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# Cellular and Humoral Immunity in Cartilage-Hair Hypoplasia

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## Summary

**Cartilage-hair hypoplasia (CHH), a rare autosomal recessive syndrome of short-limbed growth failure, is unexpectedly common in the Finnish. An unselected series of 28 1- to 23-year-old subjects with CHH were studied for their immunologic capacity. All had been immunized with BCG as newborn, and most with vaccinia, without complications. Eight of the subjects had a history of unusually frequent infections, although none had had exceptionally severe ones. The tuberculin skin reactivity was markedly depressed: 2 of 23 subjects tested with 10 TU reacted, against two-thirds of the general population of the same age. Average counts of circulating lymphocytes were subnormal in 14 of the 28 subjects, this was more frequent among the younger subjects. The counts of circulating neutrophil granulocytes were mostly normal. The serum levels of IgG, IgA, and IgM were normal, and on the average, above the mean levels for age. Sixteen of the 28 had antibodies against cow's milk, which is a very significantly increased prevalence. The numbers of immunoglobulin-containing cells in the jejunal mucosa and the concentrations of IgA and IgM in the intestinal juice were normal. The phytohemagglutinin (PHA)-induced lymphocyte transformation was below the 95% confidence range for the age in 25 of the 28 subjects, and concanavalin A (Con A)-induced transformation was similarly depressed. A significant positive correlation was present between the counts of circulating lymphocytes and the PHA-induced lymphocyte transformation: the lymphopenia is presumably predominantly due to a decrease in the number of PHA-responding lymphocytes. The PPD-induced lymphocyte transformation was also depressed; this was in significant correlation with the depression of skin reactivity to tuberculin. The proportion of T cells of circulating lymphocytes, as judged with the E rosette test, was decreased but still unexpectedly high in view of the degree of depression of PHA response. Varicella was observed in the subject who previously had the lowest PHA response. Her disease was clinically mild and her response with an increase in the circulating lymphoblasts was normal. Impairment of cellular immunity, although variable in degree, is evidently an integral part of the CHH syndrome, and at least as constant as the hair abnormality.**

## Speculation

**A defect in the cellular immunity is an integral part of CHH. The basic biochemical defect, which remains to be detected, should explain both the metaphyseal dysplasia and the immune deficiency.**

Cartilage-hair hypoplasia, a condition caused by an autosomal recessive gene, is a growth failure syndrome in which the subjects are short limbed and often have abnormally fine and sparse hair (20). Its prevalence is high among the Amish in the United States (20), but few cases have been reported from other populations. The unusual susceptibility of some of the subjects to certain viral infections, especially varicella and vaccinia (9, 19, 20), suggests a defect in cellular immunity. Other syndromes have been described in which a deficiency of the immune system is associated with growth failure and/or abnormalities of hair and skin (5, 6, 27). Lux *et al.* (19) made an immunologic study of two children with CHH, selected because of a history of severe varicella. Persistent lymphopenia, diminished delayed skin test sensitivity, and decreased *in vitro* responsiveness of lymphocytes to PHA were observed.

The prevalence of CHH is exceptionally high in the Finnish population (21, 22). We have carried out a clinical and immunologic study of 28 consecutive cases of CHH referred to our clinic for growth disorders. Some of them had suffered from recurrent respiratory tract infections, but none from very severe viral infections. The immunologic findings are presented in this report. The clinical and genetic findings will be described elsewhere (15).

## MATERIALS AND METHODS

### SUBJECTS

The series comprises all the 28 Finnish subjects with CHH who were known at the time of this study, and is thus unselected with respect to the subjects' immunologic capacity. The age range was 1–23 years (Table 1). Informed consent was obtained from the patients or parents. Four pairs of siblings were included. The pattern of occurrence in the families was compatible with the autosomal recessive mode of inheritance (15). In height all the subjects were more than 3 SD below the mean for age and sex, and their limbs were abnormally short for the length of the trunk. The changes of the metaphyses of the tubular bones ranged from mild irregularities to scalloped, sclerotic, and cystic areas in radiographic appearance. The skull (23), epiphyses, and vertebral column were normal. In five subjects the hair was normal (15), but in all other respects the clinical picture was typical. In two (J.T., M.K.) of these subjects the diagnosis was evident from the complete syndrome in the family. In the other three (H.A., T.U., M.Ma.) the diagnosis was based on other clinical and radiographic criteria (15). A careful history of past infections was taken and physical signs of chronic infections were recorded. The immunologic studies were done when signs of infection were absent.

### PERIPHERAL BLOOD LEUKOCYTES

Total leukocytes were counted and differential counts of 100 leukocytes were made in two to eight (average 4.9) samples taken on different days. Counts made in the same laboratory from an age-matched group of 152 healthy children were used as normal reference (17). Means and 95% confidence limits were calculated for 6 age groups after logarithmic transformation of the counts.

Proliferative activity of blood leukocytes was assayed by short *in vitro* labeling with tritiated thymidine ([$^3$H]TdR), as described previously (28).

### IMMUNOGLOBULIN DETERMINATIONS

The serum levels of IgG, IgA, and IgM were measured by single radial immunodiffusion and expressed in percent of the mean for

Table 1. *Blood leukocyte counts and immunologic findings in subjects with CHH*

| Subject[1] | Sex and age (yr) | Susceptibility to infections | History of varicella | Blood leukocytes/mm³ | | Tuberculin skin test (TU) | Antibodies to cow's milk | Lymphocyte transformation (% blasts) | | Rosette-forming lymphocytes (%) | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Neutrophils | Lymphocytes | | | PHA | PPD | EAC | E | |
| H.A. | F1 | – | – | 1273 | 2377[2] | 100+ | + | 71 | 5.0 | 14 | 70 | Normal hair |
| P.H. | F1 | – | – | 2440 | 2364 | 100+ | + | 46 | 1.4 | 24 | 57 | |
| L.S. | M1 | – | – | 2278 | 4750 | 1– | + | 51 | 0.5 | 20 | 63 | |
| T.U. | M1 | – | – | 6053 | 1824 | 100+ | + | 25 | 1.2 | 18 | 46 | Normal hair |
| A.M. | F2 | – | – | 790 | 1360 | 100– | + | 18 | 0.0 | 15 | 73 | Gluten antibodies (+) |
| K.N. | F2 | + | – | 4857 | 2243 | 1– | – | 37 | 0.0 | | | |
| M.E. | F3 | + | + | 1401 | 1841 | 100– | – | 9 | 0.0 | 40 | 58 | [3] |
| J.S. | M3 | – | – | 2035 | 2605 | 1– | + | 71 | 0.5 | 13 | 78 | |
| V.-P.S. | M8 | – | + | 3345 | 1914 | 10+ | + | 46 | 2.2 | | | |
| E.K. | F8 | – | + | 3052 | 988 | 100– | – | 15 | | 21 | 70 | |
| H.S. | M8 | + | + | 2541 | 1837 | 100– | – | 18 | 0.2 | 48 | 63 | |
| M.Ma. | F9 | – | – | 3658 | 1907 | 100– | + | 18 | 0.0 | 24 | 66 | Normal hair |
| M.Mi. | M9 | – | – | 4201 | 1066 | 10– | – | 22 | 0.0 | | | Hirschsprung's disease |
| M.K.-R. | M10 | – | – | 3345 | 2565 | 100+ | + | 60 | 13 | 7 | 74 | |
| V.-M.S. | M10 | – | + | 2188 | 1960 | 100– | + | 53 | 1.8 | | | |
| K.L. | F10 | + | + | 1158 | 1783 | 100– | – | 21 | 0.9 | 27 | 75 | |
| Ra.M. | F11 | – | – | 3612 | 1863 | 100+ | + | 17 | 0.9 | 53 | 52 | Gluten antibodies (+) |
| I.Sa. | F11 | – | – | 2605 | 1560 | 1– | + | 19 | 0.8 | | | IgG ↑ |
| I.Si. | F12 | – | + | 1395 | 1457 | 1– | + | 53 | 0.1 | | | IgG ↑, antinuclear antibodies (+) |
| J.T. | M12 | + | + | 3728 | 2261 | 100+ | – | 61 | 0.2 | | | IgG ↓, normal hair |
| T.J. | F12 | – | – | 3514 | 1637 | 100+ | – | 18 | 1.4 | 38 | 72 | |
| K.T. | M13 | + | + | 4388 | 2700 | 100+ | – | 52 | 0.8 | | | |
| L.F. | M15 | – | + | 3984 | 1452 | 100– | – | 10 | 0.0 | 38 | 75 | IgE ↑ |
| P.K. | M16 | + | + | 3573 | 2271 | 100– | + | 39 | 0.0 | 47 | 68 | Rheumatoid factor (+) |
| M.K. | F17 | + | + | 4572 | 1761 | 100– | – | 11 | 0.0 | 34 | 66 | Normal hair |
| M.Me. | F17 | – | – | 2829 | 1428 | 100– | + | 37 | | 23 | 78 | |
| L.M. | F21 | – | + | 5465 | 1465 | 100+ | – | 21 | 1.1 | 44 | 83 | |
| Re.M. | M23 | – | – | 3238 | 2289 | 10+ | + | 24 | 6.1 | 23 | 76 | |

[1] The following pairs of subjects are siblings: L.S. and J.S., V.-P.S. and V.-M.S., Ra.M. and Re.M., J.T. and K.T.

[2] Underlined figures indicate values outside 95% confidence limits of control subjects.

[3] Two siblings with probable CHH died at the ages of 3 and 6 months.

healthy children studied in the same laboratory (12). IgE was quantified by a radioimmunoadsorbent test (Phadebas, AB Pharmacia, Uppsala, Sweden). The isohemagglutinins, antinuclear antibodies, rheumatoid factor and precipitating antibodies to cow's milk and gluten (12) were studied by routine methods. Complement fixation titers to viruses were measured on microtitration plates by conventional methods (4, 7) (Institute of Virology, University of Helsinki, Helsinki, Finland).

Immunoglobulin-containing cells in biopsy specimens of the jejunal mucosa were quantified by direct immunofluorescence after staining with appropriate antisera (24). The immunoglobulin levels in jejunal juice were measured as described previously (24).

### SKIN TESTING

Serial intracutaneous tuberculin testing was performed starting from 0.1 TU of purified protein derivative (PPD) tuberculin (State Serum Institute, Copenhagen, Denmark). Of the affected subjects, 22 were tested with up to 100 TU or until a positive reaction was observed.

### LYMPHOCYTE CULTURES

Ten to 30 ml of venous blood was drawn into a heparinized syringe (50 IU heparin/ml). The blood was diluted with 3 parts of tissue culture medium RPMI 1640 (Oriola Pharmaceuticals, Helsinki, Finland), and mononuclear cells were separated by the Ficoll-Isopaque gradient centrifugation method of Böyum (2). The supernatant, which contained about 15% plasma, was collected and used as culture medium. The interphase cells were washed twice with phosphate-buffered saline (PBS), and resuspended in a small amount of RPMI 1640. Cells were counted in a hemocytometer, and preparations were made with a cytocentrifuge (Shandon Scientific Co., Ltd., London, England). On the average, 70% of the cells were lymphocytes and 27% monocytes. An aliquot of the cells was suspended in the plasma-containing supernatant, and cell density was adjusted to $0.8 \times 10^6$/ml. Next, 2.5-ml cultures were set up in 35-mm plastic Petri dishes (Falcon Plastics, Los Angeles, CA). Cultures stimulated with PHA and control cultures with autologous plasma were prepared from all patients. Depending on the cell yield, additional cultures were set up with PPD and Con A as stimulants, as well as cultures with 20% pooled human AB plasma in RPMI 1640, and the different stimulants. PHA (PHA-P, Difco, Detroit, MI) was used in a final concentration of 1:250, PPD (preservative-free, State Serum Institute) 10 μg/ml, and Con A (AB Pharmacia), 4 μg/ml. PHA- and Con A-stimulated cultures were harvested after 3 days, and PPD-stimulated and control cultures after 6 days. One hour before harvest, 1 μCi [$^3$H]TdR (spec act 6.7 Ci/mmol, New England Nuclear Corp., Boston, MA) was added per ml culture fluid. Cells

were prepared for May-Gruenwald-Giemsa staining and for scintillation counting as described previously (11).

### ROSETTE FORMATION

From the mononuclear cells separated by the Ficoll-Isopaque method monocytes were removed by mixing $10^7$ cells in 10 ml PBS with 2% calf serum and incubating the suspension for 30 min at 37° in a glass bottle with a bottom area of 50 $cm^2$. The incubation was repeated once, and the nonadherent cells were collected, washed, and resuspended in serum-free RPMI 1640. The average purity of the lymphocytes was 96%.

The relative numbers of lymphocytes bearing receptors for C'3 (EAC rosettes) and for sheep red cells (SRC) (E rosettes) were determined by the method of Jondal *et al.* (14), slightly modified (29). In addition to the lymphocyte purification method, the main modifications were counting of the E rosettes in a hemocytometer after incubation overnight in the cold, and use of crystal violet to facilitate determination of the percentages of rosette-forming cells (RFC).

## RESULTS

### SUSCEPTIBILITY TO INFECTIONS

All of the subjects had been immunized with BCG and most of them with vaccinia. No complications had resulted from the vaccinations. At least 13 had had varicella infection, but none in a hemorrhagic or unusually severe form. Eight of the subjects were considered to be exceptionally susceptible to infections. The criterion for this was a relative one: they had had six or more uncomplicated upper respiratory tract infections or two or more prolonged purulent infections, such as otitis or sinusitis, during the past year. None of these eight patients had a permanent handicap caused by infections.

### SKIN TEST SENSITIVITY

None of the subjects gave a positive skin reaction to 1 TU of tuberculin. Only 2 of 23 tested with 10 TU were reactive, and 11 of 20 tested were nonreactive even to 100 TU. As compared with the tuberculin skin test reactivity of reference subjects derived from the same population, that of the subjects with CHH was significantly diminished (Fig. 1).

### PERIPHERAL BLOOD LEUKOCYTES

As compared with the age-matched reference subjects, the number of circulating lymphocytes was significantly decreased in the affected subjects (Table 2). The difference was more pronounced in the younger age groups: the lymphocyte counts of the affected subjects were on the same low level independently of age (Table 1), whereas in the reference series the number of lymphocytes decreased from the mean of 7080/$mm^3$ in the age group 1–2 years, to 2349/$mm^3$ in the age group 14–16 years. Although the mean neutrophil counts in the CHH subjects and the reference series were similar, the scatter of the counts was wider in the affected subjects, and some low as well as high neutrophil counts were seen (Table 1).

The proportion of DNA-synthesizing blood leukocytes was determined in 20 subjects with CHH. In all subjects the majority of cells labeled with [$^3$H]TdR were lymphoblasts or "atypical lymphocytes"; labeled immature myeloid cells were as few as in the controls. An average of 3.2 lymphoid cells/1000 mononuclear leukocytes were labeled, which was somewhat more than in age-matched control subjects (1.9, $P < 0.1$). However, counts of labeled lymphoid cells per $mm^3$ blood were practically the same in subjects with CHH and in control subjects.

In one subject (M.E.) the proportion of labeled cells was determined 4 days after she presented signs of varicella infection. The count of labeled lymphoid cells was then 26 per 1000 mononuclear cells, whereas 1 month earlier the value had been 2.9. In four otherwise healthy children the count of labeled lymphoid cells 4–6 days after the first signs of varicella was 17–28.

### SERUM IMMUNOGLOBULINS

Most of the affected subjects had serum IgG levels within the 95% confidence limits of healthy children. One had decreased and two increased serum IgG concentration for the age. All had IgA and IgM concentrations within the 95% confidence limits of normal subjects. One subject had increased serum IgE. On the average, the subjects with CHH had slightly increased levels of serum IgG, IgA, and IgM (Table 3).

The levels of isohemagglutinins were appropriate. Antibody titers to viral agents patients had suffered from, and to common epidemic viruses (*e.g.*, influenza, herpes simplex, smallpox, chickenpox, and varicella) were neither low nor abnormally high. All patients had been vaccinated parenterally with killed polio virus. The titers against one or more types of polio virus were measured and found to be in the same range as in immunologically normal children.

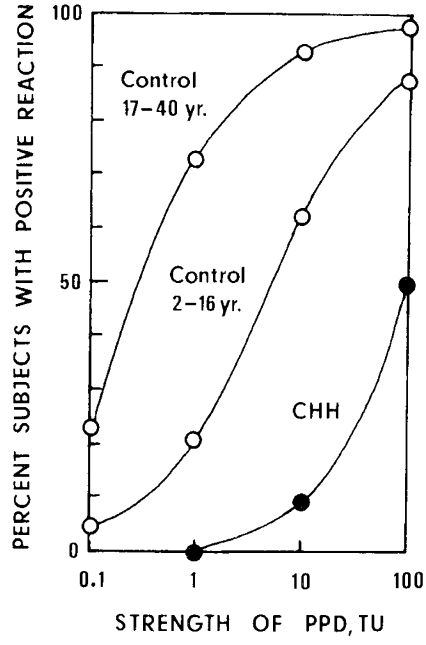


Fig. 1. Cumulative percentages of subjects with positive skin reactions to different strengths of PPD.

Table 2. *Counts of peripheral blood neutrophils and lymphocytes in subjects with CHH and in age-matched reference subjects*

| | Cells/$mm^3$ | | |
|---|---|---|---|
| | CHH ($n$ = 28)[1] | Reference subjects ($n$ = 152) | $P$ |
| Neutrophils | 3138 ± 1317[2] | 3095 ± 1310 | |
| Lymphocytes | 1982 ± 703 | 4002 ± 2065 | <0.001 |

[1] Total number of subjects.
[2] Means ± SD. Values for CHH subjects are calculated from the mean of 2–8 counts for each subject.

Table 3. *Serum immunoglobulins in subjects with CHH*

| Immunoglobulin class | % of mean for age |
|---|---|
| IgG | 120 ± 30[1] |
| IgA | 126 ± 66 |
| IgM | 126 ± 55 |
| IgE | 84 ± 152 |

[1] Mean ± SD.

Rheumatoid factor was present in one and antinuclear antibodies in another of the 25 affected subjects studied. Only 2 of the 28 subjects had precipitating antibodies to gluten, but 16 (57%) had antibodies to cow's milk (Table 1). The latter finding was significantly ($P < 0.0005$, $\chi^2$-test) different from the 17% prevalence in age-matched controls (12).

### IMMUNOLOBULIN-CONTAINING CELLS IN JEJUNAL MUCOSA

In the numbers of IgA- and IgM-containing cells in the jejunal mucosa (Table 4) and in the concentrations of IgA and IgM in the intestinal juice (Table 5) the affected subjects did not differ from the control subjects. IgG-containing cells were somewhat more frequent in the specimens from the subjects with CHH (Table 4). IgE and IgD-containing cells were as rare in the specimens from the affected subjects as in those from the control subjects.

### PHA-INDUCED LYMPHOCYTE TRANSFORMATION

In most of the affected subjects PHA-induced lymphocyte transformation was markedly depressed (Table 1, Fig. 2). In 3-day-old cultures with autologous plasma 34 ± 19% (mean ± SD) of the lymphoid cells were blasts. As compared with age-matched reference subjects the response of all affected subjects was below the mean for the age, and in all except three below the 2.5th percentile of the control subjects (Fig. 2).

The results were essentially similar when the PHA-induced response was expressed as cpm of [$^3$H]TdR incorporation. In the subjects with CHH the response was 2613 ± 460 cpm (mean ± SE), as compared with 6411 ± 632 in age-matched reference subjects. No significant differences were observed in the responses when pooled human AB plasma was used in the culture medium instead of autologous plasma.

Correlations were sought between the PHA response and susceptibility to infections, hair abnormality, skin test sensitivity, PPD-induced lymphocyte transformation, and neutrophil and lymphocyte counts in the peripheral blood. A positive correlation was found between PHA response and lymphocyte count (Fig. 3). The PHA response was significantly lower ($P < 0.05$) in subjects negative to 100 TU than in those giving a positive reaction in the tuberculin skin test. Other significant correlations were not found.

### CON A-INDUCED LYMPHOCYTE TRANSFORMATION

Con A-induced lymphocyte transformation was studied in 12 subjects with CHH. The response was 6.4 ± 3.3% (mean ± SD) blasts on the average, which was significantly ($P < 0.001$) lower than in age-matched reference subjects (13.0 ± 8.0, $n = 36$).

Table 4. *Immunoglobulin-containing cells in jejunal mucosa in subjects with CHH and in reference subjects*

| | Number of cells/mm$^2$ | |
|---|---|---|
| | CHH ($n = 23$)[1] | Reference subjects ($n = 21$) |
| IgA cells | 791 ± 167[2] | 771 ± 233 |
| IgM cells | 246 ± 132 | 184 ± 90 |
| IgG cells | 87 ± 55 | 51 ± 26 |

[1] Number of cases studied.
[2] Mean ± SD.

Table 5. *Levels of IgA and IgM in intestinal juice of subjects with CHH and of reference subjects*

| | mg/100 ml | |
|---|---|---|
| | CHH ($n = 16$)[1] | Reference subjects ($n = 21$) |
| IgA | 2.9 ± 2.6[2] | 4.0 ± 2.5 |
| IgM | 2.6 ± 2.4 | 2.0 ± 1.8 |

[1] Number of cases studied.
[2] Mean ± SD.

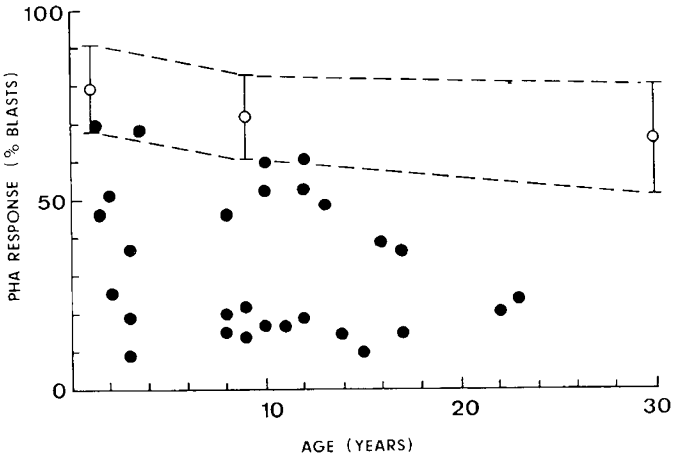


Fig. 2. PHA-induced lymphocyte transformation *in vitro* in subjects with CHH. Open circles and vertical bars indicate means and 95% confidence limits in reference subjects in the age groups 1 year ($n = 20$), 6–12 years ($n = 16$), and 18–38 years ($n = 18$).

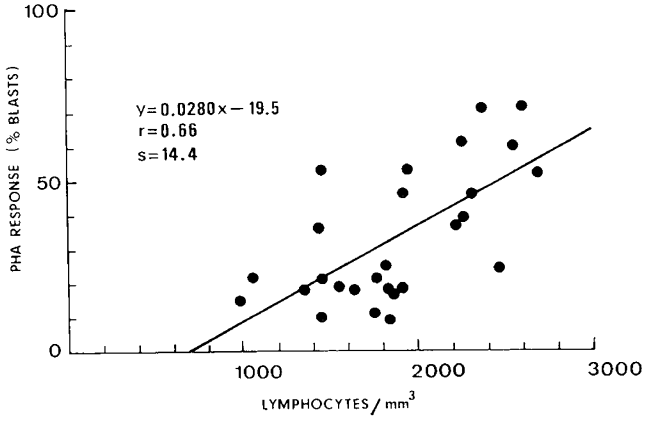


Fig. 3. Correlation between blood lymphocyte count and PHA-induced lymphocyte transformation *in vitro* in subjects with CHH. Values for subject L.S. (Table 1), in whom the lymphocyte count was outside 3SD of the mean for the whole series, have been omitted.

### PPD-INDUCED LYMPHOCYTE TRANSFORMATION

Lymphocytes from 26 of the affected subjects were tested for PPD-induced lymphocyte transformation (Fig. 4). The percentage of blasts in 6-day-old culture was correlated with the results of the skin tests (Table 1). In 11 cases no significant lymphocyte transformation was observed (<0.3% blasts). One of these patients had a positive skin test with 100 TU, the others were negative to 100 TU or not tested with this strength of PPD. No significant differences were observed between cultures containing autologous plasma and those set up with pooled human AB plasma.

### ROSETTE TESTS

The proportions of lymphocytes forming E rosettes and EAC rosettes were determined for 20 of the affected subjects (Table 1 and Fig. 5). The percentage of E-RFC was significantly ($P < 0.001$) decreased, 68.2 ± 9.4 (mean ± SD), as compared with the controls (78.7 ± 7.8), and the percentage of EAC-RFC significantly ($P < 0.05$) increased, 28.6 ± 13.3 (controls 19.8 ± 8.7). The sum of EAC- and E-RFC was close to 100% (96.7 ± 14.7), but the variation was wider than in the controls (98.6 ± 7.4). In the E rosette test RFC were differentiated into cells forming "tight" (more than 12 SRC bound to a lymphocyte) and "weak" (3–12 bound SRC) rosettes. In the average percentage of tight rosettes (30% of all rosettes) the affected subjects did not differ significantly from the control subjects (32%).

The percentage of T cells, as judged by the E rosette test, was

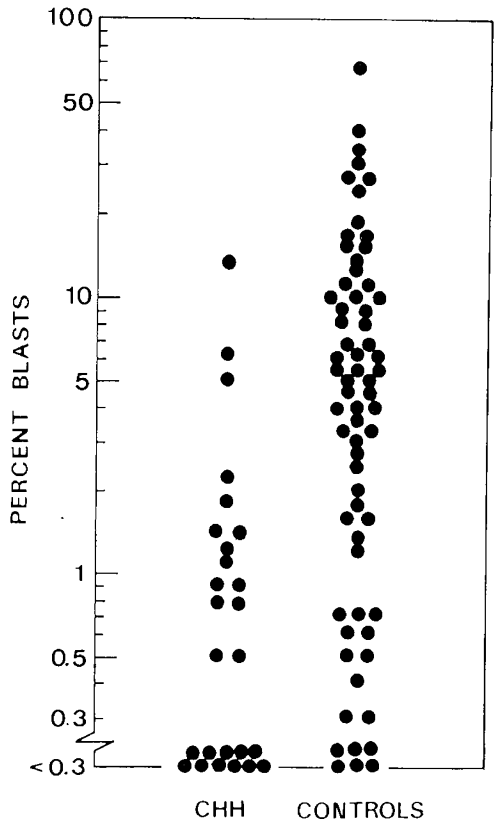


Fig. 4. PPD-induced lymphocyte transformation *in vitro* in subjects with CHH and age-matched reference series.

unexpectedly high in view of the markedly depressed PHA response. Moreover, the percentage of E-RFC was not significantly correlated with the percentage of blasts in PHA-stimulated cultures (Fig. 5). The possibility was considered that this discrepancy could be due to selective loss of B cells during the purification of lymphocytes. The following findings argue against this possibility. 1) The yield of lymphocytes after the purification procedure was only slightly smaller in affected than in control subjects, and this difference was inversely correlated with the higher proportion of monocytes in the former group. 2) The percentage of EAC- and E-RFC did not correlate with the lymphocyte yield either in the affected subjects or in the control subjects. 3) In the case of one subject with CHH the lymphocytes remaining in the second bottle were detached by vigorous aspiration and tested for rosette formation. The results did not differ from those obtained with lymphocytes purified by the regular procedure. 4) E-RFC from one subject with CHH were isolated by velocity sedimentation (29), and tested for PHA-induced lymphocyte transformation. The percentage of blasts was only slightly greater than in cultures of nonfractionated lymphocytes (49% *vs.* 39%).

## DISCUSSION

The degree of the bone and especially of the hair abnormalities of CHH is variable, and it is not always possible to make the definitive diagnosis on purely clinical grounds. In the present series all patients had short-limbed growth failure with x-ray findings of metaphyseal dysplasia, but the hair abnormality was not always manifested.

Unusual susceptibility to infections with certain viruses has suggested that cellular immunity is impaired in CHH (20). This notion was supported by detailed immunologic studies of patients selected for a history of severe viral infections (9, 19). No failure of humoral immunity was detected, but depression of cellular immunity was apparent from diminished delayed skin test reactivity, delayed rejection of skin allograft, and decreased responses of lymphocytes to PHA and to allogeneic cells (9, 19).

The present series differs from those of the other reports mentioned in being nonselected. Only 8 of the 28 affected subjects found in Finland were considered to be unusually susceptible to infections. However, Finnish subjects with CHH seem to resemble those reported elsewhere in greater susceptibility to fatal infections. A short limbed infant belonging to a pedigree which includes three of the subjects of our series died at the age of 21 months from prolonged, intractable diarrhea and was found to have thymic hypoplasia and dysplasia (15, 30). Two short limbed siblings of another member of our series had succumbed at the ages of 3 and 6 months to severe infections; one had severe anemia from birth.

Of the three subjects who had PHA-induced lymphocyte transformation in low normal range, two had normal hair. One of these two was a certain case of CHH by the evidence of presence of the complete syndrome in the brother. Otherwise, the findings in the normal-haired group of subjects were not different from the others.

Lymphopenia was observed in most of our subjects with CHH, as has been reported in CHH patients suffering from severe infections (9, 19). The absolute blood lymphoblast count did not differ from that in the control subjects. The presence of increased numbers of lymphoblasts in peripheral blood is regarded as a manifestation of an immune response (3, 28), but the relation of this response to humoral and cellular immunity is not known. However, a relation to cellular immunity is suggested by the findings that lymphoblasts were predominantly T cells both in infections caused by two herpes viruses, Epstein-Barr virus (26, 29) and cytomegalovirus (29), and in the "physiologic lymphoblast response" during the neonatal period (18). After varicella infection, one of our CHH subjects was found to be capable of a lymphoblast response of equal magnitude to that observed in otherwise healthy children. Chronic neutropenia has been reported in some subjects with CHH, and in one case of nearly fatal varicella the neutropenia was associated with failure of myeloid maturation (19). In the present series the neutrophil counts were mostly on the normal level, but in a few of the subjects abnormally low counts were recorded.

As judged by the total immunoglobulin and specific antibody levels the subjects had no derangement of humoral immune responses. The high incidence of precipitating antibodies to cow's milk was an unexpected finding. Their presence suggests abnormal penetrance of proteins of cow's milk through the jejunal mucosa in an antigenic form, as is the case in jejunal damage (celiac disease) and IgA deficiency.

Skin tests for delayed hypersensitivity were done only with tuberculin. Because of the almost 100% BCG vaccination, the

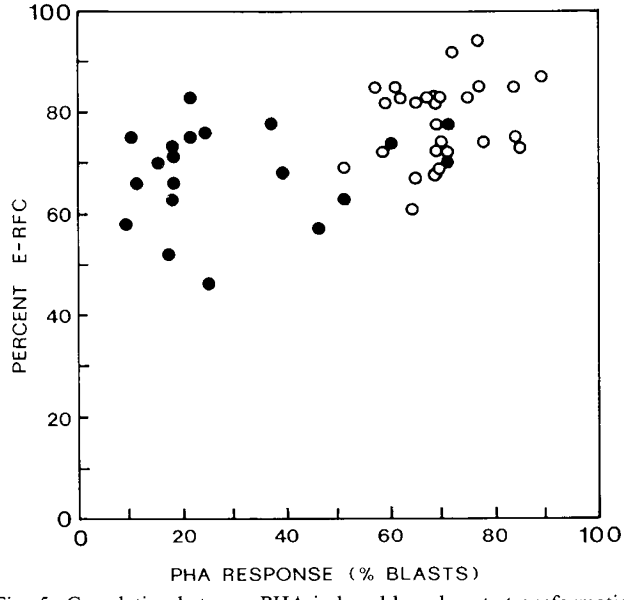


Fig. 5. Correlation between PHA-induced lymphocyte transformation *in vitro* and percentage of lymphocytes forming E rosettes in subjects with CHH (●) and reference subjects (○).

tuberculin test is a very useful indicator of the degree of delayed sensitivity in the Finnish population. More than 90% of the adults (25) and about two-thirds of the children react to 10 TU of tuberculin. A profound anergy was evident in our subjects, since only 2 of the 23 tested with this strength of tuberculin were reactive. Skin test reactivity was correlated with the PPD-induced lymphocyte transformation *in vitro,* as has been found in healthy subjects and in patients with sarcoidosis (10). This suggests that the anergy is due to lack of specifically reactive lymphocytes and not, for example, to impaired production of mediators of delayed sensitivity.

PHA-induced transformation of lymphocytes is decreased in several diseases in which cellular immunity is depressed. In the present series PHA-induced lymphocyte transformation was markedly depressed, but the degree of suppression was variable. The PHA responses observed in subjects with CHH selected for histories of severe viral infections (9, 19) seem to have been even more depressed than those of our subjects, but differences in methodology and in the expression of the data make comparison difficult. The decrease in the number of PHA-responsive lymphocytes is even more pronounced when the low lymphocyte counts in peripheral blood are taken into account. The positive correlation observed between the lymphocyte counts and the PHA response suggests that the lymphopenia is predominantly caused by a decrease in PHA-responsive lymphocytes.

Rosette tests were applied in an attempt to quantify B and T lymphocytes in peripheral blood more directly. The results of these tests were unexpected. Although the proportion as well as the absolute number of T cells was diminished, the decrease was far smaller than would have been expected from the number of cells responding to PHA which is considered to be a T cell mitogen (1, 13).

This decrepancy might be due to a defect in the T cells in CHH affecting responsiveness to T cell mitogens but not receptors for SRC. An alternative explanation would be that even in healthy individuals T cells belong to two subpopulations, both having receptors for SRC, but only one being able to give a proliferative response to stimulation with mitogens, and only this latter population being decreased in CHH. Wybran *et al.* (32) used a modification of the E rosette test in which only about one-third of the E-RFC are detected, and found a correlation between this test and cellular immune functions. They suggested that their "active rosette test" identified T cells with very high binding affinity for SRC (31). However, in our test, the binding affinity between lymphocytes and SRC seemed not to be decreased in CHH, since the proportion of lymphocytes forming "tight" rosettes was practically the same as in the control subjects.

Recently, an association has been observed between the severe combined immunodeficiency disease and deficiency of adenosine deaminase; a derangement in the reutilization of purine metabolites has been suggested as the basic defect in the severe combined immunodeficiency disease (7). The activity of adenosine deaminase in erythrocytes from patients with CHH has been reported to be normal (16). This observation was confirmed in two of our present patients.

## CONCLUSION

In our unselected series almost all subjects with CHH had subnormal responses in one or more of the tests related to the functions of cellular immunity, although most of them had no apparent unusual susceptibility to infections. A depression of cellular immunity, although variable in degree, evidently is an integral part of CHH and is a feature at least as constant as the hair abnormality.

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