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Cartilage-hair hypoplasia
immune deficiency
severe combined immunodeficiency
thymosin
thymus

Severe Combined Immunodeficiency with Cartilage-Hair Hypoplasia: *In Vitro* Response to Thymosin and Attempted Reconstitution

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Extract

The present report describes an infant with severe combined immunodeficiency and cartilage-hair hypoplasia whose lymphocytes responded to thymosin *in vitro*. Immunologic evaluation was undertaken at 4½ months of age following a history of recurrent severe infection. Family history included three cousins who died in early infancy, one from streptococcal meningitis and pneumonia, one from generalized varicella, and another from reticuloendotheliosis.

Quantitative immunoglobulins were markedly depressed: IgG 141, IgA 0, and IgM 24 mg/100 ml. There was an absolute lymphopenia, multiple skin tests were negative, and *in vitro* lymphocyte responses to mitogens and antigens were depressed. Spontaneous E rosette determinations were 21% compared with control values of 65.7%. Erythrocyte adenosine deaminase (ADA) activity was normal.

The patient's E rosette formation increased in the presence of thymosin, fraction 5, reaching a maximum of 56% with a concentration of 500 μg thymosin. Blastogenic responses to phytohemagglutinin also increased in the presence of thymosin. Transplantation of 24-week fetal thymus in Millipore diffusion chambers and subsequently transplantation of 18-week fetal thymus by intraperitoneal injection was accomplished. E rosettes increased to 35–40% and blastogenic responses to mitogens increased. Eight days after the second transplant the patient underwent a mild graft vs. host reaction which subsided after 1 week and mitogen blastogenic responses again increased to 5–8 times previous values, but still well below control ranges. Repeated episodes of pulmonary infection ensued, cor pulmonale resulted, and the clinical course was relentlessly downhill with the patient expiring from respiratory failure 5 months after transplantation.

Speculation

Reconstitution for primary cellular immune deficiency requires two steps: (*1*) provision of an adequate population of precursor lymphoid tissue which engrafts into the host and (*2*) maturation of available and/or transplanted cells. Some patients only require therapy directed toward the latter process and this can be accomplished with thymosin, transfer factor, fetal thymus in a diffusion chamber, or with fetal thymus by injection or transplant. Other patients, however, require transplantation of precursor lymphoid tissue, usually from bone marrow. Response to thymosin *in vitro* will not accurately separate these two groups of patients.

Severe combined immunodeficiency (SCID) syndrome is a disorder characterized by absence of both humoral and cellular immune competence. One variant of this fatal inherited deficiency is associated with short-limbed dwarfism and cartilage-hair hypoplasia, first described by McKusick et al in 1964 (15). Such infants exhibit severe clinical disease with many bacterial and viral agents as do others with SCID but, in contrast, they also demonstrate particular susceptibility to overwhelming varicella-zoster infection (13). These patients usually die during infancy and therefore require early immune reconstitution in order to appreciate any chance of survival (19).

Successful reconstitution of immunologic capacity in SCID has been achieved primarily with histocompatible bone-marrow transplantation (3,4,12) and recent reports have indicated that transplantation with fetal thymus (1,2,9) or fetal liver (5,11) may represent the most acceptable alternative. Administration of human transfer factor is felt to be a useful adjunct to transplantation with these tissues. Bone-marrow transplantation from histoincompatible donors has never been successful and unfortunately few patients have living siblings who might provide compatible tissue since this genetically transmitted disorder often affects such siblings.

Another approach to cellular immune reconstitution currently under investigation is the subcutaneous administration of thymosin, an extract of calf thymus. Prediction of clinical response to this mode of therapy has been demonstrated to be the increase of spontaneous E rosette formation in the presence of thymosin in vitro. To date only one patient with Primary immune deficiency has successfully reponded to thymosin; this was a young girl with thymic hypoplasia and immunoglobulin synthesis similar to Nezelof syndrome (20).

The present report describes an infant with SCID and cartilage-hair hypoplasia whose lymphocytes responded to thymosin in vitro. The patient received thymus transplantation and transfer factor therapy but subsequently demonstrated no apparent clinical benefit, dying from chronic pulmonary disease 5 months following transplantation.

Case Report

The patient, a caucasian male, was born following an uneventful pregnancy, labor, and delivery. Birth weight was 6 lb, 13 oz and his immediate neonatal course was uneventful. Physical exam revealed shortened extremities and x-rays of long bones were interpreted as compatible with achondroplastic dwarfism or cartilage-hair hypoplasia syndrome (Fig. 1 and 2). Family history revealed that the father had a history of recurrent infection thought to be secondary to "low white blood cell counts". The paternal grandfather is 63 inches tall but otherwise normal. The father's height is 68 inches and the mother is 66 inches tall. Also pertinent is that three of the patient's paternal third cousins, all male, expired during infancy. These cousins were not siblings. One died at 3½ months from streptococcal meningitis and pneumonia; another died at 4 months of age from generalized varicella and also had documented hypogammaglobulinemia. A third died at 2½ months of age from a disease felt to be reticuloendotheliosis, possibly Letterer-Siwe disease. None were known to have shortened stature.

The patient did quite well until 2½ months of age when he was hospitalized at Santa Rosa Medical Center with generalized petechiae and bloody stools. He had received his second DPT injection one week prior to admission; the first had been administered at 6 weeks of age along with oral polio vaccine. On admission, his weight was 9 lb, 15 oz and height 46 cm. He was described as a pale, achondroplastic dwarf in no acute distress. Initial laboratory studies demonstrated a WBC of 3300/mm^3 with 7% neutrophils, 60% monocytes, hemoglobin 7.0 gm/100 ml, hematocrit 31% and 7,000 platelets/mm^3. Bone marrow examination revealed the presence of immature non-platelet producing megakaryocytes, myeloid arrest at the myelocyte level, greatly decreased numbers of plasma cells, but a normal quantity of lymphocytes. This clinical episode was felt to be compatible with idiopathic thrombocytopenic purpura precipitated perhaps by a viral illness. The patient was given two units of platelets and two units of unwashed fresh packed red blood cells during hospitalization. Platelets increased to 16,000/mm^3 and the patient was placed on prednisone 2.5 mg/kg/day. The platelet count continued to rise until reaching normal values on the twelfth hospital day. However, the absolute neutrophil count remained at approximately 500/mm^3. The infant was rehospitalized at 3½ months of age with a 5 day history of irritability, vomiting, and diarrhea. Admission temperature was 101.3 degrees F and a neutropenia of less than 100/mm^3 was noted. Following a septic workup, he was placed on gentamicin, carbenicillin and methicillin. CSF, blood and urine cultures were negative so all antibiotics were discontinued after 72 hours. Platelet counts remained normal and he was gradually tapered off prednisone. At 4½ months of age, he was again hospitalized with bilateral bronchopneumonia. The same antibiotic regimen was reinstituted and at this time a complete immunologic evaluation was undertaken.

MATERIALS AND METHODS

Cell mediated immunity. In-vitro lymphocyte blastogenic responses to the mitogens: phytohemagglutinin (PHA), pokeweed (PWM), and concanavalin A (Con A), to the recall antigens: monilia and tetanus toxoid, and to mixed leucocyte cultures (MLC) were determined with the micromethods previously described (16). For some assays, thymosin fraction 5 was added to the culture materials in a final concentration of 250 µg/ml but the methods were otherwise identical. Skin tests were accomplished in the usual manner by injecting 0.1 ml of the material intradermally into the volar aspect of the forearm and skin test sites were read at 24, 48, and 72 hours; materials employed were : PHA 10 µg/0.1 ml, monilia skin test antigen 1:10 (Dermatophytin "O", Hollister-Stier Laboratories, Yeadon, PA) and fluid tetanus toxoid (Eli Lilly, Indianapolis, IN). Thymosin fraction 5 was kindly supplied by Dr. A.L. Goldstein; this material was extracted from calf thymus tissue and purified as reported (8). Spontaneous (E) rosettes for peripheral lymphocytes were determined by methods previously described (10); assays were also performed with the addition of varying concentrations of thymosin, fraction 5. Lymphocytes binding 3 or more sheep red blood cells were considered positive. A lymph node biopsy was obtained for examination of paracortical and deep cortical areas by light microscopy.

Humoral immunity. Quantitative immunoglobulin levels were determined by a standard radial diffusion technic (14). Peripheral blood lymphocytes, isolated with a Hypaque-Ficoll separation gradient, were incubated with fluorescein labelled antibodies to IgG, IgM, IgA and light chains, and examined in wet-mount preparations for surface immunoglobulins (6). Peripheral lymphocytes were also examined for formation of EAC rosettes with methods previously described (10). The cortical areas of a lymph node biopsy were also evaluated for the presence and extent of follicle and germinal center formation.

Thymus transplantation. The initial thymus was obtained from a 24 week gestation neonate immediately after death. One lobe of the organ was divided in half and each half placed in a sterile 0.45 µ pore-size Millipore diffusion chamber which was kept in cooled tissue medium until implantation 30 minutes later. The chamber was constructed from a methyl ester (Lucite) ring with an outside diameter of 14 mm, an inside diameter of 10 mm, and a depth of 2 mm; Millipore disks were glued to the outside surfaces of the rings. The chambers were implanted under the right rectus sheath (16). The second thymus was obtained from an 18 week male fetus following an intrauterine prostaglandin $F_2\alpha$ induced therapeutic abortion. A cell suspension in normal saline was made from this tissue and injected intraperitoneally into the patient. Each donor mother gave written informed consent for this use of the fetal tissue.

Transfer factor. Dialyzable transfer factor (TF_d) was prepared and purified from a single donor by the methods previously described (17). Leucocytes were obtained by leukapheresis using a Continuous-Flow Celltrifuge (American Instrument Co.). Lymphocytes were separated from the cell pack using a Hypaque-Ficoll gradient, freeze thawed in the presence of DNase 10 times and TF was then dialyzed and concentrated by lyophilization. One unit of TF_d was equivalent to 1×10^9 lymphocytes.

RESULTS

Quantitative immunoglubulins were markedly depressed: IgG 141, IgA 0 and IgM 24 mg per 100 ml. Agglutination titers to typhoid H and O antigens remained < 1:2 following typhoid vaccine administration. Assay of peripheral blood lymphocytes for surface immunoglobulins revealed the following proportion of positive cells: IgG 6%; IgA 1%; IgM 15%; and light chain 17%; controls averaged 19%, 2%, 6%, and 22% respectively. Percent and absolute numbers of EAC rosettes were 54% (416) as compared to 21.8 ± 2.4% (597 ± 265) in controls and these values remained essentially unchanged in the presence of thymosin in vitro. Determinations of total hemolytic complement and C'3 were normal. Erythrocyte adenosine deaminase (ADA) activity was also normal.

Absolute lymphocyte counts ranged from 370 to 1141 with most determinations less than 700 cells/mm^3. All skin tests were negative in spite of two previous injections of tetanus toxoid vaccine and a history of monilia diaper rash. Lymphocyte responses to mitogens yielded a blastogenic index (BI) for PHA of 3.5 (normal 57-125), while the BI for PWM and Con A were < 2 as were responses to monilia, tetanus toxoid, herpes simplex type 1, and mixed lymphocyte culture. In the presence of thymosin, fraction 5, responses to PHA increased to a BI of 11.7 but blastogenic reactions to PWM and Con A were not altered. An inguinal lymph node biopsy demonstrated depleted lymphocyte stores in both cortical and paracortical regions.

Expressed as percent and absolute numbers per mm^3, spontaneous E rosette determinations were 21% (162) compared to control values of 65.7 ± 4.2% (1725 ± 470). As seen in Figure 3, the patient's E rosette formation increased in the presence of thymosin, fraction 5, reaching a maximum of 56% (432/mm^3) with a concentration of 500 µg/ml of thymosin.

At 6 months of age, the absolute neutrophil count reached normal levels (>1500/mm^3) and remained normal until death. The reason for the increase was not apparent. Possibilities include the disappearance of neutrophil antibodies, the development of colony stimulating agent or other serum factors which enhance neutrophil production, and correction of an intrinsic neutrophil maturation defect. Specific data, however, are not available to support any of these hypotheses.

The patient was begun on immune serum globulin, 0.66 ml/kg every 2 weeks, which maintained the IgG level above 200 mg per 100 ml. At 7 months of age, following counselling and informed consent from both parents, the patient received 24 week neonatal thymus in millipore diffusion chambers as described above. At the same time, one unit of transfer factor was given by subcutaneous injection. Seven days post transplantation, E rosettes increased to 36% although the absolute number of rosetting lymphocytes was still only 292/mm^3. The blastogenic response to PHA had also increased to a BI of 14.3 with the BI for PWM and Con A - 6.3 and 11.7 respectively 8 days post transplantation; these values gradually decreased to 5.0, 3.2 and 5.5 one month after transplant although E rosettes remained 35-45%. Therefore, it was elected to offer the patient fetal thymus by intraperitoneal injection. This was accomplished without incident. A second unit of transfer factor was also administered. Eight days following transplantation, the patient experienced a mild graft-versus-host (GVH) reaction characterized by fever, a generalized macular rash, hepatosplenomegaly, and diarrhea. This subsided after 1 week and mitogen blastogenic responses again increased to 5-8 times previous values but still well below control ranges. Skin tests and in vitro blastogenic responses to antigens remained negative. Over the subsequent 3 months the patient had repeated episodes of apparent pulmonary infection with infiltrates and atelectasis but etiologic infectious agents could never be identified. Cor pulmonale resulted and the clinical course was relentlessly downhill with the patient expiring from respiratory failure 5 months after transplantation at age one year. The immediate cause of death was bronchopneumonia. Microscopic examination of the lungs at necropsy was negative for Pneumocystis carinii, bacteria, or viral inclusion bodies. Lymph nodes were sparse with architecture similar to the biopsied node. The thymus was noted to be dysplastic with absence of Hassall's corpuscles and poor corticomedullary differentiation.

DISCUSSION

Despite rapid advances in the field of immunology and transplantation research, the therapeutic approach to the infant with SCID remains unsatisfactory.

Histocompatible donors are usually not available for bone marrow transplantation in these patients. With unmatched tissue, either marrow, liver, or thymus, GVH reactions present a formidable obstacle to successful reconstitution and for this reason cell-impermeable chambers have been employed to encapsulate thymus transplant tissue and thereby prevent GVH disease. Success with this approach is predicated on the ability of thymus tissue to elaborate a hormone like compound(s), presumably thymosin, which then influences available lymphoid tissue to maturate and provide appropriate cellular immune responses. The first patient to receive fetal thymus in a diffusion chamber was an infant with DiGeorge syndrome (16); although evidence of cellular immune competence was observed, the patient died from aspiration pneumonia shortly after transplantation. More recently a child with SCID was successfully reconstituted with fetal thymus in a Millipore chamber in conjunction with immune serum globulin injections; in vitro evidence of cellular reactivity was demonstrated and after 3 years followup, has remained free from significant infection (7). Other cases of SCID with thymus transplantation have been reported (1,2,9). Transplants were accomplished either by implanting the tissue under the rectus abdominis sheath (16) or by injecting cell suspensions into the peritoneal cavity (1,2);

GVH disease remains a significant risk with these procedures although such reactions may be minimal if fetal tissue of 13 weeks gestation or less is used. With this approach, however, the thymus may be too immature to provide adequate reconstitution.

More recently, successful immunologic reconstitution has been achieved with fetal liver cells (5,11). The mild GVH reactions in 3 engrafted patients is certainly encouraging but failure of reconstitution for many other patients given fetal liver (5) points out the limitation of this approach.

In the present case, no compatible donor was available for consideration of bone marrow transplantation so thymus tissue was employed. Following the implantation of neonatal thymus in a Millipore chamber, the only observed effects were an increase in E rosettes in-vitro and augmentation of mitogen induced blastogenesis. The latter response waned rapidly and the patient continued to do poorly clinically. Therefore the thymus transplant was repeated but this time with 18 week fetal thymus given by intraperitoneal injection. One unit of transfer factor was also administered subcutaneously. Mitogen induced blastogenesis again increased but no change in clinical status could be appreciated and the patient expired.

Reconstitution of primary immune deficiency usually involves two essential steps: (1) providing an adequate population of precursor lymphoid tissue which engrafts into the host and (2) maturation of already available "immature" lymphocytes and/or transplanted cell. It has become apparent that some patients, particularly those with deficiencies in cellular immunity alone, require only therapy directed toward maturation of their own thymic dependent lymphocytes. This has been accomplished with whole thymus transplantation, fetal thymus in diffusion chambers, transfer factor, and thymosin. Other patients have even demonstrated "spontaneous" maturation. The methods for assessing progress of reconstitution have included primarily a battery of in-vitro assays. It should be emphasized that success of therapy is certainly long term followup with demonstration of normal responses to infectious agents and reasonable quality of life. Such information is not presently available for most reported efforts at reconstitution.

The present case clearly exhibited maturation of available precursor lymphocytes following the in-vitro addition of thymosin and following both attempts at thymus transplantation. The ability of circulating T lymphocytes to form E rosettes was increased as were responses to mitogens. The effects following all three procedures were quite similar and suggest that thymus transplantation without engraftment provides hormonal influences identical to those produced by thymosin either in-vitro or in-vivo. However, lymphocyte maturation in this patient was not sufficient and the need for providing an adequate population of lymphoid tissue was never achieved with thymus transplantation. The infant therefore succumbed to chronic pulmonary infection. It was felt that the GVH reaction did not contribute to the patient's demise since all recognized effects subsided long before death.

Shortly before death, efforts were being directed toward fetal liver or adult bone marrow transplantation as the next approach at reconstitution. Each procedure therefore represented one of increasing patient risk, i.e. (1) thymus in a Millipore diffusion chamber, (2) whole thymus transplantation, (3) intraperitoneal infusion of fetal liver cells and (4) bone marrow transplantation. It was hoped that with the first two steps, immune serum globulin injections would provide adequate humoral immunity as no reconstitution of the B cell population is anticipated with thymus transplantation. The addition of transfer factor was based on our recent experimental studies in immune deficient non-human primates which have demonstrated protection from infectious agents with the same lot of human transfer factor (18). Finally, therapy with thymosin was not strongly considered since it was apparent that repopulation of lymphoid tissue was essential for reconstitution in this patient.

SUMMARY

A 3 month old male infant with cartilage-hair hypoplasia also had severe combined immunodeficiency. Quantitative immunoglobulins were markedly depressed (IgG:141, IgA:0, IgM:24 mg%) as were percent lymphocytes bearing surface immunoglobulins; 54% of peripheral lymphocytes formed EAC (B) rosettes (control 21%). Lymphocyte responses to mitogens yielded a blastogenic index (BI) for PHA of 3.5 (normal 57-125), while the BI for pokeweed and Con A were < 2 as were responses to monilia and tetanus toxoid. Recall antigen skin tests were negative and a lymph node biopsy showed depleted lymphocyte stores in both cortical and paracortical regions. Spontaneous (E) rosettes were 21% (control 65%). Erythrocyte adenosine deaminase (ADA) activity was normal. In-vitro assays in the presence of thymosin, fraction 5 were as follows: 25 µg thymosin - 36% E rosettes, 50 µg - 45%, 100 µg - 45%, 250 µg - 49%, 500 µg - 56% and 1 mg - 26%. The BI to PHA also increased to 11.7 in the presence of thymosin, but responses to pokeweed and Con A did not change. Transplantation of 24 week fetal thymus in Millipore diffusion chambers and subsequently 18 week fetal thymus by intraperitoneal injection resulted in increased E rosettes to 32-41% although severe lymphopenia persisted. Responses to PHA, pokeweek and Con A also increased to 5-8 times previous values, but still well below control ranges. The patient expired 5 months after transplantation.

REFERENCES AND NOTES

1. Ammann, A. J., Wara, D. W., Salmon, S., and Perkins, H.: Thymus transplantation. Permanent reconstitution of cellular immunity in a patient with sex-linked combined immunodeficiency. New Engl. J. Med., 289:5, 1973.
2. Ammann, A. J., Wara, D. W., Doyle, N. E., and Golbus, M. S.: Thymus transplantation in patients with thymic hypoplasia and abnormal immunoglobulin synthesis. Transplantation, 20:457, 1975.
3. Bergan, J. J.: ACS/NIH organ transplant registry third scientific report. JAMA 226:1211, 1973.
4. Bortin, M. M.: A compendium of reported human bone marrow transplants. Transplantation, 9:571, 1970.
5. Buckley, R. H., Whisnant, J. K., Schiff, R. I., Gilbertsen, R. B., Huang, A. T., and Platt, M. S.: Correction of severe combined immunodeficiency by fetal liver cells. New Engl. J. Med. 294:1076, 1976.
6. Cooper, M. D., Lawton, A. R., and Bockman, D. E.: Agammaglobulinemia with B lymphocytes: specific defect of plasma-cell differentiation. Lancet, 2:791, 1971.
7. Frankel, L. D., Thong, Y. H., Steele, R. W., and Bellanti, J. A.: Familial immune deficiency and leukodystrophy: reconstitution with fetal thymus in a millipore chamber. Pediat. Res., 8:139, 1974.
8. Goldstein, A. L., Guha, A., and Zatz, M. M.: Purification and biological activity of thymosin, a hormone of the thymus gland. Proc. Natl. Acad. Sci., 69:1800, 1972.
9. Hong, R., Huang, S. W., Levy, R.: Cartilage-hair hypoplasia: effect of thymus transplants. Clinc. Immunol. Immunopathol., 1:15, 1972.
10. Jondal, M., Holm, G., and Wigzell, H.: Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. J. Exp. Med., 136:207, 1972.
11. Keightley, R. G., Lawton, A. R., and Cooper, M. D.: Developmental defects of T and B cell lines in humans. Transplant Rev., 16:15, 1973.
12. Levey, R. [illegible], Kemperer, M. R., Gelfand, E. W., Sanderson A. R., Batchelor, J. R., Berkel, A. I., and Rosen, F. S.: Bone-marrow transplantation in severe combined immunodeficiency syndrome. Lancet, II:571, 1971.
13. Lux, S. E., Johnston, R. B., August, C. S., Say, B., Penchaszadeh, V. B., Rosen, F. S., and McKusick, V. A.: Chronic neutropenia and abnormal cellular immunity in cartilage-hair hypoplasia. New Engl. J. Med. 282:234, 1970.
14. Mancini, G., Carbonara, A. O., and Heremans, J. F.: Immunochemical quantitation of antigens by single radial immunodiffusion. Immuno-chemistry, 2:235, 1965.
15. McKusick, V. A., Elderidge, E., Hoetetler, J. A., Ruangwit, U., and Egeland, J. A.: Dwarfism in the Amish. II. Cartilage-hair hypoplasia. Bull. Johns Hopkins Hosp., 116:285, 1964.
16. Steele, R. W., Limas, C., Thurman, G. B., Schuelein, M., Bauer, H., and Bellanti, J. A.: Familial thymic aplasia. Attempted reconstitution with fetal thymus in a millipore diffusion chamber. New Engl. J. Med, 287:787, 1972.
17. Steele, R. W., Eichberg, J. W., Heberling, R. L., Eller, J. J., Kalter, S. S., and Kniker, W. T.: In vivo transfer of cellular immunity to primates with transfer factor prepared from human or primate leucocytes. Cell. Immunol., 22:110, 1976.
18. Steele, R. W., Heberling, R. L., Eichberg, J. W., Eller, J. J., Kalter, S. S., and Kniker, W. T.: Prevention of herpes simplex virus type 1 fetal dissemination in primates with human transfer factor. In Transfer Factor Proceedings of the Second International Workshop on Basic Properties and Clinical Application of Transfer Factor. (Academic Press, New York, 1976.)
19. Stiehm, E. R. and Fulginiti, V. A.: Immunologic disorders in infants and children, p. 263. (W. B. Saunders Co., Philadelphia, 1973.)
20. Wara, D. W., Goldstein, A. L., Doyle, N. E., and Ammann, A. J.: Thymosin activity in patients with cellular immunodeficiency. New Engl. J. Med., 292:70, 1975.
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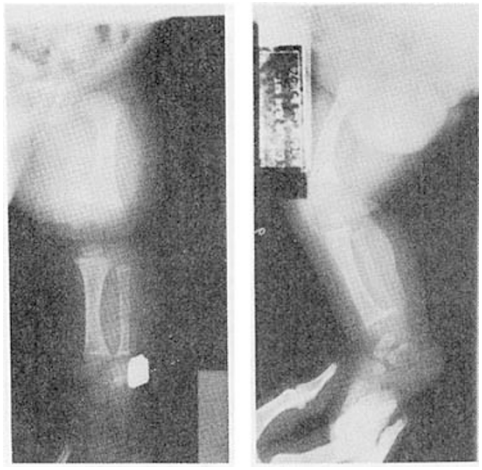

Figures 1 and 2: Frontal and lateral x-ray projections of the lower extremity. The long bones are shortened with terminal flaring, most compatible with achondroplasia. Sclerosis, cystic changes or a scalloped appearance of the metaphysis as described in short-limbed dwarfism with SCID is not present.

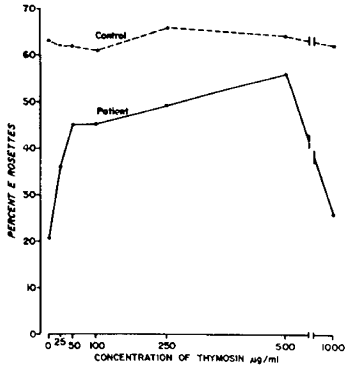

Figure 3: The influence of various concentrations of thymosin, fraction 5, on in vitro spontaneous E rosette formation in a patient with severe combined immunodeficiency.