Clin. exp. Immunol. (1982) **50**, 621–628.

Lymphocyte dysfunction in cartilage hair hypoplasia. II. Evidence for a cell cycle specific defect in T cell growth

G. F. PIERCE & S. H. POLMAR *Departments of Pathology and Pediatrics, Case Western Reserve University School of Medicine and Rainbow Babies and Childrens Hospital, Cleveland, Ohio, USA*

(Accepted for publication 23 July 1982)

SUMMARY

Defects of *in vitro* B and T lymphocyte function and impaired delayed type hypersensitivity reactions, as well as an increased risk of lethal viral infections have been reported in cartilage hair hypoplasia (CHH), an autosomal recessive form of short limbed dwarfism. We have previously found an intrinsic proliferative defect that affected several cell types from CHH individuals. In order to further evaluate it we developed continuous T cell lines (CTCL) from CHH and normal individuals. The T cells from cultures of CHH and normal individuals were indistinguishable with respect to cell surface antigens characteristic of fully differentiated T cells, as defined by monoclonal antibody analysis. However, CHH T cells produced significantly less interleukin 2 (IL2) than normal T cells and the growth of CHH CTCL in response to exogenously supplied IL2 was markedly diminished (cell cycle 120–165 hr) compared to normal CTCL (cell cycle 48–60 hr). Furthermore, the exogenous IL2 was not absorbed from growth medium by CHH CTCL at the same rate as normal CTCL. Both production and utilization of IL2 are cell cycle specific events that occur during G_1 phase before the onset of DNA synthesis (S phase). Thus, CHH T lymphocytes appear to have a defect related to G_1 phase that results in a longer cell cycle for individual cells, and leads to decreased proliferation of the population. We postulate that this G_1 phase defect is present in multiple cell types in CHH and that analysis of continuous T cell lines from CHH individuals may permit the identification of this defect.

INTRODUCTION

Cartilage hair hypoplasia (CHH) is an autosomal recessive form of short limbed dwarfism which is found with increased prevalence among the Old Order Amish of the United States (McKusick *et al.*, 1965). The Amish are a genetically and culturally isolated religious subculture descended from immigrants from the Alsace-Lorraine region and now living in Pennsylvania, Ohio and Indiana. The CHH syndrome also occurs with increased prevalence in Finland (Virolainen *et al.*, 1978; Ranki *et al.*, 1978). CHH individuals have sparse, fine hair and manifest impaired cutaneous delayed type hypersensitivity and *in vitro* lymphocyte responses to mitogens and antigens (Lux *et al.*, 1970; Virolainen *et al.*, 1978; Trojak *et al.*, 1981). Some individuals with CHH show an increased susceptibility to severe viral infections, such as varicella and vaccinia (McKusick *et al.*, 1965). In previous studies we have identified intrinsic defects in the proliferation of CHH T and B lymphocytes as well as CHH fibroblasts (Pierce & Polmar, 1982). In CHH individuals, peripheral

Correspondence: Stephen H. Polmar, St Louis Children's Hospital, 500 S. Kings highway Boulevard, St Louis, Missouri 63110, USA.

0009-9104/82/1200-0621$02.00

blood T and B lymphocyte populations were found to be decreased but there were normal proportions of T helper/inducer (OKT4) and suppressor/cytotoxic (OKT8) subpopulations. Excessive suppressor activity could not be demonstrated and macrophage accessory cell function was normal. In the present study we have extended our investigation of the defect in CHH T lymphocyte proliferation utilizing continuous T cell lines (CTCL).

For activation, T cells from peripheral blood require an initial mitogenic or antigenic signal (Ruscetti & Gallo, 1981). Binding of mitogen or antigen and macrophage derived interleukin 1 initiates production and secretion of interleukin 2 (IL2, T cell growth factor) by T cells (Smith *et al.*, 1980). Concurrently, either IL2 producing T cells or other populations of T cells develop the ability to bind IL2, which then triggers the onset of DNA synthesis (S phase) (Stadler *et al.*, 1981; Larsson, 1981; Maizel *et al.*, 1981). Thus, it is IL2, and not the initial signals, which induces proliferation of T cells. The role of macrophages, antigens, and mitogens is limited to initial activation of T cells. Activated T cells (CTCL) are fully differentiated and can be cycled indefinitely by the regular addition of media containing interleukin 2 to their cultures (Kurnick, Hayward & Altevogt, 1981; Pawelec *et al.*, 1981). Other soluble factors produced by mitogen or antigen activated mononuclear cells, such as interleukin 1 and interferon, are unable to support continuous T cell growth in culture (Ruscetti & Gallo, 1981).

In the present study, we established CTCL dependent upon IL2 for continuous growth in order to better analyse the growth defect present in CHH T lymphocytes. We report here (1) a quantitative deficiency in IL2 absorption and utilization by CHH CTCL and (2) a diminished production of IL2 by CHH peripheral blood lymphocytes. Since both of these events must occur in the G_1 phase of the cell cycle before the onset of DNA synthesis, we postulate a partial metabolic block in G_1 in CHH CTCL which results in a longer G_1 phase. This block may result in the impairment of cellular immunity and the diminished resistance to vaccinia and varicella observed in some CHH individuals.

MATERIALS AND METHODS

Patients. Blood samples were obtained from eight CHH individuals (mean age 17·9 years, range 13–26), two of their normal siblings and five unrelated controls (mean age 24·6 years, range 12–40). The diagnosis of CHH was established in each subject by field teams from The Johns Hopkins Hospital, under the direction of Dr V.A. McKusick, as described previously (McKusick *et al.*, 1965; Trojak *et al.*, 1981), and all CHH individuals were of Old Order Amish descent. All CHH individuals were clinically healthy at the time of study, with one exception (see Table 2). Six affected subjects had a history of severe varicella infection, lasting from 15 to 45 days. Blood samples were obtained in accordance with the guidelines established by the Institutional Review Board of University Hospitals of Cleveland.

Production of IL2 containing media. Peripheral blood mononuclear cells (PBMC) from normal, healthy adults were obtained after Ficoll-Hypaque density centrifugation of heparinized whole blood. IL2 was prepared by culturing pooled PBMC from 10 individuals with phytohemaglutinin P (PHA 3·75 μg/ml; DIFCO, Detroit, Michigan, USA), in 2% pooled, heat-inactivated AB serum (American Red Cross) and RPMI 1640 (GIBCO, Grand Island, New York, USA) at a concentration of 1×10^6 cells/ml for 48 hr (Ruscetti & Gallo, 1981; Alvarez, Silva, & de Landazuri, 1979). Cells were irradiated prior to culture (2,000 rad) to maximize IL2 production (Inouye *et al.*, 1980). The culture supernatant was harvested, filtered through a 0·45 μm filter, and stored at −20°C until used. This supernatant had activity designated as IL2 and was tested for potency in the CTCL microassay described below, before being used to support the growth of CTCL. Different lots of this material had IL2 activity ranging from 39–54 units/ml. To test the ability of single CHH and normal donors to make IL2, PBMC culture supernatants were generated in a similar fashion, except without the stimulus of allogeneic cells. These supernatants were also tested for activity in the CTCL microassay.

Initiation of cell lines. CTCL were initiated from PBMC (1×10^6 cells/ml) incubated with PHA (3·75 μg/ml) in 10% AB serum and RPMI 1640. After culture for 5 days, PBMC were resuspended to

a concentration of 2×10^5 cells/ml in IL2, AB serum, and RPMI 1640 in a 2:1:2 ratio (v/v). Cells were grown in 75 cm^3 flasks and counted and rediluted in fresh IL2 (16–22 units/ml final concentration), AB serum, and RPMI 1640 at 5 day intervals. For continued growth, lines were absolutely dependent upon feeding with active IL2.

Microassay for production and utilization of IL2. IL2 production by PBMC, and adsorption and utilization by CTCL were quantitated using starved IL2-dependent normal CTCL (cells that were not fed for 5 days). Five day starved CTCL that were in culture for at least 15 days were cultured (1×10^4 cells/0·1ml) in 96 well microtitre plates for 48 hr with eight $\log_2$ dilutions of different IL2 preparations. Tritiated thymidine (^{3}H-TdR; 52 Ci/mmole, 0·25 μCi/well; Amersham, Arlington Hts., Illinois) was added for the last 24 hr; cells were harvested, counted, means of triplicate samples were determined, and the percentage of maximum proliferation was plotted on probit paper as a function of increasing IL2 dilutions. The IL2 dilution required for 50% maximal proliferation was calculated from the probit regression line. A commercial source of delectinized IL2 (BRL, Bethesda, Maryland, USA) of known activity (10 units/ml) was assayed simultaneously in all experiments in order to express our IL2 preparations as units of IL2 activity. Using these methods, reproducibility between the lines and within a line tested on multiple occasions was excellent (Table 1).

CTCL in culture for more than 15 days were largely unresponsive to PHA, and therefore useful for IL2 quantitation in the microassay. The concentration of PHA in the dilution of IL2 containing supernatant required for 50% maximal proliferation in the CTCL microassay was found to account for less than 10% of the ^{3}H-TdR incorporation, and it was not sufficient for fresh PBMC mitogenesis.

Surface marker analysis of CTCL. CTCL in culture for greater than 15 days were analysed for T cell and macrophage antigens, as previously described (Pierce & Polmar, 1982). The following monoclonal antibodies from Ortho Pharmaceuticals (Raritan, New Jersey) were employed: OKT3 (pan-T), OKT4 (helper/inducer), OKT8 (suppressor/cytotoxic), OKIa1 (anti-Ia), and OKM1 (anti-macrophage; anti-natural killer cell). Fcγ rosettes, E_{AET} rosettes, latex particle phagocytosis and non-specific esterase activity were determined as previously described (Birch & Polmar, 1981; Pierce & Polmar, 1982).

Table 1. Standardization of IL2 activity using CTCL from the normal individuals*

	Max c.p.m.†	IL2 dilution for 50% max c.p.m.‡	IL2§ (units/ml)
CTCL A	5,642	1:7·0	1·43
	5,671	1:9·9	1·01
	9,203	1:7·7	1·30
CTCL B	8,315	1:8·9	1·12
	5,362	1:7·8	1·28

* The delectinized IL2 preparation was tested in CTCL A on three separate occasions, and in CTCL B twice. CTCL were used after 15 days culture.

† Maximum proliferation was obtained at the highest final concentration of IL2 used, 1:1. Cells cultured without addition of IL2 had counts less than 300 c.p.m.

‡ The point at which 50% maximal proliferation occurred was obtained from the probit regression line. Eight dilutions were tested ranging from 1:1 to 1:256.

§ IL2 units/ml required for 50% maximal proliferation were calculated based on 10 units/ml in the standard stock solution.

Statistical analysis. The two tailed, unpaired Student's *t*-test was employed to evaluate statistical significance. Correlation coefficients and regression analyses were performed using standard statistical methods and the significance level was evaluated in two tailed *t*-tests.

RESULTS

Growth of CTCL from CHH and normal individuals

The growth of CTCL from three CHH individuals, one normal sibling and one normal control is shown in Fig. 1. Growth of cells in culture is expressed as the growth index (cells recovered/cells cultured). The growth of normal control and normal sibling CTCL was significantly greater ($P < 0{\cdot}01$) than that of the CHH CTCL between days 10 and 30. Growth indices of CTCL from three additional normal individuals were similar to the normal control and normal sibling illustrated in Fig. 1. Normal CTCL had cell cycle doubling times from 48 to 60 hr which is consistent with published reports (Strausser & Rosenberg, 1978), while CHH CTCL required 120–165 hr to progress through the cell cycle. Microscopic analysis of Wright stained cytocentrifuge CTCL preparations also revealed fewer mitoses in CHH lines, compared to normal (data not shown). A marked decrease in growth in all cultures was observed between 30–40 days of culture. This decrease was most likely due to the use of IL2 containing residual PHA and the development of sensitivity to PHA by CTCL (Ruscetti & Gallo, 1981).

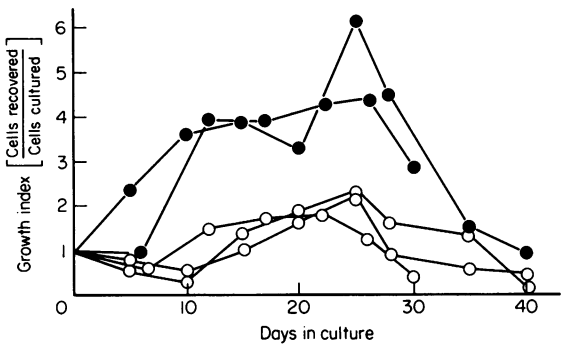

Fig. 1. Long term growth of CTCL in large flask cultures. Three CHH CTCL (o——o) and one normal and one sibling CTCL (●——●) were cultured in large flasks and fed with fresh IL2 at 5 day intervals. The rate of growth is expressed as the growth index, and was calculated from cell counts performed at the time of feeding. From days 10–30, normal lines had a significantly higher growth index, compared to CHH CTCL ($P < 0{\cdot}01$).

The T lymphocyte subpopulation composition of CHH and normal CTCL was examined using subset specific monoclonal antibodies. No differences in T cell subpopulations were detected between CHH and normal CTCL, and both had surface markers similar to those reported by others (Kurnick *et al.*, 1981; De Vries, Vyth & Mendelsohn, 1981). Eight CHH CTCL were $90{\cdot}7 \pm 4{\cdot}7\%$ (mean $\pm$ s.e.) E_{AET}-rosette positive and $96{\cdot}4 \pm 1{\cdot}8\%$ OKT3 (pan-T) positive. Seven normal CTCL were $94{\cdot}1 \pm 1{\cdot}9\%$ E_{AET} and $94{\cdot}9 \pm 1{\cdot}8\%$ OKT3 positive. Both CHH and normal CTCL contained 40–60% each of cells bearing OKT4 (helper/inducer) and OKT8 (suppressor/cytotoxic) antigens. OKIa1 (anti-Ia) was present on 75–85% of both CHH and normal CTCL. No macrophage or natural killer cell antigens were detected in any of the lines. All CTCL were latex particle, Fcγ and OKM1 negative and only focal non-specific esterase staining was observed, which is a characteristic of Tμ^+ cells (Ferrarini *et al.*, 1980). Therefore, the decreased growth of CHH CTCL compared to normal CTCL does not appear to be due to differences in their T cell subset composition.

Reproducibility of the CTCL microassay

Although starved CTCL in culture for greater than 15 days were always used for the microassay, peak IL2 induced proliferation (c.p.m. of ^{3}H-TdR incorporated) varied among lines as well as

within the same line tested on different days. For this reason the commercial IL2 was used as a reference standard in all experiments (Table 1). The point at which 50% of maximal proliferation occurred was obtained from the probit regression lines, and the IL2 units/ml required for this amount of proliferation was calculated (Ruscetti & Gallo, 1981).

IL2 production by CHH and normal individuals

IL2 production of CHH and normal PBMC in response to PHA was quantitated in the CTCL microassay. PBMC from CHH individuals produced less IL2 after culture for 48 hr with PHA than PBMC from normal individuals ($P < 0{\cdot}005$; Table 2). Furthermore, the units of IL2 activity produced at 48 hr were correlated with the PHA-induced proliferative response of the PBMC observed at 72 hr for each individual tested ($r = 0{\cdot}94$; $P < 0{\cdot}01$; Table 2). Proliferative responses toward PHA in the present series of patients were 10–40% of normal and are similar to those reported previously (Trojak *et al.*, 1981; Pierce & Polmar, 1982). The slopes of the probit regression lines of normal CTCL fed with CHH IL2 were similar to the lines obtained using normal IL2, with one exception (Table 2).

Table 2. Production of interleukin 2 by CHH and normal PBMC

PBMC source	IL2 activity (units/ml) CTCL A	CTCL B	PHA-induced PBMC proliferation (c.p.m.)
CHH 1	0·99	0·89	23,436 ± 2,106
CHH 2	0·81	0·92	nd
CHH 3	0·91	0·97	18,018 ± 1,258
CHH 4[b]	0·30	0·23	601 ± 8
Normal 1	1·47	1·31	35,432 ± 1,982
Normal 1	1·52	1·40	33,107 ± 2,830

* PBMC were incubated with PHA for 48 hr, the supernatants were harvested and tested for IL2 activity in two starved CTCL. Proliferative values were obtained from 72 hr PBMC microcultures with the same dose of PHA.

† This CHH individual is reported here for comparison only. He was severely lymphopenic (500/mm^3), had a history of severe, chronic respiratory infections, severe varicella, and has been reported on elsewhere (Lux *et al.*, 1970).

Absorption and utilization of IL2 by CHH and normal CTCL

Decreased IL2 production by CHH PBMC demonstrated above cannot solely explain the significantly decreased growth of CHH CTCL because excess exogenous IL2 does not overcome the proliferative defect. CHH CTCL may be either unable to adsorb IL2 or CHH cells may have a defect in the intracytoplasmic transmission of the IL2 signal to trigger nuclear DNA synthesis. Utilization of IL2 was measured by assaying residual IL2 activity of supernatants obtained from CHH and normal CTCL 3 days after IL2 feeding. When tested using two starved normal IL2-dependent CTCL in microassay, supernatants harvested from two CHH CTCL had $91{\cdot}6 \pm 0{\cdot}7\%$ of the initial IL2, whereas supernatants from two normal CTCL had $54{\cdot}2 \pm 1{\cdot}1\%$ of the initial IL2 activity ($P < 0{\cdot}005$). In addition, CHH CTCL increased only 50% in 3 days, while normal CTCL increased 2–3-fold, which is similar to the values shown in Fig. 1. The growth index was directly related to the removal of IL2 from the culture supernatants ($r = 0{\cdot}985$; $P < 0{\cdot}01$). Slopes of the microassay probit lines obtained using the IL2 from CHH and normal CTCL cultures were similar, suggesting that the unutilized IL2 remained potent.

Although the peak proliferative responses of 5 day starved CTCL in microassay demonstrated

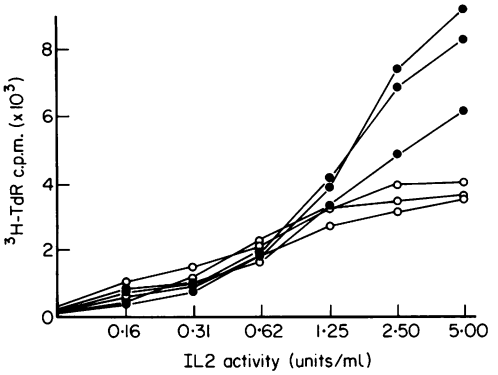

Fig. 2. Proliferative responses of CTCL to increasing amounts of IL2 in microassay. Ten thousand CTCL/0·1 ml were cultured for 48 hr with $\log_2$ dilutions of IL2 of known activity (BRL). CHH CTCL (o——o) and normal CTCL (●——●) showed similar ^{3}H-TdR incorporation at lower IL2 dilutions. With higher amounts of IL2 activity proliferation of CHH CTCl plateaued, while normal CTCL proliferation increased to significantly higher values ($P<0·01$ at 5 units/ml). Each CTCL was tested at six different IL2 dilutions, in addition to spontaneous background proliferation.

some variability, two patterns emerged. IL2 starved normal CTCL had consistently higher peak proliferative responses as well as a different dose response curve with varying IL2 dilutions, as compared to CHH CTCL ($P<0·01$ at 5 units IL2/ml; Fig. 2). Normal and CHH CTCL consistently showed comparable proliferation in response to low amounts of IL2, however, patterns of proliferation in the two groups diverged when they were incubated with concentrations of IL2 above 0·62 units/ml, with markedly increased proliferation of the normal lines at the higher IL2 concentrations. Regression analysis of probit curves using the same data presented in Fig. 2 revealed that CHH lines achieved half maximal growth on less than half the IL2 required by normal lines. Slopes of the CHH probits were significantly less than normal ($P<0·05$), suggesting a lower level of activation per unit of IL2 in CHH lines.

DISCUSSION

Diminished *in vivo* and *in vitro* cell-mediated immunity in Finnish cartilage hair hypoplasia have been reported (Virolainen *et al.*, 1978; Ranki *et al.*, 1978), and we have previously shown intrinsic proliferative defects in multiple cell types in Amish CHH individuals (i.e. fibroblasts, B cells, T cells) (Trojak *et al.*, 1981; Pierce & Polmar, 1982). McKusick *et al.* (1965), reported that biopsied metaphyseal plate chondrocytes and cartilage from a CHH child resembled the findings in normal adults in whom growth had ceased.

The initial activation of T cells by mitogens or antigens results in a cell cycle progression from the resting state, G_0, to early G_1, and the concomitant production of IL2 by a subset of activated T cells (Stadler *et al.*, 1981; Ruscetti & Gallo, 1981). Later in G_1, T cells acquire IL2 receptors with the resultant ability to adsorb IL2 from the medium (Larsson, 1981). Cell surface binding of IL2 triggers the G_1–S phase transition, and subsequent mitotic events (Maizel *et al.*, 1981). Analysis of CHH and normal CTCL growth, IL2 production, and IL2 utilization has provided a means of identifying the phase of the cell cycle in which the CHH cell activation block expresses itself. In the present study, we found that CHH PBMC produce less IL2, CHH CTCL proliferate at a lower rate in the presence of exogenously supplied IL2, and CHH CTCL remove less IL2 from the medium, when compared to normals. These defects probably explain the decreased activation observed in CHH peripheral blood T lymphocytes in earlier studies (Trojak *et al.*, 1981; Pierce & Polmar, 1982). Predictably, the addition of IL2 to PHA stimulated peripheral blood T cells did not result in an augmentation of ^{3}H-TdR incorporation (data not shown), in contrast to the results obtained with some other types of primary immunodeficiencies in which there are isolated IL2 production deficits

(De Vries *et al.*, 1981; Lopez-Botet *et al.*, 1982). Gillis *et al.*, (1981) have reported combined deficits in the production of, and the response to, T cell growth factor (IL2) in PBMC from aged individuals. However, none of these investigators used CTCL, therefore the effects of peripheral blood T cells, macrophages, and other *in vivo* derived soluble factors were not rigorously excluded.

The IL2 dose–response curves and the slope of CHH probit lines revealed that utilization of IL2 is decreased in CHH CTCL, because proliferation of normal CTCL increased markedly at higher IL2 concentrations, but proliferation of CHH CTCL remained low. These findings are consistent with the possibilities that CHH CTCL have a decreased number of cell cycle specific IL2 surface receptors, or that fewer cells in the population are able to express such receptors. The availability of purified radiolabelled IL2 is necessary in order to confirm these findings, as well as to permit more definitive conclusions on the role of IL2 in the pathogenesis of primary immunodeficiencies (Robb, Munck & Smith, 1981).

All CTCL were continuously cycling in a steady state (Ruscetti & Gallo, 1981; Brooks, Bennett & Smith, 1980). Therefore, as CTCL cultures were cut back at 5 day intervals, non-dividing (G_0) cells in CHH and normal CTCL were rapidly diluted out, and all cells in culture were the progeny of cycling cells. Since IL2 production and utilization are separable G_1 phase events, it is likely that this phase of the cell cycle is abnormal in CHH CTCL. This hypothesis is compatible with the transition–probability model of the cell cycle, which suggests that two distinct signals are required for G_0–G_1 and G_1–S phase transitions (Hume & Weidemann, 1980; Brooks *et al.*, 1980). In CHH T cells, an initial mitogenic signal does not trigger sufficient G_0–G_1 transition and concomitant IL2 production. Therefore, the second signal (IL2) cannot mediate a normal level of G_1–S phase transition in CHH CTCL.

These observed defects may reflect decreased levels of critical substrates that are required by individual G_1 phase cells in order to increase their transition–probability. Alternatively, increased levels of non-metabolizable or slowly metabolizable substrates can also affect cell cycle progression (Castor, 1980). For example, deoxyribonucleosides at high concentrations can block synthesis of DNA precursors in T cells and appear to play an important role in the pathogenesis of purinogenic immunodeficiencies (i.e., adenosine deaminase and nucleoside phosphorylase deficiencies) (Gelfand, Lee & Dosch, 1979; Polmar, 1980; Raivio, 1980). Thus, in contrast to CHH, the defect in the purinogenic immunodeficiencies is expressed in late G_1–S phase.

The G_1 phase defects identified in this report may be responsible for impaired T cell mediated immunity observed in CHH individuals. Investigation of metabolic processes occurring in the G_1 phase of the cell cycle in CHH lymphocytes is likely to provide insights into the proliferative defects in other CHH cell types as well as into the genetic and metabolic basis of cartilage hair hypoplasia.

This study was supported in part by NIH Grant AI 18527. Glenn F. Pierce was supported by Medical Scientist Training Program Grant GM07250.

The authors would like to thank Dr Patrick C. Kung of Ortho Pharmaceuticals for the gifts of OKIal and OKMl, Drs Bernice Z. Schacter and R. Neal Smith for critical reviews of the manuscript, and Claire Svet for expert secretarial assistance.

REFERENCES

ALVAREZ, J.M., SILVA, A. & DE LANDAZURI, M.O. (1979) Human T-cell growth factor. I. Optimal conditions for its production. *J. Immunol.* **123,** 977.

BIRCH, R.E. & POLMAR, S.H. (1981) Induction of Fc gamma receptors on a subpopulation of human T lymphocytes by adenosine and impromidine, an H_2-histamine agonist. *Cell. Immunol.* **57,** 455.

BROOKS, R.F., BENNETT, D.C. & SMITH, J.A. (1980) Mammalian cell cycles need two random transitions. *Cell,* **9,** 493.

CASTOR, L.N. (1980) A G_1 rate model accounts for cell-cycle kinetics attributed to 'transition probability'. *Nature,* **287,** 857.

DE VRIES, J.E., VYTH, F.A. & MENDELSOHN, J. (1981) T cell growth factor-mediated proliferation of lymphocytes from a T chronic lymphocytic leukaemia patient lacking mitogen and alloantigen responsiveness. *Clin. exp. Immunol.* **43,** 302.

FERRARINI, M., CADONI, A., FRANZIA, T., GHIGLIOTTI, C., LEPRINI, A., ZICCA, A. & GROSSI, C.E. (1980) Ultrastructure and cytochemistry of human peripheral blood lymphocytes. Similarities between the cells of the third population and T_G lymphocytes. *Eur. J. Immunol.* **10,** 562.

GELFAND, E.W., LEE, J.J. & DOSCH, H.M. (1979) Selective toxicity of purine deoxynucleosides for human lymphocyte growth and function. *Proc. Natl. Acad. Sci. USA.* **76,** 1998.

GILLIS, S., KOZAK, R., DURANTE, M. & WEKSLER, M.E. (1981) Immunological studies of aging: decreased production of and response to T cell growth factor by lymphocytes from aged humans. *J. clin. Invest.* **67,** 937.

HUME, D.A. & WEIDEMANN, M.J. (1980) Mitogenic lymphocyte transformation. In *Research monograph in immunology*. Vol. 2. Elsevier-North Holland, Amsterdam.

INOUYE, H., HANK, J.A., ALTER, B.J. & BACH, F.H. (1980) TCGF production for cloning and growth of functional human T lymphocytes. *Scand. J. Immunol.* **12,** 149.

KURNICK, J.T., HAYWARD, A.R. & ALTEVOGT, P. (1981) Helper and suppressor–inducer activity in human T cells and their cloned progeny maintained in long-term culture. *J. Immunol.* **126,** 1307.

LARSSON, E.L. (1981) Mechanism of T cell activation. II. Antigen- and lectin-dependent acquisition of responsiveness to TCGF is a nonmitogenic, active response of resting T cells. *J. Immunol.* **126,** 1323.

LOPEZ-BOTET, M., FONTAIN, G., RODRIGUEZ, M.C.G. & DE LANDAZURI, M.S. (1982) Relationship between IL2 synthesis and the proliferative response to PHA in different primary immunodeficiencies. *J. Immunol.* **128,** 679.

LUX, S.E., JOHNSTON, R.B., AUGUST, C.S., SAY, B., PENCHASZADEH, V.B., ROSEN, F.S. & MCKUSICK, V.A. (1970) Chronic neutropenia and abnormal cellular immunity in cartilage-hair hypoplasia. *N. Engl. J. Med.* **282,** 231.

MAIZEL, A.L., MEHTA, S.R., HAUFT, S., FRANZINI, D., LACHMAN, L.B. & FORD, R.J. (1981) Human T lymphocyte/monocyte interaction in response to lectin: kinetics of entry into the S-phase. *J. Immunol.* **127,** 1058.

MCKUSICK, V.A., ELDRIDGE, R., HOSTETLER, J.A., RUANGWIT, U. & EGELAND, J.A. (1965) Dwarfism in the Amish. II. Cartilage-hair hypoplasia. *Bull. Johns Hopkins Hosp.* **116,** 285.

PAWELEC, G., REHBEIN, A., MULLER, C., SONNEBORN, H.H. & WERNET, P. (1981) Human T lymphocytes grown in T-cell growth factor: Functional attributes in MLC, CML, PLT and allogeneic suppression. *Immunology*, **42,** 529.

PIERCE, G.F. & POLMAR, S.H. (1982) Lymphocyte dysfunction in cartilage-hair hypoplasia. I. Evidence for an intrinsic defect in cellular proliferation. *J. Immunol.* **129,** 570.

POLMAR, S.H. (1980) Metabolic aspects of immunodeficiency disease. *Sem. Hemat.* **17,** 30.

RAIVIO, K.O. (1980) The biochemical basis of immunodeficiency disease. *Eur. J. Pediatr.* **135,** 13.

RANKI, A., PERHEENTUPA, J., ANDERSSON, L.C. & HAYRY, P. (1978) *In vitro* T and B cell reactivity in cartilage hair hypoplasia. *Clin. exp. Immunol.* **32,** 352.

ROBB, R.J., MUNCK, A. & SMITH, K.A. (1981) T cell growth factor receptors: Quantitation, specificity and biological relevance. *J. exp. Med.* **154,** 1455.

RUSCETTI, F.W. & GALLO, R.C. (1981) Human T-lymphocyte growth factor: regulation of growth and function of T lymphocytes. *Blood*, **57,** 379.

SMITH, K.A., LACHMAN, L.B., OPPENHEIM, J.J. & FAVATA, M.F. (1980) The functional relationship of the interleukins. *J. exp. Med.* **151,** 1551.

STADLER, B.M., DOUGHERTY, S.F., FARRAR, J.J. & OPPENHEIM, J.J. (1981) Relationship of cell cycle to recovery of IL2 activity from human mononuclear cells, human and mouse T cell lines. *J. Immunol.* **127,** 1936.

STRAUSSER, J.L. & ROSENBERG, S.A. (1978) *In vitro* growth of cytotoxic human lymphocytes. I. Growth of cells sensitized *in vitro* to alloantigens. *J. Immunol.* **121,** 1491.

TROJAK, J.E., POLMAR, S.H., WINKELSTEIN, J.A., HSU, S., FRANCOMANO, C., PIERCE, G.F., SCILLIAN, J.J., GALE, A.N. & MCKUSICK, V.A. (1981) Immunologic studies of cartilage-hair hypoplasia in the Amish. *Johns Hopkins Med. J.* **148,** 157.

VIROLAINEN, M., SAVILAHTI, E., KAITILA, I. & PERHEENTUPA, J. (1978) Cellular and humoral immunity in cartilage-hair hypoplasia. *Pediat. Res.* **12,** 961.