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Age-Related Changes in Humoral and Cell-Mediated Immunity in Down Syndrome Children Living at Home

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ABSTRACT. Abnormalities of humoral and cell-mediated immunity have been described in Down syndrome but reported findings have been inconsistent. Confounding factors have included age, institutional *versus* home life, hepatitis B antigenemia, and zinc deficiency. To clarify this problem, we studied 64 children with Down syndrome (DS) compared with an age-matched control group. All children had always lived at home. All the DS children were negative for hepatitis B surface antigen. Serum zinc concentration in the DS group was on average 12 μg/dl lower than age-matched control children. They also had significantly lower levels of immunoglobulin M, total lymphocyte count, T and B lymphocytes, and T helper and suppressor cells. *In vitro* lymphocyte response to phytohemagglutinin and concanavalin A was significantly reduced at all ages in the DS group. Lymphocyte response to pokeweed mitogen increased with age in control children but decreased in the DS children. By 18 yr, the mean response for DS was 60000 cpm lower than controls. The DS group had significantly higher concentrations of immunoglobulins A and G than controls and the difference increased with age. Complement fractions C3 and C4 were also higher in the DS group at all ages. The number of HNK-1 positive cells was higher in the DS group than controls at all ages. When hepatitis and institutionalization are excluded as confounding factors, DS children still differ in both humoral and cell-mediated immunity from an age-matched control group. (*Pediatr Res* 22: 536–540, 1987)

Abbreviations

DS, Down syndrome
MSLT, mitogen-stimulated lymphocyte transformation
HBSAg, hepatitis B surface antigen
PHA, phytohemagglutinin
DNCB, dinitrochlorobenzene
AA, atomic absorption
ELISA, enzyme-linked immunosorbent assay
NK, natural killer
smIg, surface membrane immunoglobulin
FITC, fluorescein
PWA, pokeweed mitogen
ConA, concanavalin A
TLC, total lymphocyte
TC, T cells
HC, helper cells
SC, suppressor cells

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Repeated and chronic respiratory infections are an important cause of morbidity in children with DS (Trisomy 21) (1, 2). DS children are at increased risk for leukemia (3, 4) and have a greater incidence of autoimmune disorders than the general population (5). This suggests that immunodeficiency may be an important component of DS (6). Abnormalities of cell-mediated, humoral, and phagocytic functions have been described in DS (6–19) but the findings have not been consistent. This inconsistency has been variously attributed to factors such as age variability between subjects and controls, institutionalization as a cause of exposure to frequent infections, and persistence of HBsAg in the blood of DS subjects (6, 15, 19, 20).

Zinc deficiency is known to depress immune responses in both inherited and acquired deficiency states (21, 22). Low serum zinc concentration has been reported in DS (23–25) although again findings have not been consistent (26). A moderate reduction in serum zinc concentration was shown to decrease the zinc-dependent activation of thymulin, a circulating thymic hormone (25, 27), and thus affect acquisition of immunocompetence by T lymphocytes (27). Thymulin was shown to be low in children with DS (25). In a single study it was reported that 2 months of daily oral zinc sulfate therapy in 12 DS children improved the *in vitro* response of lymphocytes to high dose PHA, neutrophil chemotaxis, and skin reactivity to DNCB as well as increasing serum zinc levels (23). This supported a possible role for zinc deficiency in the immunodeficiency of DS. Differences in zinc status may also therefore have contributed to the inconsistency of reported data in DS.

To resolve these controversies, DS children who had always lived at home were studied together with age- matched controls to determine differences in zinc status, cell-mediated, and humoral immunity.

The objectives of the study were to determine whether non-institutionalised DS children differ from healthy like-age normal control children in humoral and cell-mediated immunity. If differences were demonstrated, were they related to serum zinc status or persistent hepatitis antigenemia? Did DS children with chronic respiratory problems, nasal discharge and cough differ from DS children without these chronic symptoms?

SUBJECTS

The DS study group consisted of 64 DS children, aged 1–19 yr, living in a home environment, who attended our research

clinic for the first time during a 6-month period in 1984/85. Recruitment occurred through a series of meetings at which the study was described to parents. The sole selection criteria were appropriate age and willingness of families to participate in the study. Three DS children had surgically corrected cardiac defects and three others were on treatment for hypothyroidism. None was acutely ill at the baseline visit although more than one-third reported chronic symptoms such as nasal congestion and cough. The latter are called the "symptomatic" group.

Data were obtained over the same period from 88 control children. A randomly selected subset of 30 children provided control data for the immunology tests. All controls were normal healthy children aged from 1–19 yr drawn from an unselected community population living in the same areas as the DS group who had no clinical signs of illness when blood samples were taken. Informed consent was given by the parents of all subjects. The study protocol was approved by the University of British Columbia Clinical Screening Committee for Research involving Human Subjects.

METHODS

A brief clinical history was obtained and a single venous blood sample collected aseptically into a heparinized vacutainer for immunology and protein studies, and into a tracemetal free collection tube (Becton Dickinson) for the trace metal studies. Zinc was measured by flame AA spectrometry using a Varian AA 1475. Serum copper was also measured by electrothermal AA on the Varian GTA-95 since oral zinc therapy may impair copper absorption and lower serum copper concentration. Proteins were measured by nephelometry (Behring LN Nephelometer). A blood sample from each DS child was screened by ELISA for HBsAg and anti-HBsAg.

Lymphocytes were separated by standard techniques (28) using density gradient centrifugation in Ficoll-Paque (Pharmacia). Lymphocyte cell surface marker analysis was performed by 2-color direct immunofluorescence. For determination of T cell populations we used monoclonal antibodies from Becton Dickinson Monoclonal Center (Leu-1 for T cells, Leu-2a for T-suppressors, Leu-3a for T-helpers and Leu-7 (HNK-1) for NK cells. B lymphocyte was assayed with FITC-goat-antihuman IgG + IgA + IgM (H + L) antibodies (Zymed Laboratories). A blank of mouse antibody was run in parallel and its value subtracted from the test results. Lymphocyte blastogenic response to mitogens, PHA, PWM, and ConA were determined using microtitre plate culture technique and with optimal concentration of mitogen, in triplicate, as previously described (29).

Statistical analysis. Clinical group differences and age effects on chemistry and immunology variables were investigated using analysis of variance where age was treated as a linear effect. The effect of zinc and copper on the other variables was also studied by analysis of covariance. All calculations were performed using SAS Statistical Software (30). Since a large number of variables was studied, Bonferroni corrections were taken into account when determining significance of a result.

RESULTS

All 64 DS children were negative on screening for HBsAg and for anti-HBsAg. This excluded persistent hepatitis antigenemia from further consideration as a confounding factor in this study.

The mean age of the 23 "symptomatic" DS children was lower (6.5 yr) than that of the 41 "asymptomatic" children (10 yr) ($p < 0.006$).

Zinc and copper. The effect of age on zinc and copper levels was similar in both the DS and control groups (zinc $p = 0.32$, copper $p = 0.33$) (Figs. 1 and 2). In both groups zinc levels decreased with age but the change was not significant ($p = 0.08$), while copper levels decreased significantly with age ($p < 0.0001$). Zinc levels were on average 11.78 μg/dl lower in the DS group ($p < 0.001$) while copper was on average 7.97 μg/dl higher ($p = 0.03$).

Immunoglobulins. Figures 3–5 show the effect of age and diagnostic group on immunoglobulin G, M, and A. For IgM the effect of age was the same in both groups ($p = 0.16$) while it differed significantly with respect to IgA ($p = 0.001$) and IgG ($p = 0.004$). IgM levels were on average 55 mg/dl lower in the DS group ($p < 0.0001$) and increased significantly with age in both groups ($p = 0.001$). IgA and IgG increased with age in both groups, with a more rapid increase in the DS group. IgA and IgG levels were higher in the DS group at all ages.

After adjusting for age and group effects, IgA levels were correlated negatively with serum zinc ($p = 0.02$) and positively with serum copper ($p = 0.01$) while IgM and IgG were correlated with neither.

Complement fractions. The effect of age on the logarithm of complement fractions C3c and C4 was the same in both groups. The average level of C3c increased with age ($p = 0.02$), serum zinc ($p = 0.006$), and serum copper ($p < 0.0001$) and was also significantly higher in the DS group ($p < 0.0001$). Mean (SD) C3c concentration was 82 (15) mg/dl in the DS children and 69 (11) mg/dl in controls. The average level of C4 depended on neither age ($p = 0.11$) nor serum zinc ($p = 0.52$) but was correlated with serum copper ($p < 0.0001$) and higher in the DS group ($p < 0.0001$). Mean (SD) C4 concentration was 31 (13) mg/dl in the DS children and 18 (5) mg/dl in controls. No difference was found between the "symptomatic" and "asymptomatic" DS children for mean concentrations of serum zinc

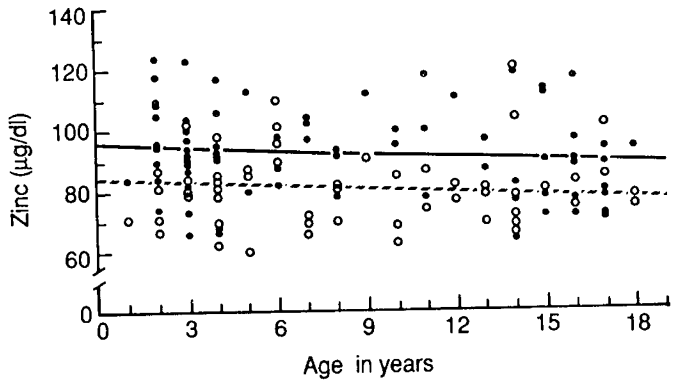

Fig. 1. Serum zinc concentration by age for DS children (O – – –) and controls (● ——). For DS, zinc = 84 − 0.37 age, for controls zinc = 96 − 0.37 age, $R^2 = 0.18$.

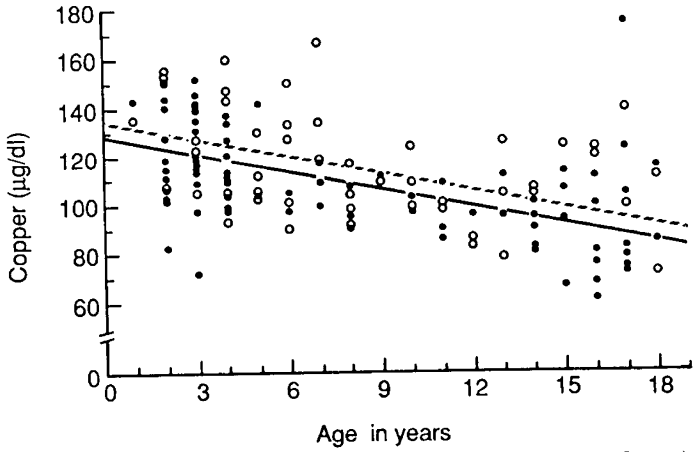

Fig. 2. Serum copper concentration by age for DS children (O – – –) and controls (● ——). For DS, copper = 133 − 2.34 age, for controls copper = 127 − 2.34 age, $R^2 = 0.36$.

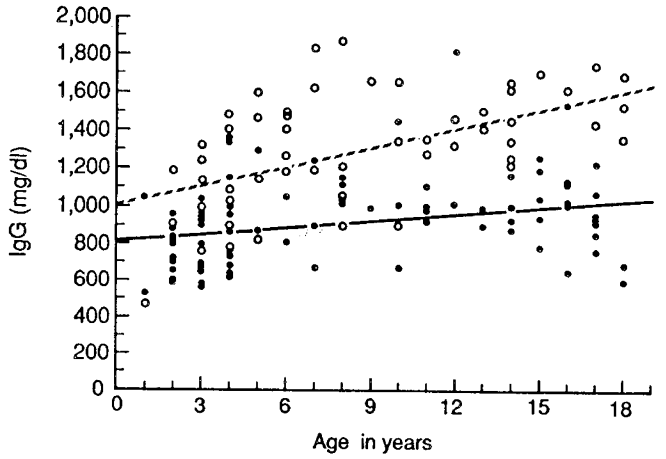

Fig. 3. Serum IgG concentration by age for DS children (O - - -) and controls (● ——). For DS, IgG = 997 + 34.17 age, for controls IgG = 813 + 11.76 age, $R^2 = 0.47$.

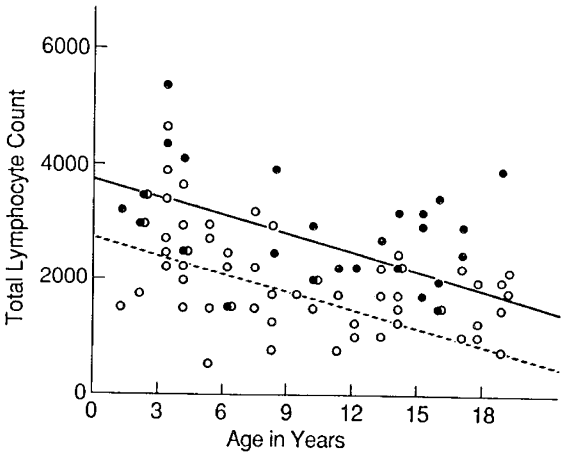

Fig. 6. Total lymphocyte count by age for DS children (O - - -) and controls (● ——). For DS, TLC = 2735 − 80.17 age, for controls TLC = 3745 − 80.17 age, $R^2 = 0.35$.

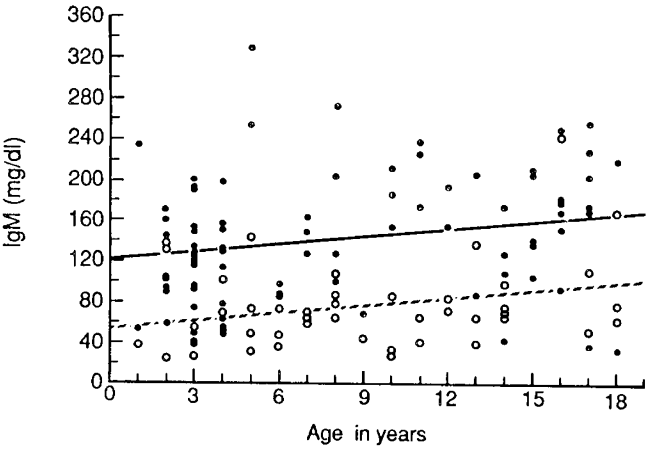

Fig. 4. Serum IgM concentration by age for DS children (O - - -) and controls ● ——). For DS, IgM = 54 + 2.51 age, for controls IgM = 122 + 2.51 age, $R^2 = 0.34$.

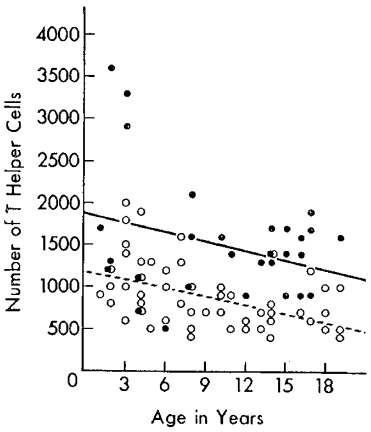

Fig. 7. T HC count by age for DS children (O - - -) and controls (● ——). For DS, HC = 1215 − 36.12 age, for controls HC = 1890 − 36.13 age, $R^2 = 0.35$.

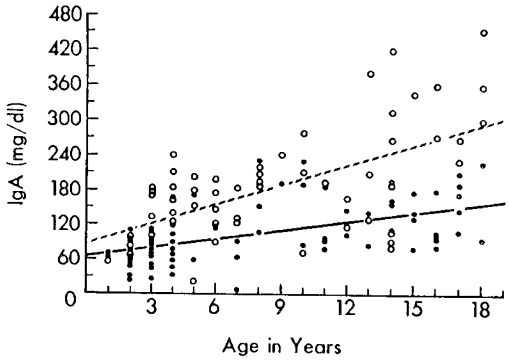

Fig. 5. Serum IgA concentration by age for DS children (O - - -) and controls (● ——). For DS, IgA = 86 + 11.57 age, for controls IgA = 63 + 5.21 age, $R^2 = 0.51$.

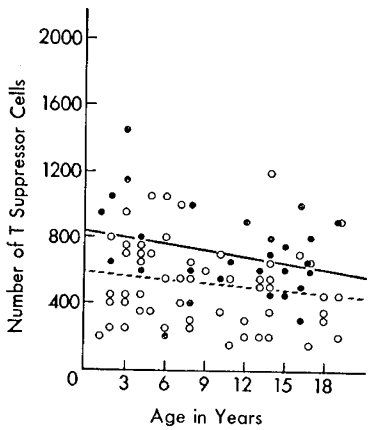

Fig. 8. T SC count by age for DS children (O - - -) and controls (● ——). For DS, SC = 630 − 12.88 age, for controls SC = 837 − 12.88 age, $R^2 = 0.16$.

($p = 0.72$), copper ($p = 0.31$), IgG ($p = 0.83$), IgA ($p = 0.11$), IgM ($p = 0.41$), or C4 ($p = 0.13$). C3c was slightly higher in the symptomatic DS group [87 (4)] mg/dl than in the asymptomatic group [79 (15)] mg/dl ($p = 0.04$).

Lymphocyte subsets. Figures 6–8 show the effect of age and diagnostic group on the TLC and helper and suppressor TC counts. For TLC, TC, T HC, and T SC, the effect of age was similar in the DS and control groups ($p > 0.42$) in all cases. TLC, TC, and HC numbers declined significantly with age ($p < 0.001$ in all cases) while the relationship was weaker for SC ($p = 0.04$). Counts in the DS group were significantly lower than in the controls for TLC, TC, HC, ($p < 0.0001$) and for SC ($p =$

0.0003). The effect of age on B cell count (Fig. 9) differed between the two groups ($p = 0.03$). In the control group B cells declined significantly with age ($p = 0.01$) while in the DS group mean levels were not different across ages ($p = 0.08$). After adjusting for age and group effects, there was no correlation of lymphocyte subset counts with zinc or copper concentration.

DS children had significantly higher numbers of cells positive for the Leu-7 (HNK-1) marker, which reacts with the population of large granular lymphocytes including NK cells ($p = 0.009$). The mean number of HNK-1 cells was 290/mm^3 in DS children and 165/mm^3 in controls.

MSLT. Figures 10 and 11 show the effect of age and diagnostic group on MSLT for PHA and PWM. The effect of age was the same in both groups for PHA and ConA but significantly different for PWM ($p < 0.0001$). A slight but not significant increase of MSLT with age was seen in both groups for PHA and Con A. The average response was 35,000 cpm higher in the controls for PHA ($p = 0.001$) and 54,000 cpm higher in the controls for Con A ($p < 0.0001$). For PWM, the average MSLT increased with age in the controls and decreased in the DS group; the average cpm were approximately equal in the two groups at 6 yr. By 18 yr the DS response was on average 60,000 cpm lower. After adjusting for age and group effects, serum copper had no effect on MSLT ($p = 0.29$) while there was a weak inverse correlation of serum zinc with MSLT ($p = 0.04$ for PWM, $p = 0.06$ for PHA, and $p = 0.14$ for Con A).

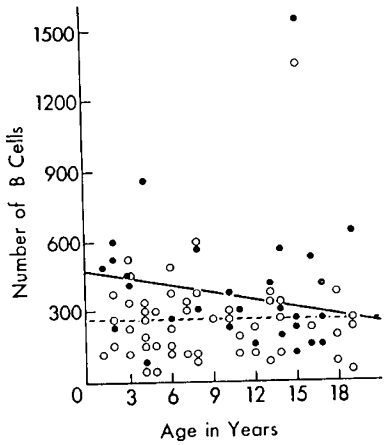

Fig. 9. B lymphocyte count (BC) by age for DS children (O - - -) and controls (● ——). For DS, BC = 235 + 2.98 age, for controls BC = 496 − 14.38 age, $R^2 = 0.14$.

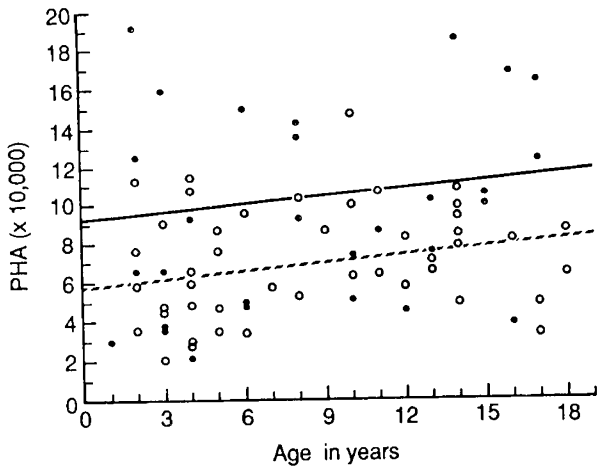

Fig. 10. *In vitro* lymphocyte transformation response to PHA by age for DS children (O - - -) and controls (● ——). For DS, PHA = 5.76 + 0.14 age, for controls PHA = 9.26 + 0.14 age, $R^2 = 0.21$.

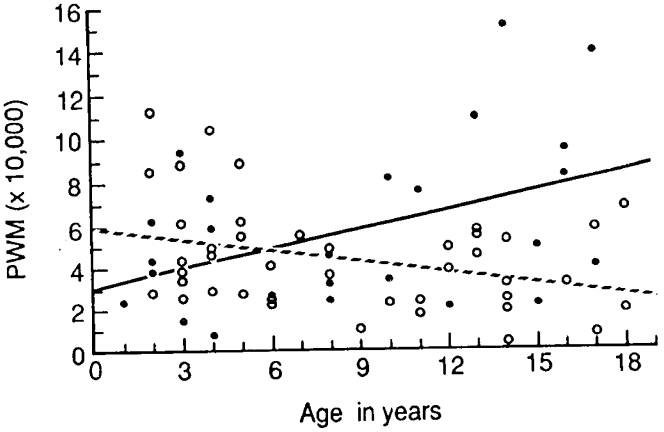

Fig. 11. *In vitro* lymphocyte transformation response to PWM by age for DS children (O - - -) and controls (● ——). For DS, PWM = 5.89 + 0.18 age, for controls PWM = 3.04 + 0.31 age, $R^2 = 0.20$.

No difference was found between the "asymptomatic" and "symptomatic" DS groups for lymphocyte quantitation or response to mitogens with one exception. The asymptomatic group had a much higher percentage of HNK-1 positive cells (18.5%) than the symptomatic (9.6%) ($p = 0.004$).

DISCUSSION

The major differences between DS and controls found herein were the markedly decreased TLC, T HC, and SC, PHA- and Con A-stimulated lymphocyte transformations, serum zinc and serum IgM levels, and the increased HNK-1 positive cells and serum IgG and IgA.

Whereas the ability of lymphocytes to respond to PWM stimulation increases with age in control children, DS children show a significant decrease in response to PWM. We have shown that distinct differences exist between DS and control children that cannot be attributed to persistence of HBsAg or to institutionalization.

More than two-thirds of the DS children in comparison to age-matched controls were moderately zinc deficient as defined by a serum zinc level more than 10% below the mean of the control group. No significant correlation was found for serum zinc with lymphocyte subset counts or MSLT.

Plasma or serum zinc has been reported to be significantly lower in DS children (23–25), while studies comparing adult groups or a wide age range of subjects found similar zinc or copper concentrations in DS and non-DS subjects (26). The reduction of mean serum zinc to about 88% of the level in age-matched controls suggests that a chronic state of moderate zinc deficiency may be present in our DS children. The reason for the low serum zinc in DS is as yet unexplained. Possible causes include inadequate dietary intake of zinc, abnormal absorption, redistribution in response to stress or infection, or increased needs. Dietary zinc intake was not specifically assessed in this study but based on parental contact, we have no reason to believe that dietary inadequacy is the explanation for the low zinc in the DS children. Mean serum zinc was no different in the "symptomatic" (81 μg/dl) and "asymptomatic" (82 μg/dl) DS groups so that the stress of chronic infection was not a factor in the low serum levels in this study.

Fabris *et al.* (25) suggested that a 20–30% reduction in normal serum zinc level could increase the ratio of inactive to active circulating thymic hormone, thymulin, and thus affect acquisition of immunocompetence by T cells. Although all lymphocyte subsets were lower in our DS children, the difference was most

marked for the T cell subset, particularly HC. However, thymulin was not measured in our study.

Studies of cell-mediated immunity in DS have shown varied results with respect to numerical and functional variation from normal. Reasons may include failure to adjust for age effect or use of assays reacting to different cell surface markers to determine similar cellular subsets.

Other groups using surface markers have reported similar increases in HNK-1 cells in DS (16). Others (18) studying NK function rather than surface markers have shown no significant difference between DS and control groups for NK cytotoxicity and antibody-dependent cellular cytotoxicity in unseparated lymphocytes although the DS lymphocytes did show somewhat stronger activity. The Leu-7 marker is not exclusive for the NK cell population. This may partially explain the apparent inconsistency.

Reports of immunoglobulin and protein status in DS have also varied. Generally, hypergammaglobulinemia has been thought to reflect persistent or recurrent antigenic stimulation. A high prevalence of HBsAg positivity in several studies of DS appeared to provide a candidate antigen (5, 19, 20). The importance of institutionalization for this problem has been controversial. HBsAg positivity in DS was reported as 28% in a large institution *versus* 3% in non-DS subjects in the same institution compared with 1.5% in DS subjects in a small institution (19). A study comparing DS and non-DS subjects matched for age and residence (home or institution) found that the presence of antithyroglobulin antibodies was positively correlated with the presence of HBsAg (5). In our study, no child was positive for HBsAg including nine children with raised titers of thyroid microsomal antibody. Etiologies other than persistent HBsAg must be sought to explain the marked variation in immunoglobulin profile in our DS group compared to controls.

Despite failure to show correlation of current serum zinc levels with the severely depressed total lymphocyte, TC and T HC counts and the depressed MSLT, the possibility that these problems were due to a chronic state of moderate zinc deficiency in the DS children in our study could not be excluded. A prospective study of zinc supplements, serial zinc levels and lymphocyte subset distribution, MSLT, and infections in DS children is in process to resolve this issue.

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