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RECENT APPROACHES TO THE TREATMENT OF ACUTE LYMPHOCYTIC LEUKEMIA IN CHILDHOOD

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INTRODUCTION

Acute lymphocytic leukemia (ALL) is the most common malignancy in childhood accounting for 35–40% of cancers in this age group. At the time of diagnosis approximately a trillion (10^{12}) leukemic cells are present throughout the body (1). The bone marrow has been replaced and suppressed by leukemic cells causing a cessation of normal blood cell production. Previously both physicians and parents equated a diagnosis of acute lymphocytic leukemia with a certainty of rapid death. This dire prognosis has changed dramatically in the last 20 years. Now a complete bone marrow remission can be induced in 95% of children with ALL. Moreover, one half of these children will have a prolonged leukemia-free survival and probable cure.

This chapter reviews the therapeutic advances that have led to this great improvement in survival, the current problems associated with this treatment, and approaches to achieve more specific and effective therapy. Recent articles have summarized the first two topics (1–7), so we concentrate on new directions for therapy. Innovative new approaches are needed since the percentage of patients who achieve long-term leukemia-free survival has not increased appreciably in the last 10 years.

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THERAPEUTIC ADVANCES

Modern chemotherapy of acute lymphocytic leukemia began in 1947 with the demonstration that a folic acid antagonist could induce remissions (8). In the next two decades corticosteroids, 6-mercaptopurine, cyclophosphamide, vincristine, L-asparaginase, cytosine arabinoside, daunorubicin, and doxorubicin were shown to be effective single agents in this disease. Complete remissions (defined as normal bone marrow morphology and function plus no clinical evidence of leukemia) were induced in 20–70% of children with each of these drugs, but the leukemia almost always recurred in a few months (1).

The first major conceptual advance was the use of combination therapy. This strategy derived from studies with transplantable mouse leukemia that established the following principles: 1. Cell-kill hypothesis. A single drug can kill a certain proportion of cells and this proportion is largely independent of the initial number of cells (9). The increase in survival time is related to the number of remaining viable cells after treatment (10). If two drugs have additive or more than additive antitumor effects and less than additive toxicity, the combination of both agents will produce therapeutic benefit over each drug alone. 2. Cell cycle specificity. Certain drugs are equally cytocidal to proliferating and nonproliferating cells while other drugs are cytocidal only in the proliferative phase of the cell cycle. These drugs were called cell cycle–nonspecific and cell cycle–specific agents, respectively. Certain cell cycle–specific agents are maximally cytocidal during only one phase of the cycle (11). 3. Growth kinetics. As the size of a tumor increases, its rate of growth slows as a result of a decrease in the proportion of proliferating cells, an increase in the duration of the cell cycle, or an increase in the death rate of the tumor cells. Consequently, cycle-specific agents are least effective when the tumor is large. 4. Drug resistance. Cells with resistance to a drug have a selective growth advantage versus sensitive cells. Thus, the proportion of cells resistant to a single drug increases with each successive administration of that drug. Eventually the tumor becomes completely resistant to that drug. Combinations of active agents may delay or prevent the overgrowth of sublines of the tumor that are resistant to one or more of the drugs in the combination (12). 5. Recovery from sublethal injury. Cells have various mechanisms to repair damage to macromolecules by radiation or drugs. Combinations of drugs may delay or prevent this repair.

In childhood acute lymphocytic leukemia, a combination of prednisone, vincristine, and L-asparaginase induces complete remissions in 90–95% of children (1). These drugs are selected because they are the most active single agents, have different mechanisms of action, for the most part have nonad-

ditive host toxicity, and are not toxic to the already compromised bone marrow. Daunorubicin may be substituted for asparaginase but it is myelosuppressive.

Another concept learned from the animal studies and early clinical trials was that residual disease was present even after the bone marrow appeared normal. In humans as many as a billion leukemic cells may remain after remission is achieved. The duration of the remission depends on the actual number of residual cells and the rate of their proliferation in an individual patient. Because of emergence of resistant leukemic cells, continuation of the same drugs used for induction usually does not produce prolonged remissions. The most effective drugs for maintenance of remission are methotrexate and 6-mercaptopurine. Most current regimens include these drugs.

The very important concept that more therapy may not be better therapy was demonstrated in Study VIII of St. Jude Children's Research Hospital. After achieving remission, children were randomly assigned to groups that received one to four drugs. Nine of 20 children receiving methotrexate alone in maximum weekly doses developed severe neurological toxicity and only four were long-term leukemia-free survivors. The combination of two drugs, methotrexate and 6-mercaptopurine, produced the best rate of continuous complete remission. Addition of one or two other drugs (cyclophosphamide and cytosine arabinoside) to this regimen did not improve the antileukemic effect but increased the number of hospitalizations, infections, and deaths during initial complete remission (4). Other studies have shown that intensification of the induction regimens does not significantly improve survival or duration of remission but can also increase toxicity.

Fewer than one sixth of children treated with these effective combination drugs for remission induction and continuation therapy achieve long-term disease-free survival. One reason is that over half develop central nervous system leukemia (4). These observations led to the concept that the blood-brain barrier prevents the transport of otherwise effective drugs into the central nervous system (CNS) allowing the small number of leukemic cells initially present to proliferate in a pharmacologic sanctuary. Local therapy is needed to kill these cells. Cranial-spinal irradiation or cranial irradiation plus intrathecal methotrexate are the best current treatments to prevent the development of leukemia in this site. The testicle may be another sanctuary in boys with leukemia because of a blood-testicle barrier (13).

The next advance was the recognition and availability of intensive supportive care. This care includes intravenous fluid and allopurinol to aid the renal excretion of breakdown products from lysed leukemic cells; blood products to treat anemia, thrombocytopenia, and granulocytopenia; antimicrobial agents for treating infections; and radiation therapy to treat compro-

mised airways and renal obstruction. Optimal treatment also requires dental care, nutritional support, laboratory facilities, and a team specially trained to treat the social, psychologic, and medical problems that arise.

In summary, current therapy of childhood acute leukemia includes an intensive period of therapy with three drugs to induce a remission. After remission is achieved the child receives "prophylactic" local CNS treatment and starts on continuation therapy with two or more drugs. This maintenance phase is continued for 30 months or longer. During this entire period the child who stays in remission rarely needs hospitalization and can lead a nearly normal life. Fifty to sixty percent of children with ALL remain in remission throughout 2.5 years of maintenance therapy. About 20% of children stopping treatment at this time relapse within first four years off therapy. Relapse is rare after this time (14).

PROBLEMS

Two major challenges remain in the treatment of childhood ALL. First, the 50% of children who relapse must be identified early in their disease and receive more effective therapy. Second, the children who will respond well to current therapy must be identified so that their treatment can be modified to decrease short- and long-term toxicity.

As presented above, administering more drugs to all patients is not the answer to the first problem. Fortunately, methods are developing that can help identify the children who respond poorly to chemotherapy. These methods and new treatment approaches with drugs and with bone marrow transplantation are discussed in the next sections.

The long-term effects of chemotherapy and radiotherapy can now be studied since many patients are surviving. Some children have developed brain abnormalities on CAT scan, learning disabilities, and delayed growth and maturation. The eventual effects of this therapy on the aging process, incidence of other cancers, and on their offspring is unknown.

Reduced therapy may be possible for the child with good prognostic factors. A recent study reported that such children had a reduced incidence of infectious complications if the maintenance drugs were given intermittently rather than continuously (15). Another area of intensive study is the type and extent of CNS treatment needed to prevent leukemia in this site. These investigations are summarized in the last part of this chapter.

SUBCLASSIFICATION

Traditionally, prognosis in childhood ALL has been based upon patient characteristics at diagnosis as well as treatment variables. Favorable prognostic features in most studies include age 3 through 6 at presentation,

initial WBC $< 10{,}000/mm^3$, and female sex (16). The latter is only partly explained by testicular recurrences in males. However, some children with multiple poor prognostic factors survive, and among children with multiple favorable presenting features, about 20% relapse despite optimal therapy. This variability may be explained by differences in subclasses of leukemic cells.

During the past decade, the existence, development, and functions of human lymphocyte subpopulations have been progressively defined (17). Lymphoid stem cells initially appear in blood islands of the embryonic yolk sac with subsequent migration to the fetal liver and bone marrow. Lymphocyte development and maturation are characterized by sequential changes in function and surface markers as shown in Table 1. T lymphocyte differentiation occurs within the thymus while B lymphocyte differentiation occurs in the marrow, lymph nodes, and intestinal lymphoid tissues. T lymphocytes are involved in proliferative and cytotoxic responses to foreign antigens, lymphokine production, and helper or suppressor effects on antibody production. B lymphocytes are involved in antibody production, lymphokine production, stimulation of allogeneic T cells, mitogen-induced cytotoxicity, and proliferation in response to Epstein-Barr virus.

Lymphoblasts from children with ALL can be classified as T-like, B-like, and non T–non B subtypess based upon the markers in Table 1 (18). The non T–non B cell subtype has several subclasses. The most prevalant subclass, Common ALL (cALL) reacts with antisera directed to an antigen on the surface of non T–non B lymphoblasts (abbreviated $cALL^+$). Another non T–non B subclass does not react with antigen ($cALL^-$) and is called Null (26). Relative frequencies of the T-like, B-like, and non T–non B subtypes (Common and Null) are listed in Table 2 (18–22). Other subclasses exist within each subtype based on differences from the typical phenotype in reactivity to one or more of the markers or in the temperature stability of the E rosettes (18, 24, 25). The clinical significance of these variants is currently under active study.

Subclassification of ALL should be performed on marrow blasts or both peripheral blood and marrow blasts. If peripheral blood alone is used, incorrect classification as T-like may occur because of residual normal T lymphocytes (23).

Patient characteristics at presentation and prognosis have been correlated with lymphoblast subtypes (Table 3). Sen & Borella were the first to delineate the typical patient characteristics and poor prognosis of children with T-like ALL (20). This has been confirmed and a higher incidence of extramedullary relapses noted in these children (19–21). Among non T – non B leukemic patients, those with common ALL have longest disease-free survival (19). B-cell leukemia responds rarely, and then usually only transiently to typical therapy for ALL; prognosis may be better if treatment

Table 1 Markers associated with normal human lymphoid cells differentiation

Pre T cells	Human T lymphocyte antigen (HTLA)
	Terminal deoxynucleotidyl transferase (TdT)
	Peanut agglutinin (PNA)
T cells	Human T lymphocyte antigen
	Sheep red cell receptors (E rosettes)
	Fc receptors for IgG ($T\gamma$) – suppressor
	IgM ($T\mu$) – helper
	IgA ($T\alpha$)
	IgE ($T\epsilon$)
	Histamine receptors
	TH_2 antigen
	α-Napthylacetate esterase (ANAE)
Pre B cells	Cytoplasmic IgM (CIg)
	Alloantigens (Ia)
B cells	Surface immunoglobulin (SIg)
	Alloantigens
	Mouse red cell receptors (MRBC)
	Fc receptors
	Complement receptors (C3)
	Epstein-Barr virus receptors (EBV)
Plasma cells	Cytoplasmic IgM

effective for patients with Burkitt's lymphoma is given. Finally the prognosis of patients with Null ALL appears intermediate between that of children with T-like and Common ALL.

Prognosis based on lymphoblast subtyping reflects basic biologic properties of the leukemic cells. Prognosis based upon patient age, sex, and presenting WBC reflects host characteristics and/or extent of disease at diagnosis. The combination of lymphoblast typing and host characteristics may be more helpful prognostically than use of either alone (19–21). Studies involving larger numbers of patients followed for longer periods of time are necessary to confirm this suggestion.

Leukemic lymphoblasts probably reflect phenotypes of normal lymphoid cells frozen at different stages of differentiation. Common ALL antigen–positive cells can be detected in the marrow and other hematopoietic tissues of fetuses and young children (26). The antigen is detected regularly in the marrows of leukemic children after cessation of chemotherapy (whether the original diagnosis was ALL or acute myelogenous leukemia), of patients with neonatal leukemoid reactions, and of bone marrow transplant recipients during early marrow regeneration. Typical Common ALL may represent the leukemic equivalent of this small normal marrow cell subpopulation.

Table 2 Subtypes of childhood ALL

Subtype	Markers	Percentage of childhood ALL
T cell	E^+, $HTLA^+$, TdT^+ $cALL^-$, Ia^-, SIg^-	18–25%
B cell	Ia^+, SIg^+ $cALL^-$, Ia^-, E^-, $HTLA^-$	0–2%
Non T, NonB cell		
Common	$cALL^+$, Ia^+, TdT^+ E^-, $HTLA^-$, SIg^-	75–85%
Null	TdT^+ or $^-$ $cALL^-$, Ia^-, E^- $HTLA^-$, SIg^-	2–5%

Other leukemic equivalents of specific normal lymphocyte subsets have been reported. A pre-B cell phenotype characterized by the presence of cytoplasmic immunoglobulin and surface markers for Common ALL has been reported in 4 of 18 leukemic children otherwise classified as having Common ALL (27). T-cell leukemias can be divided into those with markers of suppressor T cells (TH_2^+, T^+) or helper T cells (TH_2^-, T^+). Patients with helper T-cell leukemia have the typical presenting characteristics and poor prognosis previously cited. In contrast, children with suppressor T-cell leukemia have presenting features and prognosis more characteristic of common cell ALL (28). Since tumor cells of children with T-cell mediastinal lymphoma are TH_2^+, the above data suggest that a portion of T-cell leukemias may not result from leukemic conversion of the mediastinal malignancy.

An alternative explanation for the various leukemic cell phenotypes is the loss or gain of markers by evolution of a basic leukemic stem cell. Loss or gain of major histocompatibility (HLA) antigens by leukemic cells is well recognized. In addition, shifts have been noted when cell markers at diagnosis are compared to markers at the time of relapse (29). However, such shifts could also represent selection by therapy of a minor population of cells present at diagnosis or an arrest of differentiation of the original leukemia cells at an earlier stage of development. Further studies designed to detect minor subpopulations of leukemic cells at diagnosis are necessary to resolve this problem.

Studies of leukemic cell subtypes may be important for reasons other than prognosis. By raising monoclonal antisera to leukemic subtypes the immunodiagnosis of residual or recurrent disease might be possible. The in-

Table 3 Lymphoblast subtypes, typical patient characteristics at presentation, and prognosis in ALL

Type	Typical patient characteristics	Percentage of long-term disease-free survival
Common	Male = female, age 3–6 years, WBC <20,000	60+
T-like	Male, 10 years old, WBC >50,000, mediastinal mass	10–20
B-like	Male, all ages, variable WBC, gut/pelvic masses	rare
Null	High WBC	?

ability to monitor adequacy of therapy except by light microscopic review of marrow aspirates is a major limitation of current therapy of ALL. Finally, different subtypes may have particular metabolic requirements or membrane characteristics that are therapeutically exploitable.

MORE EFFECTIVE CHEMOTHERAPY

Since long-term leukemia-free survival can be achieved with standard regimens in up to 80% of children aged 3–6 at diagnosis with Common ALL, low initial lymphoblast count, and no CNS leukemia at presentation, major improvements in disease-free survival should not be expected in this subgroup. Instead, efforts must be directed toward finding better treatment of patients with less favorable prognostic factors. Three approaches are currently being investigated: more intensive combination therapy, specific drugs for each subclass of ALL, and more effective timing of the drugs.

Several studies suggest that the rate of bone marrow response and the intensity of chemotherapy during the induction phase may influence the duration or remission (6). In contrast, St. Jude Children's Research Hospital study IX showed that the addition of a fourth drug during induction did not improve the duration of remission in standard or high risk patients. Furthermore, their studies VIII and IX showed no benefit from early CNS treatment for patients with CNS leukemia, mediastinal irradiation for patients with mediastinal masses, 2 to 4 weeks of daunorubicin and prednisone for patients with delayed bone marrow response, or an intensive phase of therapy with asparaginase and cytosine arabinoside during the first 2 weeks of remission (2). Study VI at St. Jude Children's Research Hospital showed that the addition of one week of intensive therapy (6-mercaptopurine, methotrexate, and cyclophosphamide) following induction of remission did not result in greater remission duration when the same three drug combina-

tion was used for continuation therapy. A study at our center showed that addition of a 4 week course of doxorubicin and cytosine arabinoside following induction of remission did not improve the duration of remission in children treated with 6-mercaptopurine and methotrexate for continuation therapy.

Several groups are investigating other intensified regimens for the high risk patients. For example, in Study X of St. Jude Children's Research Hospital, therapy is initiated with VM-26, a podophyllotoxin, and cytosine arabinoside in an effort to reduce the lymphoblast tumor load prior to standard induction therapy.

Analysis of mouse leukemias first indicated that subclasses can differ in sensitivity to drugs (30). L1210 leukemia has surface markers similar to common cell ALL. Methotrexate or nitrosourea treatment in mice bearing this leukemia increased survival. In contrast, these drugs were not effective in mice with the AKR leukemia, a T-cell–like tumor. Vincristine was more effective in AKR than L1210 leukemias. In parallel with childhood T-cell ALL, 6-mercaptopurine or methotrexate did not appreciably prolong remissions of AKR leukemia.

The drug sensitivity of human T- and B-cell lines has been studied in tissue culture (31–33). The T-cell lines required asparagine for growth and were killed by asparaginase while the B-cell lines did not require asparagine and were relatively resistant to the enzyme. The T-cell lines were up to 80 times more sensitive than B-cell lines to cytosine arabinoside, adenosine arabinoside, 5-azacytidine, and 8-azahypoxanthine. In contrast, the B-cell lines were 10–20 times more sensitive to 5-fluorouracil. Both types had similar sensitivity to methotrexate, hydrocortisone, vinca alkaloids, and cyclophosphamide. The results with cyclophosphamide are surprising since it is the most effective drug in the treatment of human B-cell leukemia/lymphomas and has selective toxicity toward normal B lymphocytes (34).

Recent studies have shown that the adenosine deaminase inhibitor, deoxycoformycin, has selective toxicity toward T lymphocytes and lymphoblasts (35). This increased sensitivity correlates with the T-cell abnormalities noted in children with congenital absence of this enzyme activity. The mechanism for this selective action on T-cells has not been defined.

Further biochemical studies of the subclasses of normal lymphocytes, normal stimulated lymphoblasts, and leukemic cells are needed to aid in the development of other specific chemotherapy. One possible approach is to use the surface characteristics of these cells to target cytotoxic drugs. Specifically the membrane components on sheep erythrocytes that react with T cells could be covalently linked to chemotherapeutic agents. Since many leukemic T lymphoblasts can not readily dissociate from sheep erythrocytes, such a drug complex may have selective cytotoxicity toward

these leukemic cells. Similarly, binding of drugs to whole antibodies or preferably Fab (the antibody binding portion of gamma globulins) fragments toward surface immunoglobulins could target drugs to B lymphoblasts.

Alternating or cycling different drugs has also been tried in the hope that greater cell kill would be achieved and the drug resistance retarded. Zuelzer (36) reported prolongation of remission by the cyclic administration of three drugs, but did not provide a simultaneous comparison with sequential drug therapy. Children's Cancer Study Group A did a comparative study of cyclic administration of five drugs (drugs rotated every 6 weeks) versus sequential administration (single drugs given until relapse). While a longer first remission was obtained in the cyclic group, no significant difference in survival was noted. The development of drug resistance was high; consequently poor subsequent responses were generally seen following cyclic use of the drugs (37).

Recent analysis of therapeutic protocols for treatment of murine leukemia provides some guidelines for future, hopefully more successful, maintenance therapy of acute lymphocytic leukemia (10, 12). First, combinations of drugs do not prevent the emergence of resistance to the individual agent but merely delay the onset of resistance. With each treatment a smaller proportion of cells are killed. Second, elective switching from a one- or two-drug regimen to another pair produces better cure rates than concurrent combinations of the same agents. The best time for the switch is when the first regimen has produced its maximal effect. Changing combinations too early loses some of the potential benefit of the drugs while changing too late allows regrowth of resistant clones of leukemic cells. The accurate timing of the switch depends on the rate of growth of the sensitive and resistant leukemic cells, the initial fraction of resistant cells, and any selective or mutational effects of the drugs. Mathematical models have been developed but optimal timing for individual patients will require very sensitive assays to quantitate the number of remaining leukemic cells (38). Third, to avoid cross-resistance the individual drugs must not share mechanisms of transport, pathways of metabolic activation, or pharmacologic action. In addition, two drugs should not have similar effects on nucleoproteins since a single repair mechanism may counteract the effects of both drugs.

Clinical protocols are now under way in several centers to test these therapeutic ideas. Ideally, the patients should be changed to a new combination every few months without repeating any drugs since resistant cells rarely if ever revert to sensitive ones. It is not known how long children must be treated but standard therapy lasts 30 months. Five drug pairs would be needed if the combinations were changed every 6 months. Unfortunately, not enough active, non–cross-reactive agents are available. Therefore, the planned protocols repeat some of the drugs.

One drug can affect the action of another agent by altering the cell cycle, metabolic pools, transport mechanisms, repair of cellular injury, and target enzymes. Many studies have shown that the order and timing of two drugs can modify their combined effect. Some combinations of drugs used for the treatment of childhood ALL have included drugs that antagonize the action of other drugs in the regimen. Furthermore, some of the drugs in these regimens have common pharmacologic and biochemical action so that a single mechanism of resistance affects several drugs in the combination (10). Current protocols attempt to avoid these negative interactions.

Synergistic combinations require detailed understanding of the action of the drugs in each human lymphoblast subclass. Animal studies have shown that the effects of combinations of drugs can vary in different tumors. Computer models have been developed to simulate some of these interactions and help design the order and timing of combinations (39). Current promising combinations are methotrexate or cytosine arabinoside with asparaginase; thymidine with N-(phosphonacetyl)-aspartate, cytosine arabinoside, and/or 5-fluorouracil; cytosine arabinoside with tetrahydrouridine; and glutaminase with glutamine antagonists.

BONE MARROW TRANSPLANTATION

Irreversible suppression of normal marrow stem cells is the dose-limiting toxicity for total body irradiation and for many antileukemic drugs. Bone marrow transplantation allows administration of very intensive therapy by replacing the destroyed normal marrow elements.

In the early 1950s Jacobsen and Lorenz demonstrated that infusion of spleen or marrow cells could prevent death in otherwise lethally irradiated mice. Similar attempts in human beings were usually unsuccessful since most patients failed to engraft or died of graft versus host disease (GvHD) or infectious complications (40). Recently, improved supportive care and better understanding of the regulation of human histocompatibility have renewed interest in human marrow transplantation for treatment of ALL (41).

Background

The major human histocompatibility genes, HLA-A, B, and D, are closely linked on chromosome 6. HLA-A and -B loci determine serologically detectable cell surface proteins that stimulate helper T cells. The HLA-D locus determines mixed lymphocyte and cytotoxic T-cell responses. Because of Mendelian codominant inheritance, there is one chance in four that any two siblings will be HLA-A,B,D identical. The chance of a child's having at least one HLA-A,B,D identical sibling is estimated as $[1-(\frac{3}{4})^n]$ (where n = the number of siblings). Parents cannot be HLA-A,B,D compatible

donors for their children unless the parents have one haplotype (linked HLA-A, B, and D loci) in common.

The relative importance of the HLA-A, B, and D loci for successful transplantation is not known. Initially it was assumed that D locus compatibility was critical, incompatibility inevitably resulting in graft rejection or fatal GvHD. Recent reports of successful marrow grafts, without significant GvHD, between HLA-D–incompatible individuals suggest cautious reappraisal of this concept (42). ABO blood group compatibility is not essential for successful marrow transplantation (43).

Pretransplant regimens for patients with leukemia must produce immune suppression to prevent graft rejection, make space for the new marrow, and decrease the total body burden of leukemia cells. Both total body irradiation (TBI) and high dose chemotherapy are effective immunosuppresant, antitumor, and space-making modalities. However, when used alone, either 1000 rad TBI (5–10 rad/min) or high dose cyclophosphamide (45–50mg/kg/day X 4 days) results in a high rate of recurrent leukemia (41, 44, 45). A combination of 1000 rad TBI preceded by cyclophosphamide (60mg/kg/day X 2 days) produces only a modest decrease in recurrent leukemia (46). Multi-drug pretransplant regimens decrease recurrent leukemias but are highly toxic (45, 47). The best results are seen in patients who have achieved complete remissions prior to the transplantation pretreatment.

Marrow graft rejection usually results from prior sensitization to minor transplantation antigens which are inherited independently of the major HLA complex (48). The major sources of these antigens are blood product transfusions. However, because of immune suppression by prior chemotherapy, rejection is rare in leukemic recipients.

Despite HLA-A,B,D compatibility 70% of marrow graft recipients develop graft versus host disease (GvHD). Primary target organs in man are the skin, intestines, liver, lymphoid tissue, and marrow (49). Acute GvHD appears to be due to autocytotoxic donor lymphocytes. It ceases when appropriate suppressor cells develop (50). Chronic GvHD develops in 10–20% of recipients and is characterized by sclerodermatous skin involvement, chronic liver disease, and delayed return of normal immune functions. Minor transplantation antigens may be the targets of immune attack. Methotrexate, cyclophosphamide, and antilymphocyte globulin decrease GvHD in animals (51). Their efficacy as prophylactic agents in man is poorly defined. Most allogeneic transplant recipients receive intermittent methotrexate (10 mg/M^2/dose) for 100 days post-transplantation. Other approaches to prevention or treatment of GvHD include use of cyclosporin *a* (52), donor recipient sex matching (53), elimination of lymphocytes from donor marrow (51), total lymphoid irradiation (54), and germ-free protective environments.

TBI and chemotherapy each have a high capacity for killing leukemic cells (2–6 $\log_{10}$) but little specificity. An immunologic action of donor cells against residual host leukemic cells (graft verus leukemia effect, GvL) is more specific but has a low capacity. In mice, GvL requires major (H-2) histocompatibility differences (55) unless donors are preimmunized against leukemic cells (56). In recent exciting work GvL (with increased GvHD) was produced by preimmunizing H-2–compatible donor animals with white cells from H-2–incompatible strains (57). This suggests that some leukemic antigens may be altered histocompatibility antigens. Despite donor-recipient HLA identity, recent sophisticated statistical methods have associated a GvL with GvHD in human marrow transplants (58).

Established immunity is not carried from donor to recipient. Transplanted patients are immunologic neonates who must redevelop immunity via exposures and immunizations once their capacity to respond has returned (59). Immunoglobulin levels, lymphocyte numbers, T- and B-cell lymphocyte subtypes and phytohemagglutinin responsiveness normalize within 3 months. Specific mitogen responsiveness may not occur for 6 to 24 months, and specific antibody production may be absent for 6 to 18 months. An impaired switch from IgM to IgG synthesis is common during this time. Transplantation per se, GvHD, and prophylaxis versus GvHD each contribute to delayed return of immunity. Infectious morbidity due to neutropenia may be decreased in the immediate post-transplant period by use of protected environments or granulocyte transfusions (60). Interstitial pneumonias (cytomegalovirus, pneumocystis carinii) produce high morbidity (50%) and mortality (30%) in leukemic transplant recipients from 2 to 8 months postengraftment.

Results

SYNGENEIC TRANSPLANTS Twelve of 22 patients transplanted by the Seattle team have relapsed. Life table analysis predicts 33% long-term disease-free survival. Many recipients also were given additional donor lymphocytes and irradiated autologous leukemia cells post transplantation (58).

ALLOGENEIC TRANSPLANTS In 1977, the Seattle team reported 46 patients (38 less than 20 years old) transplanted for ALL (46). Only 8 were in remission when transplanted. Life table analysis predicted 15% long-term disease-free survival; only 6 patients have been continously disease-free.

Transplantation in remission might improve results by decreasing the number of leukemia cells and improving patients' general clinical status (40). In a recent report, transplantation of 22 patients with ALL during a

second or subsequent remission resulted in a projected disease-free survival of 50% (61). Other smaller series show similar results (62, 63). Further follow-up suggests, however, that this improvement is due primarily to decreased nonleukemic mortality. Whether transplantation in first remission will reduce the rate of recurrent leukemia (as has occurred in patients with myelogenous leukemia) is not known. This approach might be explored in ALL patients with poor prognostic factors.

AUTOLOGOUS TRANSPLANTS Harvesting of marrow during remission for reinfusion after later intensive therapy is being studied. The obvious limitation to this approach is the possible presence of residual leukemic cells in the cryopreserved marrow. Even if residual leukemic cells were present, autologous marrow infusions might permit more intensive therapy.

LEUKEMIC TRANSFORMATION OF ENGRAFTED MARROW CELLS In two cases recurrent ALL has been documented in donor cells by chromosome and fluorescent Y-body analysis (64, 65). The donors remained healthy. Activation or persistence of a leukemogenic agent in the recipient probably explains those cases. Inappropriate regulation of lymphoid development in an immune-compromised host is possible but unlikely because of the lack of similar cases in allogeneic marrow recipients with aplastic anemia.

Conclusions

Bone marrow transplantation is clearly the treatment of choice for patients who relapse on therapy since it can produce some long-term remissions whereas chemotherapy cannot. It requires a suitable donor and should be done only after a second remission has been achieved. This procedure might have application during the first remission in patients with poor prognostic factors if more effective chemotherapy is not found.

CNS LEUKEMIA

The prophylaxis and treatment of CNS leukemia have been reviewed in a recent symposium (66–69). Standard prophylaxis consists of 2400 rad to the cranium plus five 12 mg/M^2 doses of intrathecal methotrexate given early in remission. Cranial spinal irradiation or intrathecal methotrexate administered throughout the period of systemic chemotherapy produces comparable results, namely, 5–10% of children relapsing in this site. Methotrexate administered intrathecally only in the early phase of therapy or 1200 rad radiotherapy produce clearly inferior results.

CNS toxicity from combined radiotherapy and chemotherapy includes leukoencephalopathy and a mineralizing microangiopathy (69). Both complications appear to be caused by the combined effects of radiotherapy, intrathecal chemotherapy, and systemic chemotherapy. Therefore, studies have attempted to answer the following questions: Is cranial radiotherapy necessary? Do all subclasses of ALL need the same prophylactic CNS therapy? Can systemic high dose methotrexate chemotherapy produce adequate levels in the cerebrospinal fluid for prophylaxis? Can the dose of radiotherapy be decreased in patients with good prognostic signs? Is intraventricular chemotherapy through an Ommaya reservoir better than intrathecal administration for high risk patients? No conclusive answers are available but the current data suggest that 1800 rad cranial irradiation is sufficient for at least the patients with good prognostic signs. Systemic high dose methotrexate plus intrathecal methotrexate may be adequate prophylaxis for this group. For the high risk groups an Ommaya reservoir may allow better monitoring of the cerebrospinal fluid and more effective therapy (67).

Clinically evident CNS leukemia can be treated with intrathecal methotrexate, cytosine arabinoside, and/or cranial irradiation. If the CNS leukemia appears after 2400 rad of cranial radiation has been administered, the therapeutic options are very limited. Further cranial or craniospinal irradiation can be administered but it may lead to severe CNS toxicity. Intraventricular methotrexate can delay recurrence of CNS leukemia but may not eradicate the disease (67, 68).

New modes of therapy are needed. Recent work by Poplack and coworkers with a monkey model may provide data to optimize the administration of cytosine arabinoside, methotrexate, and asparaginase for treatment of CNS leukemia (68, 70, 71). One exciting possibility is to utilize tetrahydrouridine to allow the maintenance of high levels of cytosine arabinoside in the cerebrospinal fluid after systemic administration. Another approach is to develop new agents for intrathecal administration. Theoretically, the best candidates would be cell cycle nonspecific agents, because very few of the leukemia cells in the CSF are undergoing cell division. Unfortunately, such agents may also be highly toxic to brain cells which are also not dividing.

CONCLUDING REMARKS

The 1960s saw great advances in the treatment of childhood ALL. Although no further improvement in long-term survival occurred in the last decade, our understanding of the biology of this disease and the pharmacology of the chemotherapeutic agents was expanded during this period. The

1980s should see further improvement in the survival of children with ALL as this new knowledge is applied in the clinics.

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