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LYMPHOHEMATOPOIETIC RECONSTITUTION

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MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

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INTRODUCTION

Lymphohematopoietic reconstitution is more than a mouthful. It is a polysyllabic idea whose time has come, and with the first case (Case 1) I would like to initiate this discussion in a manner similar to the one in which I was introduced to lymphohematopoiesis.

This unfortunate animal (and untolled numbers since then), was suffering from the devastating effects of an immunologically procured disease: Graft-versus-host (1). It has been known for some time now (2, 3) that there are cells present in suspensions of normal bone marrow which are capable of inducing this disease, assuming they are provided with an appropriate immunogenic target. It is reasonable to state that while many of the problems that attend clinical bone marrow transplantation are diverse and generally unresolved, the single major deterrent to successful hematopoietic reconstitution today is the spectre of graft-versus-host disease. Only the faintest glimmers of hope are now on the horizon that suggest a way out of this dilemma; the past is littered with the remnants of dashed hopes and might-have-beens. Yet, the potential therapeutic usefulness of lymphohematopoietic reconstitution is so great that the worthwhile struggle goes on.

The early history (4) of attempts at bone marrow therapy is perhaps not a very distinguished one: reports of oral administration of marrow first appeared in the 1890s when it was claimed that such therapy improved the condition of patients with a variety of hematologic malignancies or defective blood formation. Except for its usefulness in providing iron to deficient patients, the procedure was valueless. In 1923, saline extracts of marrow were employed orally and intravenously as hematopoietic stimulants, but again any positive value could ultimately be ascribed to the mineral content of the extracts.

From the vantage point of 4 decades later, it is difficult to conceive that it was initially thought that living bone marrow cells must be injected into the medullary cavity; yet the only firm conclusion regarding the effects of such administration was that most of the injected material passed into the general circulation. While some reports enthusiastically endorsed this therapy, the course of the severe hematologic disorders for which it was intended remained unchanged. By the early 1950s, radiobiology had burst onto the experimental scene and for the first time a sound scientific basis was growing beneath the clinical desire to utilize bone marrow transplantation in the therapeutic armanentarium.

BONE MARROW TRANSPLANTATION: HOW TO DO IT

There are certain "nuts and bolts" aspects of bone marrow transplantation which might best be considered at the outset: how are the cells procured from the donor? How are they processed? How many are needed for successful engraftment? How are they administered to the recipient? These problems and their solutions are set out in a technical paper authored by E. D. Thomas and

R. Storb (5). Essentially, the donor is taken to the operating room, placed under general anesthesia (or heavy sedation and then under local anesthesia), and of the order of 50-60 aspirations, each of 1-3 ml volume, are made in the sternum, anterior and posterior iliac crests. Individual aspiration volumes are kept small to minimize admixture with peripheral blood. The aspirates are pooled and sterily passed through progressively smaller stainless steel sieves (the French omit this aspect!) to remove large particulate material. The number of nucleated cells is determined and a correction made for the estimated contamination with peripheral blood cells. From adult donors, 15 to 30 billion cells are the expected yield. The marrow suspension is then administered intravenously to the recipient with as little delay as possible. While it is difficult to arrive at a precise ratio that would describe the minimum number of hematopoietic stem cells required to reconstitute an individual devoid of hematopoietic tissues, animal experiments utilizing irradiation-induced aplasia suggest that administration of a number of cells approximating 0.1 to 0.7% of the total hematopoietic cellular mass for that individual should suffice. In man, the rough figure given is 11-74x10^6 bone marrow cells per kilogram body weight, i. e. about 1-10 billion cells (4).

Again, with the superior vision of hindsight, it is hard to imagine not knowing that bone marrow contains hematopoietic stem cells, yet workers in the late 1940s and early 50s were engaged in a major controversy as to whether irradiation-induced marrow aplasia could be reversed by clones of stem cells (6, 7, 8, 9) or by the influence of a non-cellular"radiation recovery factor" (10) which circulated in the peripheral blood. Through a series of careful observations (11, 12), involving shielding of spleens and/or long bones during lethal irradiation and reconstitution experiments using cellular suspensions or extracts of a variety of tissues, the idea generally emerged that stem cells did in fact exist within the bone marrow population in adult animals, although its cohorts could also be demonstrated in the adult spleen, in foetal liver (12), and even in the peripheral blood. Till and McCulloch (13) then placed the investigation of these cells on a firm experimental footing in 1961 by providing an assay system, the spleen colony formation assay, that allowed quantitative and qualitative measurements to be made.

ISOGENEIC BONE MARROW TRANSPLANTATION

In Irradiation Accident Victims

The patient who has accidentally received a lethal exposure to x-irradiation presents the least complicated clinical situation to resolve. Ostensibly normal, i. e. with no antecedent hematologic, or other, disease, his therapy should be aimed at tiding him over until his own hematopoietic stem cells can recover - which they doubtless will, given ample time. On October 4, 1967, a 36 year old man, employed at a nuclear reactor power plant near Pittsburg, Pa. was exposed to approximately 600 rads when four safety valves failed simultaneously (15). By providence close to divine, he happened to have an identical twin brother who was recruited as a bone marrow donor on October 12, 1967. Within 12 days the patient's peripheral WBC began to rise; platelets and WBCs had risen to

normal by one month. By contrast, another man, who in the same accident received only 400 r (and no marrow transplant), recovered much more slowly. The transplanted patient is alive 5 years later, hematologically well, but, secondary to radiation burn, is a quadruple amputee. Obviously, when both donor and recipient are genetically identical, there is no way in which recovery can be ascribed unequivocally to the attempted marrow transplant; there are in fact no markers that would identify donor cells from host cells.

Efforts have been made, utilizing studies in experimental animals and data from human irradiation accident victims, to establish rough rules of thumb regarding the effects in man of a given dose of x-rays (16). It is generally agreed that the recipients of more than 450 r are at great risk of bone marrow failure, and those receiving in excess of 1000 r will probably die a "gut" death. If an individual has the benefit of hospital care during the post-irradiation interval, the human LD_{50} seems to be about 700 r; without benefits of modern hospital care, the LD_{50} is nearer 500 r (17, 18). All of these figures are made on the assumption that bone marrow transplantation would not be carried out.

In Aplastic Anemia

E. D. Thomas has collated his series of bone marrow transplants carried out in donors and recipients who were identical twins (19). A typical case report (Case 2) indicates that for the patient with aplastic anemia, provision of isogeneic marrow may indeed be life saving (20). In this 18 year old boy it is unclear what pathogenic mechanism underlay his aplasia, i. e. drug related or not; what is clear, is that directly after the infusion of marrow cells from his twin, he underwent prompt recovery, and the recovery seemed to be sustained.

There has been much controversy raised over the pathogenic mechanisms in aplastic anemia; undoubtedly, rather than being a single disease entity, aplastic anemia is a syndrome that can be evoked by diverse pathogenic means. J. J. Trentin has introduced the concept of HIM (21), i. e. hemopoietic inductive microenvironment, to account for the fact that the colonizing ability of an hematopoietic stem cell is a function, not only of its own generative capacities, but of the microenvironment in which it happens to lodge. By definition, a hemopoietic inductive microenvironment would sustain the clonal growth of such a cell. While a pathologic derangement of HIM in the medullary cavity might account for some patients with aplastic anemia, it is difficult to see such a mechanism as being operative in the cases reported by Thomas. It would appear that in Case 2, and several others, the defect responsible for aplasia resided in the stem cell pool, rather than in the presumed HIM.

In Acute Leukemia

When all therapeutic measures fail, which they invariably do, the patient with leukemia becomes a candidate for desperate clinical maneuvers. Attempts at providing such patients with bone marrow transplants have been included in these trials, but, unlike after benign disease, failure has generally ensued. There is no dose of irradiation, short of that necessary

to fry tissues, that can with certainty destroy all leukemic cells. Consequently, the use of bone marrow transplantation in this situation is predicted upon several considerations: (1) a marrow transplant may provide a buffer that can tide a patient over the difficult interval immediately after massive chemotherapy, when his own normal hematopoietic tissues have temporarily failed; (2) if immunologic competence ensues from successful grafting, then perhaps an immunity developed by donor cells against postulated leukemia-specific antigens could lead to eradication of the disease. In Case 3 (22), such a trial has been carried out. Because of the absence of identifying markers it is still impossible to say categorically that the marrow graft "took", but the circumstantial evidence strongly favors that hypothesis. The 12 weeks post-irradiation interval during which the patient was objectively free of her disease, may represent a remission, but the responsible mechanism is unclear. Had the irradiation induced it? Was tumor immunity operative? In the final analysis, the story ends similarly to all other cases of isogeneic marrow transplants for acute leukemia: the disease recurs (19).

ALLOGENEIC BONE MARROW TRANSPLANTS

Immunologic Considerations

While the problems associated with isogeneic bone marrow grafting are formidable, those attending allogenic marrow grafts are overwhelming. The simple introduction of a new variable - immunogenetic disparity between donor and recipient - raises a host of new issues, most of which have not been resolved. If one considers a bone marrow graft in the same manner as any other solid tissue allograft, such as kidney or skin, the immunologic problem is that the cells of the graft bear antigens - transplantation antigens-which are alien to the recipient, and as such, elicit an immune response on the part of the host which, utilizing specifically sensitized cells, and isoantibodies, brings about the destruction and rejection of the graft (23). As most other tissues of the body, hematopoietic cells bear transplantation antigens (24) and early clinical experiments (in the 1950s) employing allogeneic bone marrow transplants failed, primarily because the grafts did not take, or if they did establish residence, were summarily rejected before their therapeutic potential had been realized (25). In an effort to counteract this unwanted rejection process, Mathe and his colleagues (26), and the Cooperstown, N. Y. group (27) employed whole- body x-irradiation to the recipient prior to grafting, since at that time this was the only respectable immunosuppressive maneuver available. Since then so-called immunosuppressive chemotherapeutic agents have became available, and over the past 6 years, Santos has developed an ingenious protocol for inducing "tolerance" to subsequent bone marrow grafts by employing cyclophosphamide at a critical stage in a patient's developing immunity to donor antigens (28). Based on animal experiments, in which non-transplantation as well as transplantation antigens were used, it was shown that if an individual receives an inoculum of donor leukocytes on day 0, followed within 24 hours by a lethal dose of cyclophosphamide, he will exhibit a degree of tolerance of a subsequent graft of hematopoietic tissues. The theoretical assumption is that those immunocompetent cells able to respond to that antigen will undergo blast transformation during the first 24 hours, thus rendering them particularly

susceptible to the cytotoxic action of this radiomimetic agent. In Case 4, this protocol has been utilized in a patient with acute leukemia prior to bone marrow transplantation (29). When allogeneic donors are involved, genetic markers, such as erythrocyte antigens, chromosomes, and immunoglobulin allotypes, can be used to document the presence of donor cells in the blood and tissues of the recipient, i. e. cellular chimerism can be established and documented. Clearly, in this patient the marrow graft did in fact become established. Moreover, the experience in Santos' hands (30), and in other centers has been that Cyclophosphamide in this protocol can generally lead to the establishment of a successful transplant.

Graft-Versus-Host Disease

Clinical Description

Nonetheless, the outcome of this case, with severe diarrhea and hepatitis leading to death by infection with opportunistic organisms underscores the major problem confronting bone marrow transplantation today: lethal graft-versus-host disease. Experimentally, graft-versus-host disease can be induced when the following conditions are met: (a) the donor inoculum contains immunologically competent lymphoid cells, (b) the host tissues express transplantation antigens foreign to the donor cells, (c) the host, for any of a number of reasons, is unable to destroy the grafted donor cells (31). When these conditions are satisfied, the disease that ensues can be described in two phases: an acute phase, during which the human host generally suffers severe dermatitis, exhausting diarrhea, and fulminant hepatitis; followed by a chronic phase during which the acute symptoms subside, and the host is left with only partial immunologic competence, being most deficient in cell-mediated modalities (32, 33).

It has been established in experimental animals, ranging from chicken to mouse to dog, that if donor and host differ at the major histocompatibility locus operative in that species, graft-versus-host disease will be severe and usually lethal (34, 35). If donor and host share the same major antigenic determinants, but differ at few to many minor loci, the incidence and severity of the GVH process is much reduced. One of the spurs to increased clinical bone marrow transplantation in the past 5 years has been the incredibly fast and sophisticated advance made in human tissue typing. Within 15 years, the major human histocompatibility locus, HL-A, has been described (36, 37, 38), its two sub-loci (4 and LA) identified, the thirty odd antigenic specificities ascribed to either sublocus, and the means of establishing the parental origins of these genes devised. The ABO isohemagglutinin locus has also been documented as a transplantation antigen system, but its role in GVH disease is obscure (39).

On a smaller scale, a similar advance has been made in dog research, and Thomas' group (40) has clearly established that tissue typing makes a critical difference in the success of bone marrow transplantation in dogs, both in terms of graft "take" and chimera establishment, and ameliorating graft-versus-host disease. In Case 5 (41), a child receiving a bone marrow transplant in an effort to reconstitute a potentially lethal cell-mediated immune deficiency disorder is described. The graft from the HL-A identical sibling became established, reversed the immunologic deficiency, and induced a moderately severe, but transient, non-lethal graft-versus-host process.

Lest a tyranny emerge that allows mouse data to be translated directly to man, Case 6 (42) is presented in which a patient with aplastic anemia has been given an allogeneic bone marrow transplant from an HL-A matched sibling. The success of the transplant was apparent in the return of platelets and the resolution of the leg infection. However, the appearance of diarrhea on day 28 served as a harbinger of a severe and ultimately lethal graft-versus-host disease - despite the fact that donor and host were determined to be by present day criteria HL-A identical! Why is man so unlike the mouse in this regard? Why is lethal GVH still possible even when donor and host are matched at the major locus? One could ascribe the difficulty to incorrect or inadequate tissue typing, but the number of cases now in the literature are too numerous to allow that interpretation. Could it be due to the admixed peripheral blood lymphocytes which considerably contaminate human marrow aspirations, but are insignificant passengers when, as is done in mice, the femur is amputated and the marrow plug removed?

Pathogenesis

Whatever the reason for the discrepancy between GVH disease in laboratory animals and man, the resolution of the problem will depend at least in part on an understanding of the pathogenic mechanisms underlying GVH disease (43). In a brief summary of what we know to date, it seems certain that the attacking cells in GVH disease are immunocompetent lymphoid cells (31) and that isoantibody, which may be directed at the same tissue antigens, plays little or no destructive role. When transplantation immunology was born two decades ago, its chief wet-nurse, Sir P. B. Medawar, stated that, with rare exception, transplantation antigens were expressed on all tissues of the body, and were identical from tissue to tissue within the same individual (44). Thus, when studies were carried out on the immunogenetic specificity of the cutaneous lesions in graft-versus-host disease, it was found that skin allografts of the same genotype as the attacking donor cells develop no cutaneous lesions when the GVH disease is ravaging the host's own integument (45, 46). When hemolytic anemia accompanying GVH disease was studied, some reports indicated that only host erythrocytes were lysed (47, 48), while others reported that erythrocytes of both donor and host genotype were subject to the hemolytic process (49). The issue of immunogenetic specificity was challenged directly by Ramseier and Billingham (50) who demonstrated that in hamsters local GVH reactions incited in skin by the inoculation of homologous lymphoid cells depended for their antigenic challenge completely upon circulating mononuclear cells, while the cells of the skin and their antigenic components were irrelevant. In studies on toxic epidermal necrolysis in Syrian hamsters, a syndrome characteristically evoked in that species by a systemic GVH process, it was unequivocally shown that destruction of the epidermis was obtained irrespective of the transplantation antigens it expressed, and depended exclusively on the antigenic specificities born by leukocytes circulating in the peripheral blood of the host (51). Similarly, while hamsters with GVH disease exhibit accelerated red cell destruction, the destructive process pays no mind to the antigenic identity of the erythrocytes on which it preys (52).

Since graft-versus-host disease is primarily a cell mediated immune phenomenon, one can construct hypothetical models to explain the non-specific aspects of tissue destruction. We now know that activated lymphocytes elaborate a number of pharmacologically active substances: (a) lymphotoxin - a non-antibody macromolecule that kills innocent bystander cells by an unknown mechanism (53); (b) lymph node permeability factor - capable of generating a typical delayed hypersensitivity-like lesion in the absence of antigen (54); (c) macrophage migration inhibitory factor - which accelerates the accumulation of macrophages at the site of its secretion (55). Moreover, disseminated intravascular coagulation has been documented in some animals suffering graft-versus-host disease (56), just as localized intravascular coagulation has been implicated in the pathogenesis of solid tissue allograft rejection.

Attempts at Abrogating GVH

Based on this rudimentary understanding of the GVH process, certain attempts have been made to abrogate the GVH nemesis.

1. By treating the host with non-specific immunosuppressive agents such as cyclophosphamide (57) and methotrexate (58). There are proponents of each drug (59), but the hard facts are that neither is very effective at controlling a difficult GVH situation.

2. By employing anti-lymphocyte globulin, a (relatively-speaking) more specific immunosuppressive agent. Monaco and Russell (60) were one of the first to show in a systematic study that this material could blunt the development of transplantation immunity in mice. Other reports have indicated that the GVH reaction could be suppressed especially if the donor, or the donor cells in vitro, were treated with ALG (61, 62). Mathe (63) was the first to utilize ALG in a clinical setting for bone marrow transplantation and his first experiments involved treating both donor and recipient. His reports of these cases are impressive in that little or no GVH disease was seen. However, the rest of the world shied away from treating the donor, primarily because of the possibility of developing lymphatic neoplasm.

3. Dicke and van Bekkum (64) have championed the idea of selectively removing immunocompetent cells from donor marrow suspensions by discontinuous density gradient centrifugation. And in fact, they have reported the successful grafting of one child with lymphopenic immune deficiency using bone marrow cells treated in this manner (65). Investigators in Toronto, Canada (66) have employed a no-centrifuge, 1XG method of selective separation, in an effort to increase the yield of hematopoietic stem cells, many of which are lost in the Dicke-van Bekkum procedure. In other hands, neither technique has been particularly successful, and unless a new wrinkle comes onto the scene, it is unlikely that there is much hope in this direction of research.

4. To date, no selective inhibitors of any of the lymphocyte-derived, pharmacologically active mediators has been devised, but this seems a useful avenue of approach. Systemic heparin administration has been employed in hamster graft-versus-host disease (64), and has not ameliorated the disease.

If anything, it has resulted in increased loosening of the dermal-epidermal junction such that epidermal necrolysis is more complete and synchronous. Its effect on the hemolytic process remains unexplored.

5. By removing the target tissue from the host. There is much circumstantial evidence that indicates that host lymphohematopoietic tissues bear the major, if not exclusive, brunt of the donor cell attack (51), and if these tissues can be reduced in quantity, the disease is abated (68). While there seems no rational way to reduce permanently the total lymphohematopoietic mass in man, an alternative method would be to mask host target tissue antigens. For example, Fab fragments of isoantibodies directed at host tissue antigens can mask the presence of these determinants, but yet not lead to the lysis of the host tissue cells since the complement activating F_c fragment has been removed. Batchelor (69) has in fact done this kind of experiment by treating a potential renal allograft recipient with Fab fragments directed at the antigens present only on the graft, and has thereby secured prolonged tenure for a graft that conventionally would have been summarily rejected. In addition, the Hellstroms (70) have identified a blocking antibody in the serum of canine recipients of allogeneic bone marrow grafts that demonstrate stable cellular chimerism and little or no evidence of GVH disease. This kind of observation had been predicted by Voisin (71), and others (72, 73), who showed that isoantibody directed at either host or donor cells, in a GVH set-up, could abrogate the disease.

Case 7 represents the first attempt to bring this experimental evidence to bear at the clinical level (74). In the absence of a histocompatible sibling Buckley et al were forced into considering the use of maternal bone marrow cells despite the fact that the mother's serum contained cytotoxic antibody directed at HL-A antigens on both the father's and the patient's leukocytes. By producing Fab fragments of this maternal antibody, and by pretreating the patient and the donor cells with this preparation, circumstantial evidence was obtained showing that engraftment had taken place with only very minimal evidence of graft-versus-host disease. Whether the graft did in fact "take" in this case is less important than that the principle had been clearly understood by the clinical investigators and applied to the fullest extent of our experimental knowledge (75).

GVH and Leukemia

The existence of a mechanism that can potentially abrogate the graft-versus-host process raises yet another problem. Early in Mathe's work on human bone marrow transplantation, he voiced the idea that while severe GVH was clearly to be avoided, mild GVH might be important and perhaps essential to the success of bone marrow transplants done in leukemic subjects (76). He reasoned that leukemic cells, by some ill-defined means, might be particularly susceptible to the destruction of the GVH process and be destroyed preferentially, vis-a-vis the normal hematopoietic cells. He suggested that leukemia-specific antigens might be involved. Experimental evidence in support of this hypothesis has been slowly forthcoming in animal leukemia models (77, 78, 79), and as of now the evidence is far from overwhelming. Case 8 (80) represents an example of a patient with leukemia who sustained a remission for a considerable period after grafting with HL-A identical marrow. One wonders whether in this particular case the history of 7 previous remissions isn't at least as significant a determinant as the transplant.

It was only a matter of time before a patient, such as Case 9 (81) would be reported. A patient with acute lymphoblastic leukemia was given a bone marrow transplant from an HL-A sibling of the opposite sex. While evidence favoring a remission was scant, when the leukemia did recur, it was almost certainly found in cells of donor origin. The donor remained free of leukemia throughout. For this and other reasons, it would appear that the patients least likely to benefit from bone marrow transplantation are those with leukemia, despite the fact that they now constitute the bulk of patients undergoing this procedure.

CURRENT STATUS OF HUMAN BONE MARROW TRANSPLANTATION

In 1970, M. M. Bortin (82) provided an invaluable service by painstakingly collating all of the reported instances of human bone marrow transplantation published to that date and published the compendium in the journal Transplantation. Essentially, the compendium includes transplants done before the era of sophisticated tissue typing and improved patient care facilities and procedures. By dividing the list into four general categories, an interesting association emerges.

The Results of 203 Reported Human Bone Transplants

Disease	No. of Patients	No. with no Engraftment	No. with Secondary Disease	No. of Allogeneic Chimeras
Aplastic anemia	73	66	5	0
Leukemia	84	33	32	3
Malignant disease	31	23	1	1
Immune deficiency	15	3	11	7
Total	203	125	49	11

The incidence of graft "takes" was very low in patients with aplastic anemia or non-hematologic malignancies. A much higher percentage of "takes" was seen in patients with immune deficiency disorders or with hematologic malignancies. These findings doubtless reflect variation in the native immunological competence of these patient groups. As expected, the presence of engraftment carried with it the almost exclusive guarantee of lethal graft-versus-host disease.

Since Bortin's report, 5 major groups performing clinical bone marrow transplantation have reported summaries of their findings (30, 80, 83, 84, 85). While there are individual differences in technique and patient care, there is enough homogeneity to come to certain general conclusions: 1. Bone marrow transplantation is a superb form of definitive therapy for the patient with marrow aplasia, whether x-ray induced, drug-induced, or idiopathic, if an identical twin donor is available.

2. Bone marrow transplantation offers little hope for the leukemic, even with an identical twin. If means are found to take advantage of the immunologic competence of the graft and direct its activity preferentially toward the leukemic cells, this view would have to be altered.

3. Bone marrow transplantation is a reasonable form of therapy for the child with immune deficiency disorder, especially of the lymphopenic type, if an HL-A identical sibling is available as donor. Permanent reconstitution is a distinct possibility.

4. Bone marrow transplantation can be a last ditch attempt to reconstitute the patient with aplastic anemia who has an HL-A identical sibling available as donor.

THE FUTURE OF BONE MARROW TRANSPLANTATION

If among other problems GVH disease is licked, the clinical usefulness of bone marrow transplantation would be considerably expanded. Not only would patients with immune deficiency disorders and aplastic anemia be benefited, but it is reasonable to expect that certain congenital disorders of erythrocytes and hemoglobin could be corrected by wiping out the patient's own marrow and providing him with a marrow containing the normal array of genes. Since hematopoietic tissues and their progeny comprise a significant bulk of actively metabolizing tissues, it is even possible that certain inborn errors of metabolism whose expression depend upon the accumulation of a non-metabolizable molecule, such as in Phenylketonuria, could be averted by providing the affected individual with a population of cells that could rescue the phenylalanine and phenylpyruvic acid block by converting them into tyrosine and beyond.

Lastly, and certainly on the very distant horizon, is the hope that certain genetic molecular defects could be corrected by providing the missing enzyme(s). It is already possible in vitro to create cellular hybrids from two genetically disparate individuals by fusing their cells with Sendai

virus (86). Hematopoietic stem cells of patient origin, fused with cells of another individual possessing the normal gene for the trait in question, could then be returned to the patient where it could clone out, without care that it also carried the other individual's transplantation antigens.

Case 1. OCCUPATIONAL HAZARD

[redacted] was a 4 month old agouti hamster female who was first seen in [redacted], 1967 with the chief complaint of painful skin. She had been well until 3 days p.t.a. when she noted malaise, fever, and erythrodermia. General pruritis ensued, and on the day of admission her cage mates complained of her being obstinate and irritable. With the exception of mild arthralgias, her review of systems was negative. On family history it was discovered that her mother was an albino MHA and her father a member of the CB agouti strain. Pregnancy and delivery were uneventful. The patient was employed in a [redacted] at the [redacted] where 7 days prior to admission she had undergone skin testing as part of an ongoing investigation into the mechanisms of delayed hypersensitivity. On physical examination she was well-developed and well nourished, but very argumentative - to the point of having bit the hand of the examining physician. Nonetheless, it was determined that she had a temperature of 39.6, tachycardia, generalized lymphadenopathy, massive hepatosplenomegaly, and a generalized cutaneous erythema. At the site of the previously placed skin tests, the overlying skin was devitalized and could be peeled off with ease. Within 24 hours, a positive Nikolsky sign could be elicited over her entire body. Despite efforts at therapeutic intervention, the patient proceeded to shed her entire epidermis, became disoriented, and stopped eating and drinking. Her weight dropped precipitously. She was found dead in her cage 10 days after admission. Post-mortem examination revealed atrophy of all her lymphatic organs, and evidence of terminal pneumonia.

Case 2. APLASTIC ANEMIA RECONSTITUTED WITH ISOGENEIC BONE MARROW

An 18 year old boy developed sore throat on [redacted], 1964. His physician found a few enlarged, tender posterior cervical lymph nodes. Heterophil was 1:100. After slight improvement on Tetracycline, his fever returned and his pharynx was coated with purulent exudate. Chloramphenical was given for 4 days without improvement. His gums swelled and began to bleed; he was admitted to the hospital on October 13 with these findings There was no hepatosplenomegaly, but a small ecchymotic area was seen over the left iliac crest. WBC was 900/mm^3, 99% lymphocytes; hematocrit - 20%; platelets -10,000/mm^3; reticulocytes - 0.1%. Aspirated sternal marrow contained numerous particles filled with stroma, a few plasma cells and lymphocytes but no other normal marrow elements. Further review of history was noncontributory.

A twin brother was found by blood grouping to have a high statistical likelihood of monozygosity and on the 2nd hospital day, the normal twin was anesthetized and marrow aspirated from sternum and both iliac crests. 2.17×10^9 nucleated marrow cells were administered to the patient that day. The patient remained febrile for 4 days, but then the inflammation and swelling of the pharynx and gums subsided. Gum bleeding stopped within 2 days. By 6 days evidence of new cellular elements appeared in the peripheral blood, and marrow then revealed regenerative clumps of erythroid and myeloid elements with scattered megakaryocytes. By December 11 the marrow was normal as was the peripheral blood and the patient remained well 11 months later.

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Case 4. "TAKE" OF ALLOGENEIC MARROW IN PATIENT WITH ACUTE LEUKEMIA

This patient was a 33 year old female with a diagnosis of acute monocytic leukemia who had never been pregnant nor transfused. By lymphocytotoxicity typing, no leukocyte antigen differences were found between a brother donor and the sister patient, however mixed leukocytes cultures were not performed. On Day 0, 500 ml of donor (brother) whole blood was administered to the patient intravenously. Cyclophosphamide (60 mg/kg) was then given on days 1, 2, 3 and 4. On day 5, bone marrow was aspirated from the donor and 26×10^9 nucleated marrow cells delivered intravenously to the patient. Hematologic recovery was evident at 10 days, and in the circulating leukocyte population were cells cytogenetically identified as being of donor origin, as well as RBCs bearing antigens unique to the donor. At one month the patient appeared well and was sent home. One week later she returned with progressively severe dermatitis, diarrhea and jaundice. Enzymatic evidence of extensive hepatitis was documented in the serum. The patient developed herpes zoster and died with bilateral pneumonia. At the time of death, all cells cultured were of male karyotype. At autopsy, the lymphoid tissues were depleted, but there were good cellular elements in the marrow. Massive hepatic necrosis was found, as was necrosis in pancreas, and myocardium.

Case 5. CELLULAR IMMUNE DEFICIENCY TREATED WITH HISTOCOMPATIBLE ALLOGENEIC BONE MARROW

[redacted], a female infant, was first evaluated at 2 weeks of age. The only positive physical finding was a mild seborrheic rash. Laboratory studies revealed a hemoglobin of 18, WBC -6850/mm^3 with 33% polymorphonuclears, 49% lymphocytes, 11% monocytes and 7% eosinophils. Immunoglobulins were (mg/100ml), IgG 700, IgM <2, IgA <2. The lymphocytes responded subnormally to phyto-hemagglutinin (PHA). Subsequently, the lymphocytes became completely unresponsive to PHA. Peripheral eosinophilia and thrombocytosis appeared. No thymus shadow was seen on tomograms of the mediastinum. Over the next 6 months a maculopapular erythematous, desquamating eruption appeared, initially involving only the scalp and then spreading to the entire body. Hair on the scalp and eyebrows became scant.

At 6 months of age the patient was admitted to the hospital for detailed immunologic evaluation and possible bone marrow transplantation. The physical examination revealed a chronically ill infant. She was in the 10th percentile for height and weight.

Studies showed deficient humoral and cellular immunity. A bone marrow biopsy revealed megakaryocytosis, eosinophilia and the presence of plasma cells. Biopsy of a stimulated node showed generalized depletion of lymphocytes and poor follicular formation.

A normal 6-year-old sister (the only surviving sibling) was found to be histocompatible with the patient as determined by cytotoxic assay and mixed leucocyte culture.

Case 5 (continued)

The patient received an intraperitoneal infusion of 45 ml of bone marrow from the normal sibling consisting of approximately 1.25×10^3 nucleated cells.

Over the next 5 months the patient improved. Evidence of engraftment was obtained: 1-increased responsiveness of the lymphocytes to PHA, 2-development of cutaneous reactivity to DNFB. A decrease in the peripheral eosinophilia and generalized improvement in the appetite and disposition occurred. Nevertheless, no specific humoral antibody responses could be demonstrated. These findings were interpreted as an indication that only partial immunologic reconstitution had been accomplished. Accordingly a second bone marrow transplantation from the same donor was performed at 11 months of age. Marrow from the normal HL-A matched sibling was again utilized. Approximately 9×10^9 nucleated cells were given. One week following the second transplant the eosinophils decreased to 2%, the platelets decreased to 326,000 the lymphocytes now transformed normally when stimulated by PHA, and the immunoglobulins were (mg/100ml) IgG720 IgM43, IgA110. Evidence for further immunologic reconstitution following this marrow transplant was thus obtained.

The patient continued to do well until the 40th day following the second transplant. At this time a faint erythematous, maculopapular rash was observed primarily on the trunk. During the following weeks the rash increased in severity and extent and was associated with eosinophilia and thrombocytosis. It was felt at this time that the patient was experiencing a mild graft versus host (GVH) reaction. Over the next 4 weeks an episode of otitis media, upper respiratory illness, and DPT immunization, each occurring as separate events, resulted in an exacerbation of the rash, eosinophilia and thrombocytosis.

Eight weeks following the second transplant the patient was admitted to the hospital with fever and cough. Physical examination revealed erythematous, waxy skin with marked desquamation, loss of hair, eyebrows, and eyelashes and splenomegaly. Laboratory studies revealed a 40% eosinophilia, 926,000 platelets/mm^3. A skin biopsy revealed marked epidermal infiltration with lymphocytes, basal cell layer vacuolization, dyskeratosis and epidermal thickening, all characteristic of a graft versus host reaction.

Thirteen weeks following the second transplant the patient continued to show desquamation of the skin and had several febrile episodes with temperatures up to 104-105°F. Prednisone (5 mg/day) was given in an attempt to decrease the GVH reaction. However, because of hypertension (160/110), the steroid was discontinued after 3 days. Nevertheless, gradual improvement occurred with a cessation of desquamation of the skin, no further hair loss, return of temperature to normal and decrease of platelets, eosinophil counts and OCT to normal. The only indication of liver involvement was the transitory elevation of the OCT.

Gradual improvement in the patient continued. Twelve weeks following the second transplant no evidence of skin rash was present and scalp hair, eyebrows, eyelashes and new nail growth was present. The patient continues to do well 1 year following the second transplant.

Case 6. SEVERE GRAFT-VERSUS-HOST DISEASE FOLLOWING RECONSTITUTION OF APLASTIC ANEMIA WITH HISTOCOMPATIBLE BONE MARROW

A 60-year-old woman noted easy bruisability in [redacted], 1969. She was found to have pancytopenia, which was treated first with red-cell transfusions and later with platelet-transfusions as well. In [redacted] and [redacted] 1970, she received a course of oxymethalone without benefit. She became refractory to random donor platelets, which survived less than 1 hour. She then developed an indurated erythematous right lower leg, from which gram-positive cocci were cultured; this infection was treated with penicillin. On October 1, a platelet-transfusion from the intended marrow-donor showed a normal increment and a 6-day survival.

The patient was admitted on [redacted] 1970, for marrow transplantation from a brother. Her temperature was 38.2°C, and physical examination was negative except for the infection of the right lower leg. The hematocrit was 22, platelet-count 4800 per c.mm, and white blood-cell count 2300 per c.mm, with 15% granulocytes and 85% lymphocytes. Marrow aspiration showed acellular particles, and cytogenetic preparations showed no metaphases. She was given cytoxan, 50 mg. per kg, on the evenings of [redacted] On the morning of [redacted] she was given 21.1×10^9 marrow cells from her brother. Donor and recipient were red-blood-cell type O and HL-A identical. Successful engraftment was indicated by the rising white-blood-cell count beginning on day 7 and followed by evidence of production of platelets and reticulocytes. Cytogenetic studies showed the normal 66-year-old brother to have only XY cells in the blood but a mosaic of XY and XO in the marrow. After engraftment the recipient showed the same pattern as the donor and no XX metaphases were found. With the reappearance of granulocytes the infected area of the right lower leg became fluctuant, and incision and drainage disclosed pus and micrococci. The infection cleared rapidly after drainage. On day 26 after marrow-grafting, a fine maculo-erythematous rash was noted over the lower back. Skin biopsy on days 29 and 34 both showed focal basal layer vacuolization and necrosis, hyperkeratosis, superficial dermal necrosis, and moderate superficial dermal mononuclear-cell perivascular exudation. On day 28 the patient developed mild diarrhea. The skin rash and diarrhea rapidly became more severe, and S.G.O.T. rose to 340 L.U. per ml and bilirubin to 4.3 mg per 100 ml. The patient had intermittent low-grade fever, and on day 36 Escherichia coli was cultured from her blood. She was treated with ampicillin. The platelet-count and white-blood-cell count then dropped progressively. Her condition continued to deteriorate, with severe diarrhea and a rise in bilirubin to 10 mg per 100 ml. On day 45, an ampicillin-resistant enterobacter was isolated from the blood, bacteremic hypotensive shock developed, and she died.

Necropsy showed granulocytic hyperplasia of the marrow, marked lymphoid depletion, and necortising esophagitis with many gram-negative rods. The gut showed changes typical of graft-versus-host reaction, with patchy mucosal ulceration and chronic inflammation, and dilatation and cellular atypia of deep mucosal glands.

Case 7. PROBABLE RECONSTITUTION OF LYMPHOPENIC IMMUNOLOGIC DEFICIENCY WITH HISTOINCOMPATIBLE BONE MARROW AND ENHANCING (BLOCKING) ANTIBODY

The patient was born on [redacted] 1968, to a para 16-0-8 female. Pregnancy was full-term and uncomplicated; birth weight was 6 lb. 8 oz.; sex, female. The infant appeared to be well until two months of age when she developed pneumonia necessitating admission. Seven of the infant's 15 siblings had died prior to four months of age with infection. There was no history of early death in paternal relatives, but two maternal uncles and four maternal first cousins died in infancy of unknown cause.

On admission the patient weighed 4.1 kg and measured 56.5 cm in length. Numerous darkly pigmented circular lesions were noted over her forehead and body. Oral moniliasis was marked. Tonsils were not seen and lymph nodes were sparse. White blood cell count - 4,300/mm^3 with 17 large lymphocytes and no small lymphocytes observed on the differential count. Culture of a tracheal aspirate grew many gram negative organisms. P. carinii cysts were not seen. Chest x-rays showed an infiltrate in the right middle lobe. Thymic and adenoidal shadows were absent by x-ray. Serum antibodies and immunoglobulins A, M, D, and E could not be detected. The serum IgG concentration was 165 mg/100ml. Skin tests with undiluted goat anti-IgE antibody and 50 PNU Candida albicans antigen failed to elicit immediate and delayed cutaneous responses respectively. The infant's cultured peripheral blood lymphocytes were unresponsive to phytohemagglutinin, allogeneic cells, or candida antigen.

Histocompatibility testing of the patient, her parents, and her eight healthy siblings by LCT surprisingly demonstrated that no siblings were HL-A compatible with the patient, although there were several instances of HL-A compatibility between various of the healthy siblings. MLC studies, however, unexpectedly revealed a failure of maternal leucocytes to respond to stimulation with mitomycin-C treated paternal or patient leucocytes. Further experiments demonstrated this to be related to the presence of maternal plasma in the culture medium. Antibody cytotoxic to paternal and patient lymphocytes was detected in the mother's plasma.

In view of the infant's rapidly deteriorating clinical condition immunological reconstitution with maternal bone marrow cells was attempted. The infant was given small volumes of maternal plasma subcutaneously and intravenously - all with no evidence of local or systemic effect. A dose of 25 ml/kg body weight (85 ml total) of plasma was then infused intravenously over a two hour period with careful monitoring of vital signs and blood cellular elements. The only observed effect was a transient lowering of the polymorphonuclear count with return to normal within 12 hours. Twenty-four hours after the plasma infusion, $5x10^6$/kg body weight ($17.6x10^6$) immature maternal marrow cells were obtained from fraction #2 of a discontinuous albumin gradient and administered to the infant intravenously in 35 ml of maternal plasma on [redacted]/69. Plasma infusions were empirically continued at a dose of 10 ml/kg body weight on alternate days for the first 39 days post-transplant. Beginning one week after the marrow cell infusion the infant's clinical status began to improve. Growth increased dramatically during the first three months during which time no overt signs of GVH disease were manifested. Thrush disappeared by the 17th post-transplant

Case 7 (continued)

day, and the pneumonia gradually cleared. Although very little increase occurred in the absolute lymphocyte count and PHA responsiveness of her peripheral blood was not significant, a strongly positive delayed cutaneous response developed following skin testing with C. albicans antigen by the 13th post-transplant day. Beginning 22 days following the marrow cell infusion, a sharp increase occurred in the absolute eosinophil count and persisted for approximately one month. Mild elevations of liver enzymes were noted during this same period of time.

Leukopenia developed at approximately two months post-marrow cell infusion and persisted for two months. The patient developed pneumonia at 5 months post-transplantation and thrush recurred. Resumption of maternal plasma infusions and intensive antibiotic therapy at that point had little effect on the progression of the pneumonic process.

Because of her worsening condition, a repeat bone marrow infusion was performed five months after the first, on [redacted]/70, when the infant was 11 months of age. Her course following this transplant was similar to that following the first in that resolution of infections and a significant gain in weight occurred. Maternal plasma infusions were again empirically administered on alternate days for six weeks. Beginning at 5 days post-transplant a marked eosinophilia developed, similar to that following the first transplant. Photophobia and leukopenia also occurred transiently during the period of eosinophilia.

Pneumonia recurred at 15 months post-maternal marrow infusion #1, when the patient was then 18 months of age. A lung biopsy was performed and the microscopic appearance was compatible with Pneumocystis carinii pneumonia. Despite institution of therapy, the infant died suddenly following a grand mal seizure two days later. Permission for postmortem examination was not granted.

Case 8. PROLONGED REMISSION IN LEUKEMIC TREATED WITH HISTOCOMPATIBLE ALLOGENEIC BONE MARROW

A 10-year-old boy had the diagnosis of acute lymphoblastic leukemia made in [redacted] 1964. Therapy with 6-MP resulted in a complete remission, which was maintained with oral 6-MP until [redacted] 1965.

At time of admission on [redacted], 1970, the patient had a history of seven complete remissions spanning a period of 6 years. He had not required a previous transfusion. Physical examination showed no hepatosplenomegaly or lymphadenopathy. The hematocrit was 34%, and the total white blood cell count was 19,900/cu mm, with 3100 granulocytes, 4200 lymphocytes, and 12,000 lymphoblasts. The platelet count was 156,000/cu mm. A marrow aspirate showed 90% lymphoblasts. To reduce the load of leukemic tissue he was given a single dose of 15 mg/kg of CY and 5 daily i.v. doses of prednisolone, 500 mg/sq m. This decreased the lymphoblasts in the peripheral blood but had no effect on the marrow. On [redacted] 1970, the patient received whole-body irradiation and,

Case 8 (continued)

24 hours later, marrow from his sister.

The first 30 days were complicated only by esophagitis in the first week, which responded to oral antacids. The patient's platelet count was maintained above 50,000/cu mm by transfusion of platelets obtained from his parents. He was removed from isolation on day 25. On day 29 the marrow was hypocellular but showed islands of normal marrow elements.

The patient was discharged on day 37 and followed as an outpatient. Cutaneous and gastrointestinal manifestations of GVH were not observed. However, an elevation of serum bilirubin and alkaline phosphatase were first noted on day 37. Over a period of 2 wk the bilirubin rose to 9.5 mg/100 ml, the alkaline phosphatase to 610 IU, and the SGOT to 471 IU. The patient noted malaise associated with poor appetite. The liver was palpable 5 cm below the right costal margin. On day 65 his bilirubin had fallen to 2.5 mg/100 ml, his liver was smaller, and the SGOT had dropped to 220 IU. The alkaline phosphatase remained elevated. He was symptomatically improved. The hepatic dysfunction was accompanied by an hemolytic anemia with a rise in the corrected reticulocyte count to 7%, and a titer of 1:1000 of a cold reacting anticomplementary RBC agglutinin with I specificity. The patient's marrow was examined on day 86 and no evidence of leukemia was present.

The patient then continued to be clinically well except for some fatigability. Peripheral blood counts have been within normal limits except for a hemoglobin of 10 g. Marrow examination on day 167 showed normal cellularity but there were scattered islands of lymphoblasts. A repeat marrow examination on day 195 was interpreted as being within normal limits.

Cytogenetic studies showed that all 20 mitoses examined on day 86 and all 18 examined on day 167 in preparations of uncultured marrow had the female donor XX sex chromosomes. Also on day 167, marrow was cultured with phytohemagglutinin and all 40 mitoses examined were of donor type.

The patient continues to do well 310 days after grafting. His performance has been normal including school and sports. He has no skin rash and no diarrhea. The liver is palpable 2 cm below the right costal margin, but liver function tests are normal. The white blood cell count ranges between 9700 and 12,000/cu mm with 29%-45% lymphocytes of normal morphology. The hemoglobin is 12.6 g/100 ml and the platelet count 200,000/cu mm. The marrow is of normal cellularity without evidence of leukemia.

Case 9. LEUKEMIC TRANSFORMATION OF ENGRAFTED ALLOGENEIC BONE MARROW CELLS

In [redacted] 1969, acute lymphoblastic leukemia was diagnosed in a 16-year-old girl on the basis of a blood-cell count of 53,000 per c. mm. with 44% lymphoblasts and a marrow extensively replaced with lymphoblasts. From [redacted] 1969, to [redacted] 1970, inclusive, intermittent treatment with various combinations of drugs, induced several brief remissions. She was admitted to

Case 9 (continued)

the hospital on [redacted], 1970, for evaluation of decreased vision. Bilateral papilloedema and a sixth-cranial-nerve palsy were noted. The peripheral white-blood-count (WBC) count was 3900 per cu. mm. with a normal differential, but 15% of the marrow cells were lymphoblasts. She was considered to have active central-nervous-system leukemia, and an early marrow relapse.

On [redacted] 1970, she received 1000 rad midline tissue dose of total-body irradiation over a 4-hour period. The next day 14.6×10^9 nucleated marrow cells from her 10-year-old HL-A matched brother were infused intravenously. 2 weeks after the marrow infusion the WBC count reached a nadir of 20 cells per cu. mm. and then rose progressively to 1500 per cu. mm. on day 19. At that time focal areas of regenerating normal marrow were seen. On day 27 she had a moderate skin lesion typical of graft-versus-host reaction which was apparently controlled by methotrexate. By day 52 she was well enough to be discharged from the hospital.

10 days later marrow examination showed normal cellularity with islands of lymphoblasts occupying 20-30% of the marrow. By day 76, 80% of the marrow cells and half of the 19,700 per cu. mm. peripheral WBC were considered to be lymphoblasts. Therapy with vincristine, prednisone, and 6-mercaptopurine led to severe marrow hypoplasia with persistence of lymphoblasts. Despite support with platelet and granulocyte transfusions septicemia developed, and the patient died on day 102.

The marrow donor had no symptoms or significant abnormalities on physical examination. No abnormalities were noted on examination of peripheral blood or marrow in [redacted], 1970. 6 months after the donation of marrow there was no evidence of leukemia in marrow or peripheral blood.

Marrow and peripheral blood mitoses were 46,XY in the normal male donor.

The marrow and/or blood of the patient was sampled eight times beginning on day 15 and ending on day 84. All 240 mitoses examined in preparations of uncultured marrow and blood had sex chromosomes compatible with 46,XY and the great majority (96%) of cells contained 46 chromosomes. At a time of florid leukemia, each of 45 dividing uncultured cells from the marrow on day 79 had a Y chromosome as judged by morphological and fluorescent criteria.

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