Chediak–Higashi Syndrome: Reversal of Increased Susceptibility to Infection by Bone Marrow Transplantation


Transplantation of normal bone marrow to mice with the Chediak–Higashi syndrome (CHS) resulted in normal granulopoiesis and a reversal of their increased susceptibility to challenge with intravenous Candida albicans. These findings suggest that (1) the leukocyte defect in CHS can be reversed by marrow transplantation and (2) the mechanism for increased susceptibility to infection in these animals is due to a bone-marrow-derived cellular defect. Because of similarities between murine and human CHS, bone marrow transplantation might be considered as a mode of therapy in selected cases of the human disease.

The Chediak–Higashi Syndrome (CHS) is a rare autosomal recessive disease characterized primarily by pigmentary dilution, frequent pyogenic infections, and characteristic giant lysosomal granules present in all granule-containing cells. The disorder was initially described in man, and a similar syndrome has been noted in mink, cattle, and mice. Mice with this syndrome have been shown to have an increased susceptibility to bacterial and fungal infections, as well as defective granulocyte chemotaxis and abnormal granulocyte bactericidal activity similar to that seen in the human syndrome. The fundamental defect which leads to an increased susceptibility to infection has not been delineated, but it is considered to be related to impaired cellular function. In order to investigate further the cellular defect in CHS, as well as to explore a mode of therapy potentially applicable to the human disease, we have transplanted bone marrow cells from normal mice into mice with CHS and, subsequently, have challenged them with Candida albicans intravenously. The marrow transplant resulted in chimeras which had a significantly increased survival when compared with controls.

MATERIALS AND METHODS

Animals

The donor recipient pairs were highly inbred strains of male and female black NIH stock C57Bl/6J mice (normal) and beige NIH stock C57Bl/6J Bg animals (Small Animal Section, NIH) that had the pigmentary dilution and granulocyte functional abnormalities with increased susceptibility to pathogenic microorganisms characteristic of the CHS. The C57 and beige strains have been bred through ten backcross cycles and are considered to be congenic. Animals 16–20 wk old, weighing 20–25 g, were housed six to eight per cage, and were fed lab chow and water ad lib.
Mixed Leukocyte Cultures

One-way mixed leukocyte cultures were performed by the method of Gebhardt, with minor modifications. Single cell suspensions of spleen cells were prepared from freshly killed C57 and beige mice. Cells were suspended in minimal essential medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 0.02 M L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (NIH Media Supply Section), and 10% fetal calf serum (Grand Island Biological) and brought to 1 x 10^6 cells/ml. After x-irradiation of cells from one donor spleen with 5000 roentgens, cell suspensions were mixed and incubated in microtiter plates (Cooke Laboratory Products, Cooke Engineering Co., Alexandria, Va.) at 37°C in 5% CO_2 for 5 days. Each well contained 0.2 ml of cell suspension at a concentration of 0.5 x 10^6 leukocytes/ml and studies were done in quadruplicate. The day prior to harvesting 4 μCi of tritiated thymidine (6.7 Ci/mM, New England Nuclear, Boston, Mass.) were added to each culture well. Cells were collected on fiberglass filters by a semiautomated microharvesting device. The filters were washed with 10% trichloroacetic acid and 95%, ethanol and placed in 10 ml of Aquasol (New England Nuclear). The filters were counted in a liquid scintillation counter (Model LS-350, Beckman Instruments, Inc., Fullerton, Calif.).

Bone Marrow Transplantation

One day before transplantation, 16-20-wk-old recipient mice were exposed to 700 R total body x-irradiation between two opposing x-ray tubes operated at 200 kvp, 15 mA. This irradiation dose was lethal to control mice before 10 days with gradual reduction in leukocyte counts. The long bones of a donor animal were aspirated and 10-15 x 10^6 bone marrow cells were injected into the tail vein of an irradiated recipient mouse; the recovery of the engrafted animals was uneventful.

Leukocyte Studies

Peripheral blood leukocytes (WBC) of recipient animals were studied before infectious challenge by Sudan Black B staining and electron microscopy to determine granule morphology. Leukocyte counts (WBC/cu mm) were performed with a Coulter Counter Model Fn (Coulter Electronics Inc., Hialeah, Fla.).

Infectious Challenge

Candida albicans, strain B311, was prepared from organisms grown in Sabouraud broth for 18 hrs at 37°C with constant agitation. Yeast cells were harvested, washed 3 times with sterile 0.85%, phosphate-buffered saline at pH 7.4, and adjusted to a concentration of 1 x 10^6 organisms/0.2 ml. Thirty days after transplantation when the chimera state was established, animals were challenged with a 0.2-ml intravenous inoculum of 10^8 C. albicans. This inoculum was lethal to both C57 and Bg mice.

RESULTS

One-way mixed leukocyte cultures were nonstimulatory in all cases when CHS and C57 spleen cells were studied prior to transplantation. Engraftment of the donor marrow in each case was evident by 6-8 days. Figure 1 shows the characteristic changes in leukocyte counts of C57 mice transplanted with beige marrow and untransplanted C57 controls. Each point represents the mean ± SEM of the observed counts. Control mice showed a gradual reduction in leukocyte counts, and by day 6 all animals were dead. C57 mice transplanted with beige marrow showed an initial decrease in WBC counts, but by day 6-8 leukocyte counts began to increase and were comparable to initial values by day 16-18. The chimera state was felt to be established at
this time. Similar results were also seen with untransplanted beige mice and beige mice transplanted with C57 marrow.

Leukocyte granule morphology studies with Sudan Black B and electron microscopy showed evidence of donor marrow engraftment before infectious challenge. Circulating leukocytes in C57 mice which had received beige marrow contained large peripherally staining granules when studied with Sudan Black B and pleomorphic granules lacking internal structure when viewed by electron microscopy. Leukocytes from beige mice which had received C57 marrow showed no evidence of abnormal granule structure.

Thirty days after transplantation when the chimera state was established, animals were challenged with Candida albicans. The pattern of survival in each transplant group is shown in Fig. 2. Beige mice transplanted with normal C57 marrow showed a significantly increased survival compared with untransplanted beige mice (p value < 0.00001) or beige mice transplanted with beige marrow (p value < 0.0001). Normal C57 mice transplanted with beige marrow showed an increased susceptibility to the same infectious challenge and resembled beige mice which had not been transplanted. These experiments were done on three separate occasions with the same results obtained in each instance; therefore, data in Fig. 2 are pooled from these experiments. The rates of survival were compared for significant differences using the Wilcoxon test.
Fig. 2. Cumulative mortality is shown for groups of Chediak and C57 mice, transplanted versus nontransplanted controls, following intravenous challenge with $10^6/0.2$ ml Candida albicans. The rates of survival are compared using the Wilcoxon test. The number of mice in each group is given in parentheses. Chediak mice transplanted with C57 marrow (■) show a significantly increased survival compared with untransplanted mice (○) ($p < 0.00001$) or Chediak mice transplanted with Chediak marrow (□) ($p < 0.0001$). These experiments were done on three separate occasions with the same results obtained in each instance; the data in this figure are pooled from these experiments.

**DISCUSSION**

The present experiments show that normal granulopoiesis occurs when lethally irradiated mice with CHS are transplanted with normal bone marrow. In addition, the resulting chimera shows a significant increase in survival compared with nontransplanted beige mice, beige mice transplanted with beige marrow, and C57 mice transplanted with beige marrow when challenged with *C. albicans*. This finding suggests that the mechanism of the increased susceptibility to infection of CHS is related to impaired cellular function and is not humoral in origin. Because intact unfractionated bone marrow was transplanted in these experiments, we have not defined the particular marrow element responsible for correcting the defect in CHS.

Bone marrow transplantation is recognized as a potential mode of therapy for genetic abnormalities such as hereditary spherocytosis, cyclic neutropenia, and immunologic deficiency states. In patients with disseminated malignant neoplasms which require total eradication by high dose chemotherapy and radiotherapy, bone marrow transplantation has been used to treat secondary bone marrow toxicity. It should be emphasized that the C57 and beige mice used in this study are congenic and presumably matched at histocompatibility loci. However, human donor–recipient pairs that are HL-A
matched and nonreactive in mixed leukocyte culture still have a high incidence of graft-versus-host disease when bone marrow transplantation is performed. One must be cautious when relating results of animal studies to human situations. Yet this study suggests two potential applications of bone marrow transplantation for human CHS in reversing the leukocyte defect of severely affected individuals with frequent, life-threatening infections, and in aggressive chemotherapy of the lymphoma-like accelerated phase.

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REFERENCES

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JA Kazmierowski, RJ Elin, HY Reynolds, WA Durbin and SM Wolff