is the granulocyte count and platelet count.

Because the prognosis in severe aplastic anemia is so bleak with other forms of therapy, a prospective study of bone marrow transplantation was recently completed by the Seattle group to determine its relative therapeutic worth (15). Patients presenting with severe aplastic anemia were assigned randomly to transplant and nontransplant groups and their clinical courses compared. The study was conducted in such a manner that bone marrow transplantation was studied as an early therapeutic maneuver. Thirty-six patients entered on the transplant arm of the study, while 31 were conventionally treated. Of the latter, 12 were alive at 12 months following entry into the study, 6 having improved somewhat. The pretreatment patient characteristics of both groups of patients are listed in Table 8. It can be seen that the patients were relatively comparable with regard to the characteristics

TABLE 8

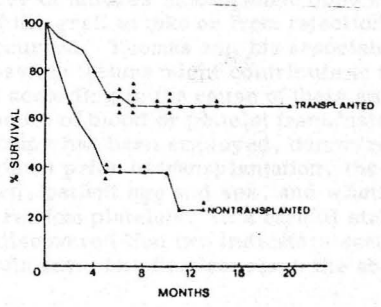
PRETREATMENT PATIENT CHARACTERISTICS

	TRANSPLANTED	NONTRANSPLANTED
AGE (YR)	16 (1-43)*	13 (0.5-77)
MALE/FEMALE	19/17	21/10
ETIOLOGY		
IDIOPATHIC	26	23
PASTHEPATITIS	4	4
DRUG-INDUCED	3	4
INSECTICIDE	3	4
INTERVAL OF SYMPTOMS-DIAGNOSIS (WK)	3 (0-17)	3 (1-17)
INITIAL HEMATOLOGIC VALUES		
PMN cu mm	200 (0-1500)	240 (0-2000)
PLATELETS/cu mm	$6x10^3$(1-23)	$5x10^3$(1-25)
RETICULOCYTES (%)†	0.1 (<0.1-1.4)	0.3 (<0.1-1.5)

*NUMBERS IN PARENTHESES REPRESENT THE RANGE OF VALUES.

†CORRECTED FOR HEMATOCRIT.

measured. The overall survival of the 2 groups of patients is presented in Figure 6. It is instructive to examine the clinical outcome of bone marrow transplantation in these patients, as a measure of the relative successfulness of the 2 modes of therapy.



Life table plot of the effect of treatment on survival in severe aplastic anemia. Triangles indicate duration of follow-up of current survivors.

FIGURE 6

The results listed in Table 9 indicate that in 34 grafted patients, 4 died with infection including 2 in which infection was superimposed on graft-versus-host disease. Nine grafts were rejected; 7 patients received second transplants from the same donors, and 2 achieved successful grafts. In total, 24 transplant patients are alive with full marrow recovery. The striking improvement in survival of patients with severe aplastic anemia treated with early bone marrow transplantation indicates that this form of therapy is the most effective treatment modality currently available and fully justifies the attempts of the house staff and attendings caring for patient JL to arrange for allogeneic bone marrow grafting.

TABLE 9

OUTCOME OF TRANSPLANTS

36 ENTERED	2 DIED DURING IMMUNOSUPPRESSION
34 ENGRAFTED	4 DIED: INFECTION
	2 SEPSIS, GVHD (DAY 63, 133)
	1 PNEUMONIA, SEPSIS (DAY 89)
	1 TUBERCULOSIS (DAY 140)
9 GRAFT REJECTION	1 DIED BEFORE RETRANSPLANTATION
	1 SPONTANEOUS RECOVERY
	7 RETRANSPLANTED
	5 DIED: NO TAKE
	2 ENGRAFTED
24 ALIVE WITH FULL MARROW RECOVERY	

An analysis of factors associated with graft rejection

It is apparent that a significant number of failures in allogeneic bone marrow transplantation result either from failure of the graft to take or from rejection before significant hematologic improvement has occurred. Thomas and his associates have evaluated in a series of 68 patients what possible factors might contribute to this problem (21). The patients were analyzed according to the cause of their aplasia, its duration prior to treatment, the total amount of blood or platelet transfusions, whether or not androgen or prednisone therapy has been employed, donor/recipient match characteristics, the conditioning regimen prior to transplantation, the number of donor marrow cells that were transplanted, patient age and sex, and whether the patient had demonstrated refractoriness to random platelets. In a careful statistical analysis of data from these patients it was discovered that two indicators seemed to be closely associated with successful engraftment. The first concerns the absolute

number of marrow cells transplanted. Positive engraftment occurred more frequently as the number of transferred donor cells was increased. In fact, there seemed to be a threshold effect: if $3x10^8$ donor cells (or more) per kilogram body weight were infused, engraftment was usually insured. The second variable that seemed positively correlated with rejection was the so-called relative response index (RRI). This in vitro variant of the mixed leukocyte culture pits the patient's lymphoid cells as "responders" against donor cells as "stimulators." The magnitude of proliferative response is then compared to the response of patient lymphoid cells against "stimulator" cells from unrelated individuals. The conceptual basis of this test is that prior sensitization of the patient to weak (non-HLA) transplantation antigens can be detected, sensitization resulting from prior platelet and blood transfusions. When the Relative Response Index was considered (Table 10), an inverse correlation was found.

TABLE 10

MARROW GRAFT REJECTION, RRI AND NUMBER

OF INFUSED MARROW CELLS

RRI	NUMBER OF CELLS (X 10^8/KG)		
	0.9-2.9	3.0-4.9	>5.0
NEGATIVE(<1.6%)	3/9	0/8	0/8
POSITIVE(>1.6%)	8/10	2/3	1/3

(NUMBER REJECTED/STUDIED)

Negative RRIs were associated with a high likelihood of engraftment if sufficient number of donor cells were given. Alternatively, positive RRI tests usually predicted that comparable numbers of donor cells would be less likely to "take." These two observations, namely, the number of donor cells transplanted and the RRI, indicate two important research avenues that need to be explored experimentally if greater success in bone marrow transplantation is to be expected.

Storb (22) and others have shown in dogs that prior sensitization of the potential recipient to weak (that is, non-MHC) transplantation antigens seriously impairs the effectiveness of engraftment subsequently. Much more heroic immunosuppressive maneuvers are necessary in order to overcome a prior state of sensitivity to the weak antigens. The PAPAPA-CY regimen incorporating procarbazine, and anti-lymphocyte globulin on top of cyclophosphamide is designed to overcome presensitization and make engraftment possible. Since prior sensitization seems to be an important determinant of the success of bone marrow transplantation, then the decision to offer bone marrow transplantation to aplastic patients at the earliest possible time and with the least possible prior exposure to blood and blood products becomes mandatory.

It is very interesting that cell dose should play such a prominent role in determining marrow graft success. It is well documented that there is a threshold number of cells required in order to achieve successful bone marrow transplantation

even among syngeneic donor and recipients (23). More than a decade ago a group of investigators working in GVH systems reported a very unconventional finding: certain F_1 hybrid mice, derived from inbred, histoincompatible strains, failed to permit hematopoietic stem cells from one parent to establish residency in spleen and bone marrow (24). (AxC57BL)F_1 hybrids treated with total body irradiation could not be rescued from radiation death with bone marrow cells from C57BL donors unless the number of transferred cells was increased 10 to 100 times compared to A strain cells or F_1 cells. This phenomenon, which has come to be known as hybrid resistance, has now been described in rats (25) and several other rodent species and may very well be operative in man. This peculiar resistance to bone marrow grafting extends not only to allogeneic cells but to xenogeneic cells; as a consequence, the more general term "genetic resistance" has been coined. There are several unusual features about high genetic resistance which separate the phenomenon from conventional immunity. The inciting antigens have not been identified. Some insight into the genetic basis for resistance can be obtained by observing the results presented in Table 11 (26). A series of murine F_1 hybrid recipients were

TABLE 11

H-2 COMPLEX CONTROL OF HYBRID RESISTANCE IN MICE

F_1 HYBRID RECIPIENT	MARROW DONOR	RESULT	H-2 REGION
bbbbbb	bbbbbb	RESISTANT	ENTIRE
kkkddd	kkkddd	SUSCEPTIBLE	
kkkddd	kkkddb	RESISTANT	D(Hh-1)
kkkddb	kkkddd	SUSCEPTIBLE	
kkkddb	kkkddb	SUSCEPTIBLE	K,I-A,I-B, 1-C,S
bbbbbb	bbbbbb	SUSCEPTIBLE	
dddddd	dddddd	SUSCEPTIBLE	K,I-A,I-B
kkkddd	kkkddd	SUSCEPTIBLE	
bbbbbb	bbbbbb	RESISTANT	ENTIRE
dddddd	dddddd	SUSCEPTIBLE	

irradiated and inoculated with bone marrow cells from donors that were congenic with each other and differed only at the H2 complex (murine HLA equivalent). It can be seen that genetic resistance is dictated by genes within the H2 complex; the genes responsible seem to be located near the D end of the complex. We have no idea what the nature of the gene product is: whether structural protein or regulatory molecule. It has been suggested that the product of the putative gene is the D region antigens. The locus responsible for hybrid resistance is designated Hh (hybrid histocompatibility). If the result of the gene action is an antigen, it is a very unusual transplantation antigenic system in that the antigens do not express themselves as codominants (as all other transplantation antigens appear to do). Instead, the antigens appear to act as corecessives, a fairly unusual genetic postulate. Other unusual features of the Hh system are that (a) prior exposure to tissues bearing the putative antigens does not lead to a hyperimmune response, and (b) using strontium-90 it has been possible to show that the mediators of the Hh rejection process are "M" cells (marrow derived), and probably represent cells of the monocyte/macrophage lineage (27). Other studies have suggested that the cell responsible for hybrid resistance is an NK cell (28,29). This intriguing phenomenon is now receiving a great deal of experimental attention and its ultimate biologic role is obscure. There is reason to believe that this unusual genetic system may play an important part, not only in determining success of bone marrow transplantation, but in affording the host resistance to the emergence of hematologic neoplasms, especially those derived from lymphoid cells (30,31,32). One would predict that a great deal more is going to be heard about Hh systems over the next several years. If a comparable genetic system exists in man, it could account for the observation that large numbers of donor allogeneic bone marrow cells have a better chance of establishing a successful graft.

To summarize this section dealing with allogeneic bone marrow transplantation for aplastic anemia, the best evidence suggests that bone marrow transplantation may be the most effective means currently available for the therapy of this disorder, and transplantation as early as possible in the course of the disease is more likely to lead to therapeutic success. Three major problems continue to hamper the success of bone marrow transplantation in this clinical setting: failure of marrow engraftment or rejection, severe graft-versus-host disease even in the presence of HLA identity, and lethal infections, most often interstitial pneumonia.

Acute leukemia

The rationale for employing bone marrow transplantation as a therapeutic measure in acute leukemia is multifaceted. As other more conventional modes of therapy gradually lose their effectiveness in these patients, bone marrow transplantation is offered as a last resort in an attempt to fend off the inevitable. More reasonably, the availability of bone marrow transplantation allows the oncologist to push, beyond the usual tolerable limits, chemotherapeutic drugs in an effort to achieve remission, toxic doses which obliterate hematopoiesis. The marrow transplant permits a relatively rapid repopulation of the marrow space and return of normal mature cells to the peripheral blood. A third reason for considering bone marrow transplantation as definitive therapy in leukemia (one less widely agreed upon), is based on the potential antileukemic effect of mild, but clinically tolerable graft-versus-host reactions. It has been suggested that the graft-versus-host destructive potential is exerted preferentially against malignant rather than normal cells (33,34). Comparatively speaking, bone marrow transplantation has become widely performed over the past 5 years; it is now possible to draw certain conclusions about its efficacy in leukemia and future potential.

There are special problems which emerge when allogeneic bone marrow transplantation is considered for acute leukemia, problems over and above those that exist in patients with aplastic anemia (See Table 12). Both types of patient have a very high risk for infection and bleeding complications. However, the patient with leukemia has usually undergone extensive antileukemic chemotherapy and irradiation which produces profound immunosuppression, far in excess of the aplastic patient. Moreover, bone marrow transplantation can only be reasonably attempted when the leukemia has gone into remission which places a further temporal restriction on the clinical situation. Once successful engraftment has taken place, there is the continued and considerable risk of developing a relapse of the leukemic process. Finally, there have been several reported instances in which leukemic transformation has occurred within donor cells (35).

TABLE 12

SPECIAL PROBLEMS WITH ALLOGENEIC BONE MARROW

TRANSPLANTATION FOR ACUTE LEUKEMIA

PREVIOUS ANTI-LEUKEMIA THERAPY

REMISSION INDUCTION

LEUKEMIC RELAPSE

MALIGNANT TRANSFORMATION OF DONOR CELLS

Identical twin bone marrow transplantation for hematologic neoplasia

In an effort to sort out problems due to histoincompatibility from problems related to the neoplastic process, one can examine the experience with bone marrow transplantations for acute leukemia when identical twin pairs are used (36). Table 13 lists the clinical results of 27 patients treated in this way.

TABLE 13

IDENTICAL TWIN BONE MARROW TRANSPLANTATION

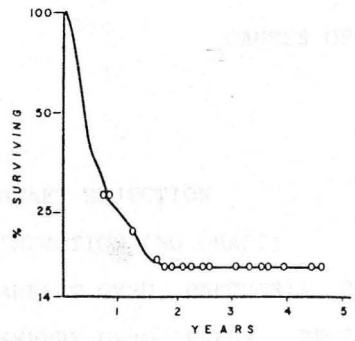
FOR HEMATOLOGIC NEOPLASIA

PRETREATMENT	NO.PTS.	IN REMISSION (>3 MOS)	DURATION (MOS)	ALIVE
X-RAY	4	0	-	0
X-RAY & IMMUNORX	3	1	10	0
CY & X-RAY	7	5	3,4,4,6,18	2
CY, X-RAY & IMMUNORX	13	10	3,4,5,7,8, 21,31,32,49	6

There are, of course, too few patients included in this analysis to make any sweeping conclusions. Since all patients received a bone marrow transplant, then the critical determinant becomes the pretreatment regimen. It would appear that significant remission induction only occurred in patients pretreated with cytoxan, and whole body x-irradiation with or without immunotherapy. The analysis unfortunately fails to compare these results with comparable patients who did not receive bone marrow transplantation. Nonetheless, effective remission inducing regimens of massive chemotherapy and whole body irradiation could be administered with the assurance that the patient can be rescued with normal hematopoietic stem cells from an identical twin donor. Thus, the incidence of remission in these patients is relatively high, and, at least in the view of the investigators, sufficiently promising to warrant continued use of this protocol in the future. Although leukemic relapse continues to plague these patients, they are at least not subject to the devastations of graft-versus-host disease with which recipients of allogeneic marrow transplants suffer.

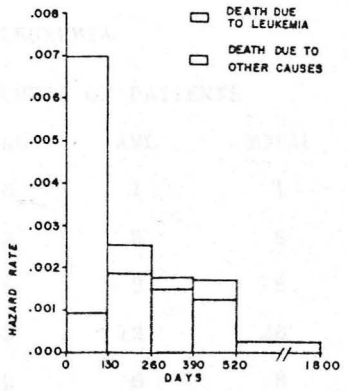
Allogeneic bone marrow transplantation in acute leukemia

Thomas et al. have recently summarized their experience with 100 patients with acute leukemia treated by chemotherapy, whole body irradiation and allogeneic transplantation (37). At the time of this report which was summarized in September, 1976, 17 of 100 patients were still alive. Figure 7 plots the Kaplan-Meier product limit estimate of the probability on a logarithmic scale of survival for 100 patients. The slope of the line changes markedly just before 2 years. In Figure 8, a histogram describes the death rate per day segregated according to whether the death resulted



Kaplan-Meier product limit estimate of the probability of surviving on a logarithmic scale in per cent for 100 patients. Living patients are indicated by open circles.

FIGURE 7



Histogram of the hazard rate (death rate per day) showing the death rate for leukemia and for all other causes.

FIGURE 8

from leukemia or other causes. In the first 4 months after bone marrow transplantation, the vast majority of deaths are due to causes other than leukemia, chiefly, graft-versus-host disease and infection. After 130 days, the incidence of leukemia as a cause of death increases, and exceeds all other causes. Interestingly, beyond 2 years, the death hazard rate, whether due to leukemia or other causes, reaches an exceedingly low value indicating that a rather stable clinical situation is achieved. Seventeen patients were alive at the time this study was compiled in September, 1976. Thirteen patients remained disease free while 3 patients had debilitating chronic graft-versus-host disease. Five patients diagnosed with acute lymphocytic leukemia and 6 with acute granulocytic leukemia were in complete remission and had returned to normal activity. They were not on chemotherapy.

It is instructive to examine a compilation of the causes of death in patients who had received bone marrow transplantation for leukemia. See Table 14. It is striking to see that only one patient out of 83 experienced graft rejection. This contrasts sharply with the experience in aplastic anemia and indicates that host immune competence is much more severely impaired in leukemic patients. As a consequence, graft rejection is a relatively unimportant concern. Three major causes of death are observed: 26 patients died of severe graft-versus-host disease complicated by pneumonia and respiratory failure, 26 patients developed a recurrence of their leukemia which as responsible for their demise. Infection

TABLE 14

CAUSES OF DEATH: BMT IN LEUKEMIA

	NUMBER OF PATIENTS		
	AAL	AML	TOTAL
GRAFT REJECTION	0	1	1
INFECTION (NO GRAFT)	3	5	8
ABSENT GVHD, PNEUMONIA, RESP. FAIL.	5	3	8
SEVERE GVHD, PNEUM., RESP. FAIL.	13	13	26
GVHD AND INFECTION	2	6	8
CHRONIC GVHD	1	1	2
RECURRENT LEUKEMIA	11	15	26
CARDIAC FAILURE	1	1	2
UNKNOWN CAUSE	0	2	2
	36	47	83

represented the other significant cause of death, whether associated with graft-versus-host disease, with no evidence of graft-versus-host disease, or with no evidence of engraftment (8 patients). In comparison with patients with aplastic anemia treated with bone marrow transplantation, only 8 of the leukemic 83 patients failed to acquire an established allogeneic graft. But similar to the aplastics, patients with leukemia that received allogeneic marrow grafts suffered from graft-versus-host disease and infections.

Infections of the interstitial pneumonitic type are particularly prominent in patients undergoing bone marrow transplantation (38). A prospective survey of interstitial pneumonia was carried out in marrow transplantation and the results are presented in Table 15. It is significant that the incidence of pneumonia was

TABLE 15

PROSPECTIVE SURVEY OF INTERSTITIAL PNEUMONIA

IN MARROW TRANSPLANTATION

RECIPIENT GROUP	TOTAL	TOTAL WITH PNEUMONIA	FATAL PNEUMONIA
HEMATOLOGIC MALIGNANCY	40	27	17
APLASTIC ANEMIA	21	6	1
TOTAL GROUP	61	30	18

found to be higher among patients with a hematologic malignancy than with aplastic anemia; moreover, the likelihood that interstitial pneumonia will be sufficiently severe as to cause death is very small in the latter group. The relationship between interstitial pneumonia and graft-versus-host disease is also an important one. Table 16 lists the experience with this complication among patients with varying clinical grades of GVHD severity. It can be seen that the development of

TABLE 16

INTERSTITIAL PNEUMONIA AND GVHD

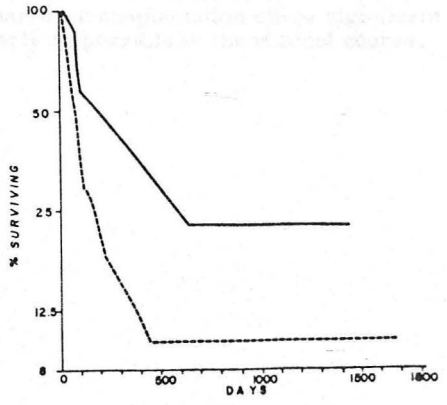
GRADE OF GVHD	NO. OF PATIENTS	WITH INTERST. PNEUMONIA	WITH LETHAL PNEUMONIA
0	19	7	2
I	24	14	6
II-IV	50	33	26
TOTAL	93	54	34

graft-versus-host disease after bone marrow transplantation carries with it an increased risk for the development of interstitial pneumonia. Impressively, if the clinical grading of the graft-versus-host process is within the range of 2 to 4, the likelihood that interstitial pneumonia will be lethal is very great. Thus there is a distinct relationship between the propensity to develop interstitial pneumonia and the emergence of severe graft-versus-host reactivity. Since GVHD imposes its own immunodeficiency on the patient (14,39), this may be enough to tip the balance in favor of facultative microorganisms.

Another point needs to be stressed concerning the relationship between clinical condition of the patient at the time of transplantation and the likelihood of survival. Thomas et al. grouped their patients into 5 categories according to the following criteria:

0. Complete remission; no history of transfusions.
1. Complete or partial remission; history of transfusions; good clinical condition.
2. Relapse, no fever, fair clinical condition.
3. Relapse, granulocytopenia, require platelet transfusions and/or febrile, poor clinical condition.
4. Advanced relapse and/or refractory to random platelets and/or febrile on broad spectrum antibiotics, very poor clinical condition.

Figure 9 depicts a Kaplan-Meier estimate of per cent patients surviving on a logarithmic scale for clinical grades 1-2, and 3-4. Not unexpectedly, patients in poor clinical state at the time of bone marrow transplantation did much less well than did patients who were transplanted in good clinical condition.



Kaplan-Meier estimate of per cent surviving on a logarithmic scale for patients of clinical grades 1 and 2 (solid line) compared to clinical grades 3 and 4 (broken line). The clinical grade was determined prospectively at the time of marrow transplantation.

FIGURE 9

Summary

Allogeneic bone marrow transplantation as a therapeutic measure for aplastic anemia and acute leukemia has been reviewed. The experience over the past 5 years in these 2 clinical situations has produced contrasting results. In acute leukemia, less than 20% of the patients achieve therapeutic benefit as evidenced by survival and remission induction. The best evidence would suggest that the extent of remission induction is related primarily to the pretransplant preparation rather than to the transplantation itself. Since there is no evidence to suggest that the graft-versus-host process has an antileukemic effect in man, the role of the transplant would appear to be relegated merely to tiding the patient over until marrow recovery takes place. While the incidence of graft rejection is very low in this situation, the susceptibility to graft-versus-host disease is great and the risk of developing a fatal interstitial pneumonia is exceedingly high. Moreover, a large number of patients develop a relapse of the leukemic process.

By contrast, allogeneic bone marrow transplantation in aplastic anemia is a comparatively effective means of therapy. Approximately 40% of patients obtain a successful graft and return to normal hematologic values and life style. Significant problems that remain in this situation are a high incidence of marrow graft rejection (perhaps related to preimmunization to weak transplantation antigens), a persisting high incidence of graft-versus-host disease (even when donor and host are HLA identical), and high risk of infection, especially interstitial pneumonia. It would appear that the clinical promise is greatest in aplastic anemia. Hope for the future rests in identification of weak (non-HLA) antigenic systems that elicit immunity following blood transfusion and platelet transfusions and which lessen the likelihood of a successful subsequent graft. The role Hh antigens and/or genetic resistance play in promoting or preventing allogeneic engraftment needs and is receiving intensive experimental investigation at this time. The etiology and pathogenesis of graft-versus-host disease still eludes us, and effective treatments aimed at blunting this devastating clinical syndrome do not exist. Nonetheless, for patients with aplastic anemia, allogeneic bone marrow transplantation offers significant hope and should be made available as early as possible in the clinical course.

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