Pediat. Res. *11:* 63-64 (1977)

A Special Report: Four-year Study of a Boy with Combined Immune Deficiency Maintained in Strict Reverse Isolation from Birth

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Summary

A 4-year study of a boy with combined immune deficiency is presented, and the impact of this disease on various aspects of his growth and development is examined. There is no evidence of immune deficiency in either parent or in the genetic background on the maternal side. Three children of a brother of the mother's father may have had immune deficiencies but two have grown to be teenagers with no problems. Another died. At autopsy, however, lymph nodes appeared normal. The deceased older brother had severe combined immune deficiency (SCID). The autopsy findings showed *Pneumocystis carinii* pneumonia to be the direct cause of death and these findings contributed to the diagnosis of SCID. After a successful germ-free birth, the male infant *(DV)* was placed in the isolator. Laboratory tests were normal except that the x-rays showed no thymic shadow, his absolute lymphocyte count ranged from 399–440/mm, and the lymphocytes showed no proliferative response to phytohemagglutinin (PHA). Specific tests showed the antibody-producing immune system and the cell-mediated immune system to be severely defective. The patient's lymphocytes elicited positive responses by lymphocytes from father, mother, and sister. Subsequent search in national and international tissue-typing laboratories has shown four HLA matches but none has been nonreactive in mixed lymphocyte culture (MLC). therefore, this patient has remained in isolation to the present; now he is 4 years old.

Approximately 35 species of microorganisms, mostly transient contaminants, have been isolated, taking into account that the same organism may have been identified under different names in different laboratories. Those isolated frequently and in sufficiently high concentration to indicate colonization have been speciated as follows: anaerobes—*Propionibacterium acnes, Lactobacillus catenaforme* (disappeared spontaneously), *Bacteroides oralis* ss. *elongatus, Clostridium (perenne, hastiforme, bifermentans), Bacteroides clostridiiformis* ss. *clostridiiformis;* aerobes—*Alcaligenes faecalis* (eradicated by antibiotics), *Staphylococcus epidermidis, Enterobacter agglomerans, Micrococcus* sp. subgroup 1, *Bacillus pulvifaciens* (disappeared spontaneously); yeasts—*Candida (tropicalis, parapsilosis).* Seven are considered to be probable components of the current autoflora: *P. acnes, C. bifermentans, B. clostridiiformis* ss. *clostridiiformis, S. epidermidis, Micrococcus* sp. subgroup 1, *E. agglomerans, C. parapsilosis.* No viruses or protozoa have been isolated. At age 3 years, the mean quantitation of anaerobic cells was 7.9×10^7 viable cells/g feces; this falls short of the mean anaerobic load from normal children. The mean aerobic concentration was 1.2×10^8 viable cells/g feces, not unlike normal children. Qualitatively his flora has abnormally few species and lacks those most common in normal subjects. This child has had no evidence of infection and has always been in excellent health even though some organisms which could be pathogenic under some circumstances have been isolated.

Phagocytic functions, adenosine deaminase (ADA) levels, and serum complement levels were normal except that C1q was 30% of normal. Thymosin assays showed adult control subject 1/4, 10-year control subject 1/128, this patient 0. To age 47 months serum immunoglobulin (Ig) M levels were generally low and IgG was not detected. No serum IgA was detected until, at 39 months, assays indicated IgA at the lowest range of sensitivity of the agar plates. Ultracentrifugal analysis of serum revealed no 19s material at 24 months but at 36 months both 7s and 19s materials were present. At 44 months these fractions were still present and an abnormal 4s component had disappeared. Radial immunodiffusion assay at 44 months indicated the presence of IgD and at that time an IgM component of normal electrophoretic mobility was detected for the first time. Before injection of keyhole limpet hemocyanin (KLH) at 1 month antibody titer was 0-0. Antibody titers and skin tests after injection were negative and remained so after further antigen injections. At 11 months, on the fifth rechallenge, the patient had an erythematous reaction of 5 mm diameter but no significant antibody responses. Two typhoid antigen injections elicited no antibody response. Using the isolated leukocyte technique, lymphocytes showed minimal or no blastogenesis in response to PHA, pokeweed mitogen (PWM), or in MLC. Using the whole blood technique, transient, low positive responses (stimulation index (SI) range 4.1–9.7) to PHA were observed but not consistently maintained. At 3 years, purified T cells showed a notable response (SI 17.4) to PHA, but this was not obtained in subsequent experiments. Transfer factor (TF) was given to this patient between 10 and 16 months of age. In skin tests to *C. albicans*, purified protein derivative (PPD) and streptokinase-streptodornase (SK-SD) administered a day after TF injection, small areas of redness appeared early and faded rapidly. Addition of TF to lymphocyte cultures obtained before administration of TF caused them to respond to *C. albicans* and PPD (SI 8 for each); after injection of TF and skin tests to these antigens, similar responsiveness could not be induced by addition of TF. After a second dose of TF, before skin test to SK-SD, the lymphocytes responded to SK-SD *in vitro* with TF (SI 13) and without TF (SI 8) added to the cultures; after SK-SD skin tests, responses were no longer elicited under the same circumstances. During the first 2 years membrane-bound immunoglobulin (SmIg), bearing lymphocytes ranged from 50–100% of the total lymphocytes whereas the percentage of lymphocytes with cells forming rosettes with sheep erythrocytes (E-RFC) markers was low, about 3–12%. Between 2.5 and 4 years striking changes occurred, representing a shift toward a normal distribution (20–40% SmIg and 19–60% E-RFC). In electron microscopic studies, new type lymphocytes which appeared at 15 months increased in number until at 4 years they represented 93% of the lymphocytes. In contrast to cells from normal donors, complement (C3) receptor-bearing cells of this patient did not express significant direct cytotoxicity; however, lymphotoxin (LT) levels 3–4 times those

of E-RFC either from patient or normal donors were detected. Consistent with the positive responses, C3 receptor-stimulated cells also produced leukocyte migration inhibition factor (MIF) activity (40 ± 16%) greater than background (15%) level. No correlation was evident between fluctuations in absolute numbers of C3 receptor-bearing cells in peripheral blood and the presence of LT and MIF responses. The expression of LT and MIF indicated that this patient was capable of nonspecific host responses. The findings suggest an impairment of the ability of this SCID patient to expand or sustain functional subpopulations essential to immunologic responsiveness.

Hematologic surveillance revealed no dramatic differences from other children with SCID. Absence of complicating infections indicated that the changes were probably attributable to the natural disorder. Thrombocytopenia appears to be related to the basic disease because occurrence and recovery did not relate in a consistent pattern with antibiotic therapy, other therapy, or bacterial contamination. Peripheral blood counts followed a pattern consistent with previously described cases.

At 3 months of age the patient showed scalp changes, loss of hair, and rough skin. He was placed on an iron-fortified formula and vitamins. The scalp problems soon cleared up. Another episode of hair loss and dry skin occurred at 15 months. He had not been given his vitamin supplements. Vitamins were given again. Subsequently scalp problems improved. This is a child who has been on low cholesterol intake from birth, yet there is no malfunction of the nervous system or delay in myelinization. At age 2 years he was excreting a larger percentage of primary bile acids (70%) than control subjects (30%). This indicated a reduced activity of bacterial flora. *DV* excreted 99.4% of his neutral lipids as cholesterol with a trace of coprostanol whereas control subjects of 1.5 years or less showed 95% cholesterol with about 5% coprostanol.

When he was 5 months, 24 days of age, using the Bayley Scales of Infant Development, the Mental Index was 116 (6.5–7.5 months) and the Psychomotor Index was 112 (6.5–7.5 months). When he was 3 years old, potential intellectual endowment was estimated to be 1–2 years above his chronologic age. He showed unusual ability in the discrimination and recognition of geometrical shapes of objects in his environment. Evaluation of general ego functioning showed rejection of some popular responses, a healthy awareness of color nuances, a rather low productivity relative to his intellectual potential, but no apparent idiosyncratic or dereistic quality in his ideation or concept formation.

At age 12 months, the child showed a marked deficit in receptive and expressive language skills. Implementation of a program of language stimulation resulted in rapid and consistent improvement. Recent objective testing revealed above average speech and language abilities, except for a mild, persistent deficit in receptive and expressive vocabulary which was related to his atypical environment.

Psychiatric evaluation in this patient demonstrates that it is possible to rear a child, under conditions of strict reverse isolation, who can respond with normal affective, cognitive, and intellectual ability to age 4 years.

Speculation

The continued maintenance of this patient in a gnotobiotic state has provided opportunity for serial studies in an uncomplicated disease state.

Although he has not remained germ free, this technology has been successful in preventing infection for 4 years in a child who, otherwise, would have been overwhelmed with infection. In addition, the studies have shown that the significantly simpler microflora in the early part of life has not been incompatible with normal growth and development.

Studies being carried out may help define more exactly the nature of the immune defect in SCID patients. Such information could increase the understanding of basic immune mechanisms and lead to information which could be useful in treatment of other immunologic disorders. Continued monitoring of the changes which have occurred in the immune system will be needed to determine whether the improvements will proceed to a stage where the immune apparatus is functioning adequately.

The role of enteric bacteria with regard to the absorption or metabolism of vitamin B_{12} and vitamin K in the human is challenged. Perhaps a few specific bacteria are involved and by chance these were implanted in this case at the appropriate time; another possibility is that the specific bacterial flora is unimportant or that bacteria are not at all critical components in nutrition with regard to vitamin B_{12} or vitamin K.

Normal people in differing countries have a wide range of intestinal function. The D-xylose absorption and fat absorption tests have different ranges of normal values. It would be interesting to know whether increases in bacterial flora are playing a role in lower blood cholesterol levels seen in patients from areas where the sanitation is poor. The patient in this study provides excellent opportunity to study sterol balance in defined flora. Continued studies are also expected to show the effects of a diet devoid of fresh and raw foods.

Continued observation of the patient's mental, psychomotor, and psychosocial development under isolator conditions can help define parameters for normal development in children.

This case particularly illustrated the importance of providing a child in an atypical environment with consistent and appropriate language stimulation, even if the child is of above average intelligence. The implementation of such a planned program for children who are hospitalized for long periods or who are in an atypical environment during language-learning years should be considered.

The effort to define elements in early experience which determine the nature of psychological development is proceeding on many fronts. The experience with this patient has been of considerable significance in this regard. Continued psychiatric evaluation will show whether psychological development will be affected in later years.