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Human Interleukin 2: Physiology, Biochemistry, and Pathophysiology in Lymphoblastic Leukemias and Immunodeficiency Syndromes *

K. Welte, S. Venuta, C. Y. Wang, S. P. Feldman, N. Ciobanu, G. Kruger, H. J. Feickert, V. J. Merluzzi, N. Flomenberg, M. A. S. Moore, and R. Mertelsmann

A. Introduction

The regulation of immune function [6] and tumor growth [16] by hormone-like factors, cytokines, has become the subject of increasing interest. Interleukin 2 (IL2) discovered by Morgan et al. [5], is produced by T-lymphocytes after antigen or mitogen stimulation and is required for the proliferation of activated T cells. IL2 is an essential mediator of the immune response [11, 15], and there is preliminary evidence that it may also be responsible for the clonal growth of human lymphoblastic leukemias [17].

Studies on the physiology and pathophysiology of IL2 are dependent on the availability of a well-defined, biochemically, and biological homogeneous molecule. We have therefore purified IL2 to apparent homogeneity [18] and have started to examine its role as mediator of the normal immune response, in human immunodeficiency syndromes and in acute lymphoblastic leukemias (ALL).

B. Methodology

Heparinized blood samples were drawn from healthy volunteers and patients after obtaining informed consent. Ficoll-Hypaque separated mononuclear blood cells were resuspended at 4×10^6 cells/ml in RPMI 1640 supplemented with 5% heat-inactivated FCS and glutamine (2 m*M*). Each sample was stimulated in triplicate microwell cultures (# 3596 culture plate, Costar Inc. Cambridge, MA) with one of the following: (a) medium alone, (b) phytohemagglutinin (PHA-M, 0.5% by volume, Grand Island Biological CO), (c) OKT3 (Ortho Diagnostic Systems, Inc., Raritan, NJ) or (d) Pan T2 (Wang et al. 1982, submitted).

C. Results

I. Physiology of lymphocyte proliferation and IL2 Production Induced by PHA and Mitogenic Antibodies

Stimulation assays were done with or without the addition of irradiated Daudi cells (5000 rads) at a final concentration of 0.5×10^6 cells/ml. At indicated time points 100 µl supernatants were removed from each well to be assayed for IL2. Identical cultures were pulsed for 4 h with tritiated thymidine [^{3}H]dT (0.5 µci/microplate well, specific activity 20 mci/m*M*. New England Nuclear, Boston, MA) and the incorporation of [^{3}H]dT measured.

The IL2 microassay, definition of units, and biochemical techniques have been published in detail elsewhere [1, 18].

1. Mitogenesis Induced by PHA, Pan T2, and OKT3

PHA as well as both T-cell specific antibodies were able to induce a proliferative response in normal PBL incubated for 4

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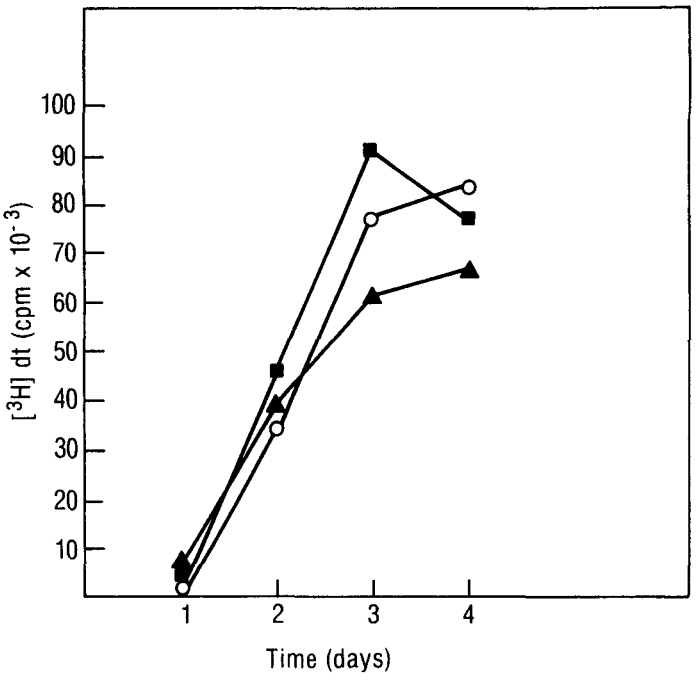

Fig. 1. Mitogenic effect of Pan T2, OKT3 and PHA on normal PBL. PBL were plated in 96-well microtiter plates at 4×10^6/ml in RPMI supplemented with 5% heat- inactivated FCS and Pan T2 (500 ng/ml), OKT3 (1.25 ng/ml), or PHA (0.5%). For each of 4 days thereafter, 0.5 μCi [^{3}H]dT was added to measure DNA snthesis. Each *point* is the average of a triplicate determination. ■, PHA; ○, Pan T2; ▲, OKT3

days a measured by incorporation of tritiated thymidine (Fig. 1). There was no significant difference between the two antibodies when used at saturating concentrations. However, the amount of Pan T2 (10^{-10} *M*) needed to induce maximum mitogenesis was 100 times more than the concentration required for OKT3 (10^{-12} *M*).

In the presence of irradiated Daudi cells we observed a twofold increase in cell proliferation with Pan T2. In contrast, the costimulation with Daudi cells on OKT3 had essentially no effect.

2. IL2 Production Induced by Monoclonal Antibodies

Pan T2 and OKT3 induced relatively low levels of IL2 production. However, when these cells were incubated in the presence of each antibody plus Daudi cells the amount of IL2 induced by Pan T2 was

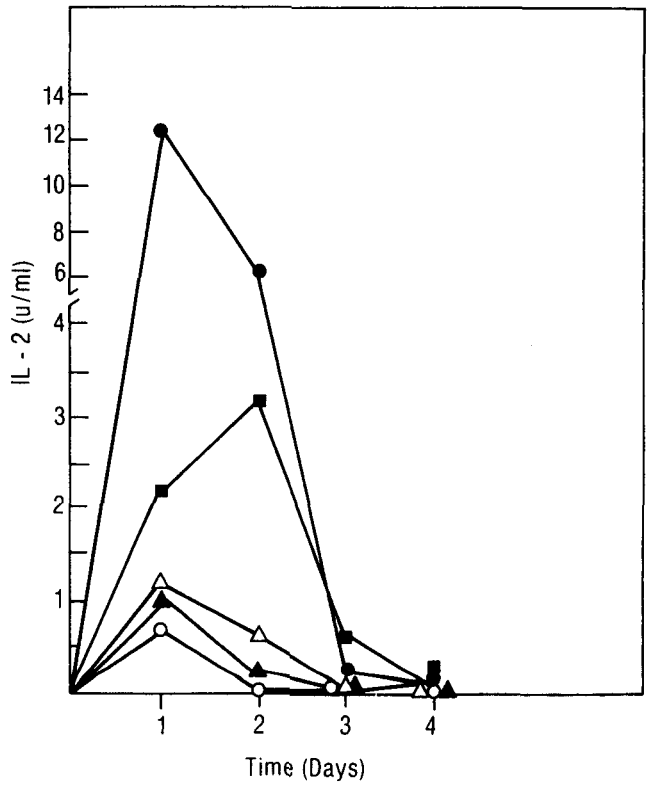

Fig. 2. IL2 production induced by Pan T2 and OKT3 on normal PBL in the presence or absence of Daudi cells. Irradiated Dudi cells were plated at 0.5×10^5/ml, where indicated. PHA (0.5%) was added to the control sample. IL2 concentration was determined in the harvested culture medium as described. ●, Pan T2 + Daudi; ■, PHA; △, OKT3 + Daudi; ▲, OKT3; ○, Pan T2

Table 1. IL 2 production by normal lymphocytes (PBL) and leukemic lymphoblasts (ALL) in response to PHA and T-cell monoclonal antibodies with and without costimulators

	PBL (U/ml)	ALL-4 (U/ml)
Media	0	0
PHA	1.2	1.4
Pan T2	1.2	0
Pan T2 + Daudi	12.5	3.0
Pan T2 + Protein A	1.5	0
OKT 3	2.1	0.8
OKT 3 + Daudi	22.2	0.9
OKT 3 + Protein A	8.5	1.6

more than tenfold higher compared with OKT3 (Fig. 2).

3. *Effect of protein A on IL-2 Production Induced by Pan T2 and OKT3*

Protein A (40 μg/ml) was added to the microwell cell supensions of normal PBL in the presene of either Pan T2 or OKT3. As shown in Table 1, protein A had only a negligible effect on the IL-2 production induced by Pan T2, while it enhanced the stimulation by OKT3 approximately fourfold. (In contrast, irradiated Daudi cells are potent costimulators for the Pan T2 response while they are without effect on the OKT3 response).

4. *Inhibition of IL2 Production and Response*

Further studies also suggest a role for HLA-DR antigens in the regulation of the IL2 production [7]. The extent of inhibition by antibodies against these structures is dependent on the mitogen used (own observation), suggesting that Pan T2 and OKT3 bind to different subunits of the T-cell activation antigen recognition complex. This has recently been continued through immunoprecipitation studies (Wang et al. 1982, submitted).

5. *Stimulation of PBL Proliferation by Anti M7 and Anti RD 114 Antiserum*

Table 2 shows that goat antisera raised against the baboon endogenous virus, M7, or RD114 were able to stimulate the proliferation of PBL, while goat anti-simian sarcoma virus (SSV) antiserum was unable to do so. Absorption of the antisera with M7 or RD114 virus removed the sera capacity to induce cell proliferation. These data suggest a common antigenic determinant shared by the T-cell activation/antigen recognition complex and M7 as well as RD 114.

II. Biochemistry

The purification of IL-2 from lymphocyte-conditioned medium (Ly-CM) has been reported in detail elsewhere [18]. Briefly, IL2, produced with or without costimulation by irradiated cells of the Burkitt's lymphoma line Daudi, was purified 37,000-fold to apparent homogeneity from Ly-CM by sequential $(NH_4)_2SO_4$-precipitation, ion exchange chromatography (DEAE-cellulose), gel filtration, and chromatography on

Table 2. Proliferation of PBL after stimulation with goat anti-virus sera

Stimulus	Proliferation [^{3}H]dT incorporation (cpm)
None	2,800
Pan T2	47,000
Anti-M7	35,000
Anti-RD114	41,500
Anti-M7 absorbed with M7	15,000
Anti-RD114 absorbed with RD114	7,800
Anti-SSV	2,700

Antisera were used at 1:125 dilutions. For absorption 1 ml of antiserum was incubated overnight at 20 °C with 1.5 mg of virus. All other conditions were identical to those in Fig. 1. [^{3}H]dT incorporation was measured 2 days after stimulation

Table 3. Biochemical characteristics of IL2 produced by PBL and leukemic lymphoblasts (ALL) in the presence and absence of Daudi cells

Method	*PBL(+Daudi)*	*PBL(−Daudi)*	*ALL(+/−Daudi)*
Molecular weight (AcA 54 Ultrogel filtration)	14,000	26,000	26,000
Molecular weight (SDS-PAGE, reduced conditions)	14,500	16,000 and 17,000	16,000 and 17,000
Isoelectric point	8.1	6.7	6.6
Hydrophobic binding to Blue agarose and Procion-red agarose	Strong	Strong	Strong
Glycoprotein	No evidence	No evidence	No evidence
pH stability	2 – 10	2 – 10	2 – 10

blue agarose and on Procion-red agarose. The purified IL2 showed a specific activity of 10^6 U/mg protein. IL2 produced in the absence of Daudi cells exhibited a native molecular weight of 26,000 as measured by gel filtration and an isoelectric point of 6.7. This IL-2 showed 16,000 and 17,000 mol wt. bands in SDS-polyacryladmide gel electrophoresis. IL-2, produced in the presence of Daudi cells, showed a molecular weight of 14,000, as measured by both gel filtration and SDS-polyacrylamide gel electrophoresis, and an isoelectric point of 8.1 (Table 3). The purified IL-2 lacked detectable activities of all cytokines tested: interferon (α and γ), granulocyte-macrophage-colony stimulating factor, B-cell growth factor, T-cell replacing factor, B-cell differentiation factor, macrophage activation factor, and thymocyte-differentiating activity. It was free of any contaminating proteins as judged by silver staining in SDS-polyacrylamide gel electrophoresis. All three molecular forms of IL-2 were biologically active, supporting the growth of human and murine cytotoxic T-cell lines at concentrations of $10^{-11}-10^{-10}$ *M*.

III. Antibody Aainst IL2

We used the purified IL2 for the production of a mouse monoclonal antibody against IL2. The fusion resulted in hybrid clones producing anti-IL2 of various subclasses (IgA, IgG-2b, IgM). All anti-IL2 antibodies inhibited the proliferation of IL2-dependent human and mouse cell lines in reponse to human highly purified IL2. One of these antibodies chosen for further characterization precipitated 14K^{125}I-IL2 as well as 16K^{125}I-IL2 and 17K^{125}I-IL2 (Feickert et al. 1982, submitted).

IV. IL2 Production by Fresh Lymphoblastic Cells and the Lymphoblastic Cell Line JM[1]

1. Production of IL2 by Leukemic Cells

Leukemic cells were cultured in the presence of PHA, OKT3, or Pan T2, and tested for IL-2 production and proliferation as described for PBL. IL-2 production induced by PHA and OKT3 stimulation continued to increase over 3 days (Fig. 3) and was not followed by a rise in cell proliferation (not shown). This was in marked contrast to the reponse of PBL to PHA and OKT3 stimulation (Fig. 2). This pattern of response was common to ALL with different phenotypes. Pan T2 was unable to induce either proliferation or IL2 production in ALL. This pattern of response was also markedly different to Pan T2 stimulation of normal PBL.

[1] The cell line Jurkat used by other investigators is a subclone of the original line JM developed by Schneider et al. [13]. Our studies have failed to show any difference between the original JM and the subclone Jurkat and therefore consider the original designation JM more appropriate

JM, a cell line derived from a T-cell ALL, is TdT+, Ia-, E+, Leu I+ and, after PHA stimulation, produces IL2 but does not proliferate. We studied the effect of Pan T2 on this line and found that this monoclonal antibody does not induce IL2 production or stimulate cell proliferation. Therefore, JM and fresh ALL cells have the same pattern of IL2 production and proliferation after PHA or Pan T2 stimulation (Table 1).

The addition of Daudi cells was able to rescue the response of ALL cells to Pan T2, and induced IL2 production. We could not detect any effect of Daudi alone on IL2 production by any of the ALL cells (Table 1) and JM (not shown).

2. *The Factor Produced by the Leukemic Population is IL2*

In order to show that the factor produced by the leukemic cell populations was indeed IL2, we tested if the factor produced by ALL cells was able to support the growth of the human cytotoxic cell line, C13.3 (kindly provided by Dr. N. Flomenberg, Sloan-Kettering Institute), which requires IL2 for survival and proliferation. The factor produced by ALL and IL2 purified from normal PBL supported the growth of C13.3 equally well.

As shown in Table 3 the biochemical characteristics of IL2 produced by leukemic cells are similar to those of IL2 produced by normal PBL. However, the molecular heterogeneity of IL2 produced by leukemic cells was not influenced by Daudi costimulation in contrast to IL2 generated by normal PBL.

Finally, IL2 produced by ALL cells binds to a monoclonal antibody prepared against rat IL2 (analysis performed by Dr. Gillis, Immunex, Seattle, WA) as well as to our own monoclonal antibody against human IL2 (Welte et al., unpublished).

A colony assay for blast cell progenitors in non-B non-T ALL has recently been described by Izzaguire et al. [3]. ALL cells were cultured in methylcellulose in the presence of Ly-CM and feeder T cells. After 5–7 days the colonies exhibited the common ALL phenotype. To test whether growth was dependent on IL2 we substituted partially purified IL2 (DEAE-cellulose fraction) and purified IL2 for the Ly-CM in the presence and absence of feeder T cells. Preliminary results suggest that partially purified IL2 alone can support the growth of ALL cells; however the highly purified

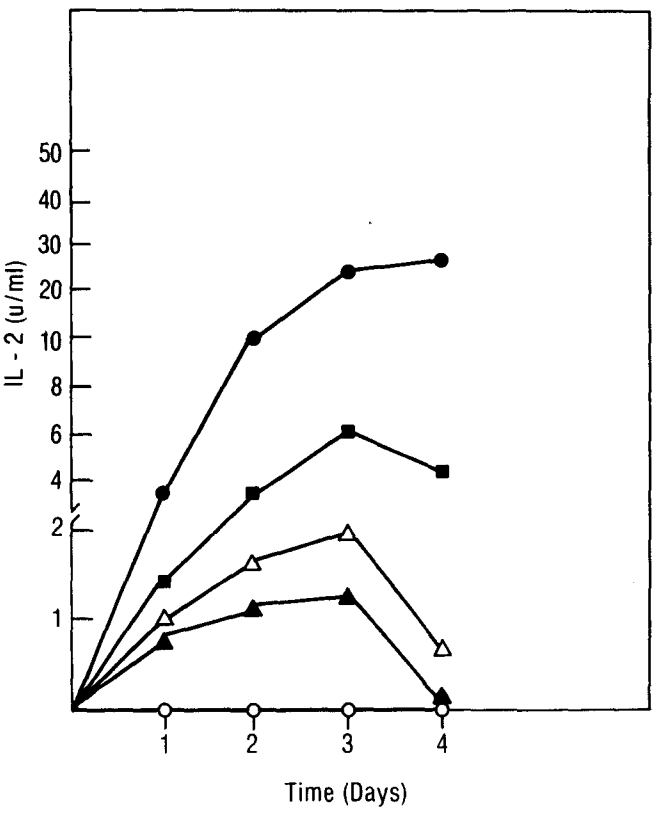

Fig. 3. Effect of Daudi cell costimulation on IL2 production induced by Pan T2 and OKT3 on ALL-4. ALL-4 PBL were plated at 4×10^5 per well. Pan T2 (500 ng/ml) and OKT3 (1.25 ng/ml) were added alone and in the presence of irradiated Daudi cells (0.5×10^5). PHA (0.5%) was added to a control sample. 112 concentration was determined in the harvested culture medium. ■, PHA; ○–○, Pan T2; ▲, OKT3; ●–●, Pan T2 + Daudi; △, OKT3 + Daudi

IL2 reuires feeder T cells for maximum colony formation (Table 4). The cell surface markers of the colonies grown in the presence of purified IL2 exhibited both pre-B and T-cell characteristics. In a more recent experiment with another ALL donor, the majority of the cells were Bl-positive. We are currently testing several additional ALL samples in this assay, and will identify the growth factor requirements of ALL cells in culture to investigate further the hypothesis of autostimulation in ALL.

V. IL2 Production and Proliferation of PBL in Primary and Acquired Immunodeficiency Syndromes (IDS).

1. Mouse model

Spleen cells from mice treated with cyclophosphamide (CY) (150 mg/kg) do not generate effective cytotoxic T-lymphocyte (CTL) responses to allogeneic tumor cells in vitro. When purified human IL2 is added to the culture system, spleen cells from CY-treated mice are able to generate normal CTL responses (Merluzzi et al. 1982, submitted).

2. Combined Varied Immunodeficiency (CVI)

Fifteen patients with CVI and one patient with a related disorder (transient hypogammaglobulinemia of childhood) had a statistically significant decreased response to mitogen stimulation when compared with a control normal population. After addition of purified IL2 the proliferative response was significantly improved with all mitogens used. Two groups could be distinguished: Group A (10/16) had full or partial normalization of proliferative response after addition of IL2, and group B (6/16) had no significant response. One patient showed a decrement in proliferative response after IL2 was added. The results are listed in Table 5 and in Kruger et al. (1982, manuscript submitted).

The production of endogenous IL2 was lower in the group of patients irrespective of the mitogen used when compared with normals (Table 5).

The anti-T-cell monoclonal antibody, Pan T2, recognized a proliferative defect in 5/16 patients which was neither recognized by PHA nor OKT3. This was not significantly correctecd by the addition of IL2. The lack of responsiveness to Pan T2, however, did correlate with the inability of the B cells of these patients to proliferate and differentiate in reponse to B-cell mitogens [12].

Table 4. Growth factor requirements of ALL cells in methylcellulose culture

	IL2 fraction[a]			
	1	3	5	None
Feeder Cells	Colonies/3 × 10^4 cells added			
–	0	308[b]	60	0
+	354[b]	318	200[b]	0
Markers	%			
J5	0	5	2	–
B1	30	10	20	–
T3	0	40	42	–

Colony assays were performed according to Izzaguire et al. [3], with deletions/additions as indicated

[a] 1, Ly-CM; 3, DEAE-cellulose purified IL2; 5, Procion-red agarose purified IL2

[b] Conditions used for marker studies

3. Kaposi's Sarcoma in Acquired Immunodeficiency Syndromes (AID)

Homosexual patients with Kaposi's sarcoma (KS) exhibited a very low proliferative response to OKT3 (15% of the normals). Four out of seven patients also had a very low proliferative response to PHA (10% of the normals) and to Pan T2 (8% of the normals). The production of endogenous IL2 was significantly lower in PBL cultures from KS patients, irrespective of the mitogen used.

Addition of purified IL2 in the presence of these mitogens was able to restore partially or completely the lymphocyte proliferation in all patients tested.

These data suggest that homosexual patients with KS have a defect in IL2 production that is correctable, in vitro, by addition of purified IL2 (Ciobanu et al. 1982, submitted) (Table 5).

Table 5. IL2 production and proliferation (in the absence and presence of 10 U/ml purified IL2) of PBL from patients with common variable immunodeficiencies (CVI), Nezeloff's syndromes, homsexual males with Kaposi's sarcoma, hemophiliacs with acquired IDS, relapsed Hodgkin's disease before retreatment, and healthy controls

Diagnosis (n = number of patients)	PHA			OKT 3			Pan T2		
	IL2 (u/ml)	Prolif. – IL2 (cpm × 10^3)	Prolif. + IL2 (cpm × 10^3)	IL2 (u/ml)	Prolif. – IL2 (cpm × 10^3)	Prolif. + IL2 (cpm × 10^3)	IL2 (u/ml)	Prolif. – IL2 (cpm × 10^3)	Prolif. + IL2 (cpm × 10^3)
CVI, group 1 (IL2 responder) $n = 10$	0.6 (0.3 – 1.7)	43 (29 – 58.6)	78 (39 – 117)	0.5 (0.2 – 1.5)	32 (6.3 – 88.1)	55 (35 – 217)	0.5 (0.3 – 1.1)	16 (1 – 88)	35 (8.2 – 128)
CVI, group 2 (IL2 nonresponder) $n = 6$	0.9 (0.3 – 7.8)	14 (8 – 51)	18 (3 – 58)	0.5 (0.3 – 1.4)	16 (7.6 – 53)	16 (7 – 65)	0.5 (0.4 – 0.6)	8 (0.5 – 21)	7 (0.5 – 23)
Nezeloff's syndrome $n = 2$	0.2	1) 1.8 2) 1.8	1) 12.7 2) 17.2	0.2	1) 1.3 2) 1.6	1) 3.9 2) 22	0.2	1) 1.3 2) 1.6	1) 5.4 2) 16.1
Kaposi's sarcoma $n = 12$	1.1 (0 – 4.2)	20 (1.6 – 72)	28 (15 – 108)	0.9 (0.2 – 1.8)	12 (3.5 – 32)	39 (13 – 80)	0.3 (0 – 1.3)	15 (0.5 – 50)	34 (0.7 – 82)
Hemophiliacs $n = 3$	1.2 (0.7 – 1.8)	12 (4.5 – 44)	18 (7.5 – 42)	0.7 (0.6 – 0.8)	8.7 (1.7 – 10.3)	16 (13 – 25)	0.7 (0 – 0.8)	0.5 (0.2 – 12)	9 (0.6 – 16)
Hodgkin's disease $n = 7$	n.t.[a]	5 (1 – 48)	13 (3 – 73)	n.t.	4 (1 – 50)	17 (3 – 83)	n.t.	2.6 (0.5.4)	14 (3 – 22)
Control $n = 20$	2.3 (0.8 – 6.8)	80 (56 – 110)	90 (52 – 127)	3.5 (0.9 – 16)	61 (37 – 88)	82 (51 – 127)	0.7 (0.4 – 24)	59 (22 – 88)	84 (50 – 125)

Results are shown as medians and ranges in parenthesis; IL2 was measured at day 1, proliferation at day 3

[a] n.t., not tested

4. Hodgkin's Disease

All patients with relapsed Hodgkin's disease before retreatment showed a decreased T-cell proliferative response to all mitogens used and had a partial normalization of T-cell proliferative response after addition of purified 112 (Table 5).

5. Hemophiliacs with Acquired Immunodeficiency

Three of five patients with hemophilia examined had an abnormal T-cell proliferation pattern purified with a partial normalization in the presence of IL2 (Bussel et al. manuscript in preparation) (Table 5).

6. Primary Immunodeficiency Syndromes

One child with Nezelof's syndrome showed in vitro restoration by purified IL2 of the proliferation in reponse to alloantigens and mitogens. After 6-day MLC in the presence of IL2, effector cells capable of NK and alloreactive cytotoxicity against PHA lymphoblasts and neoplastic cell lines were recovered. No viable cells were recovered from similar in vitro cultures in the absence of IL2. A second Nezelof's patient showed augmentation of his NK activity but no restoration of the allocytotoxic reponse. The proliferative response to PHA, OKT3, and Pan T2 is shown in Table 5. Patient 1, who demonstrated a positive in vitro response to IL2, was subsequently given IL2 subcutaneously as part of a recently initiated phase I trial. Though the patient died several days after the trial was begun due to pulmonary infection, examination of his lymphoid tissues postmortem suggested that purified IL2 may have exerted an in vivo effect on his T-lymphocytes. At autopsy, his lymphoid tissues showed only histocytes and plasma cells except in the lymph nodes draining the IL2 administration sites, where nests of lymphoid cells were identified.

These studies suggest that some primary and acquired immunodeficiencies may be caused by defects in IL2 production and/or response. In addition, they have provided some preliminary evidence that highly purified IL2 is capable of producing an in vivo effect in appropriate immunodeficient patients (Flomenberg et al. 1982, submitted).

D. Discussion

I. Physiology of IL2 Production and Response

The introduction of T-lymphocyte specific monoclonal antibodies has facilitated the ability to comprehend further the complex interaction and control of the immune response. The binding of the antibodies Pan T2 and OKT3 to specific antigenic determinants (T-cell activation/antigen recognition complex) on the surface of T-lymphocytes is able to trigger a proliferative response similar to antigenic stimulation or mitogensis. It has been observed that OKT3 is mitogenic even in the range of 10^{-12} *M*, while Pan T2 is less potent, requiring a concentration of 10^{-10} *M* for maximum stimulation. While highly costimulatory with Pan T2, Daudi cells had no significant effect on either IL2 production or cell proliferation in the presence of OKT3. Daudi cells have been used by several investigators to enhance IL2 production from normal PBL [2, 10, 11, 18]. The effect of Daudi cells could be mediated by (a) Ia antigen, (b) Fc receptors, and (c) an additional effector molecule. Both Ia antigen [7] and Fc receptors [14] have been implicated in the augmentation of IL2 production.

II. Biochemistry

The purification steps described in this study produced IL2 with a specific activity of 10^6 U/mg protein. Because the lowest molecular weight of an active IL2 polypeptide was 14,000, it could be calculated that IU/ml of IL2 was equivalent to a molar concentration of 7×10^{-11} *M*. An IL2 concentration of 1.4×10^{-11} *M*, or 4×10^5 molecules/cell, was required for one-half maximum stimulation of murine CTLL. All other purification methods [2, 4] have achieved neither a specific activity nor a yield comparable to those described here.

Native IL2 has been previously shown to exist in several molecular forms. Here, we show that the stimuli used for IL2 induction by PBL can be responsible for this heterogeneity. IL2 produced in the presence or absence of Daudi cells had a molecular weight of 14,000 and 26,000, respectively, by gel filtration and 14,500 and 16,000–17,000 repsectively by SDS-polyacrylamide electrophoresis. All molecular forms could be obtained by varying the concentration of costimulator cells.

The effect of Daudi cells on the IL2 production, however, does appear to be complicated in view of (a) the shift in molecular weight of IL2 induced by Daudi cells in PBL, and (b) the superinduction of IL2 in PBL and in human lymphoblastic leukemic cells by costimulation with Daudi cells. The possibility that different T-cell subsets or different leukemic phenotypes are responsible for the production of the two IL2 forms is currently under investigation.

III. IL2 in Lymphoblastic Leukemias

There is evidence that the growth of at least some human malignant cells is factor dependent and that the malignant cells are capable of producing these factors ("autostimulation," [16]).

We have studied the capacity of leukemic cells to produce and respond to IL2. The leukemic cells studied were either non-T, non-B ALL, or T-cell ALL. In every case, the cells produced a large quantity of IL2. This factor had physicochemical characteristics identical to that of normal IL2, with a mol. wt. of 16,000–17,000 and pI of 6.6 (Table 3) and reacted with monoclonal antibodies directed against normal IL2. These data therefore strongly support that the factor produced by ALL cells is identical or at least closely related to IL2. Costimulation of ALL cells by PHA and Daudi cell, however, failed to lead to a shift in molecular weight, suggesting a restricted expression of IL2 species in ALL. Further studies performed argue against the possibility that residual normal T cells are responsible for the IL2 production by extensive cell purification techniques (repeated E-rosetting, density gradient centrifugation of hypodiploid or hyperdiploid leukemic cells). The leukemic population studied could not have had more than 1% normal cells based on flowcytometric analysis of DNA ploidy levels.

While OKT3 was able to induce IL2 production from leukemic cells, Pan T2 alone was unable to cause the release of IL2 from ALL cells. However, the Pan T2 activation "pathway" is not completely repressed in ALL cells since it can be activated by costimulating ALL cells with Pan T2 and Daudi. The pattern of response of ALL cells to Pan T2 and Daudi suggests that the IL2 producer cell in the ALL population has an altered Pan T2 receptor complex, which could play a role in leukemogenesis.

This conclusion is supported by the study of JM, a leukemic T-cell line. The characteristics of IL2 production in this clonal population of leukemic cells were found to be similar to those of ALL cells.

How Daudi cells are able to restore the ability of Pan T2 to induce IL2 may be important in further understanding the lack of normal control mechanisms on cell proliferation in ALL.

The role of IL2 production in ALL remains to be determined. It appears unlikely that the release of this factor, critical for the proliferation of cells of T lineage, is only an epiphenomenon in ALL, irrelevant for the expansion of the leukemic clone. Recently, a clonal assay system permitting the growth of blast cell progenitors in non- T, non-B ALL has been developed [3]. In this assay, factors present in Ly-CM are required for the successful growth of the leukemic stem cells. Since (a) IL2 is present in Ly-CM, (b) IL2 produced by leukemic and normal T cells appears to be identical (Table 3), (c) peripheral blast populations of ALL do not proliferate in response to IL2, as measured by [^{3}H]dT incorporation, we hypothesize that IL2 is a factor (or one of the factors) produced by partially differentiated leukemic cells and required for the replication of the leukemic stem cells. Since leukemic stem cells represent only a small percentage of the total leukemic population, its proliferation cannot be shown in the assays used for normal PBL. The clonal assay should be able to clarify this important point. Our preliminary data suggest that highly purified IL2 is able to substitute for Ly-CM (Feldman, Izzaguire, Mertelsmann, unpublished data).

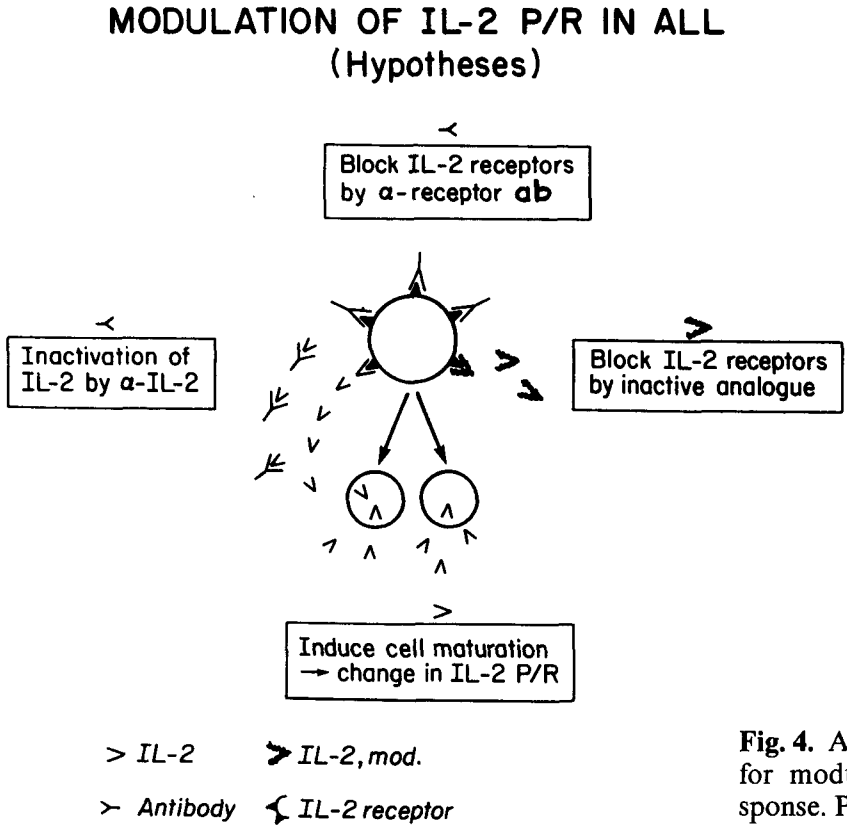

Fig. 4. Approaches under investigation for modulating IL2 production and response. P/R = production/response

In this context, it is of great interest that neoplastic T cells from patients with cutaneous T-cell lymphoma (CTCL) grow in medium containing partially purified IL2 without prior stimulation. Neoplastic T cells differ therefore from normal T cells in not requiring in vitro activation by lectin to interact with IL2 [8], suggesting induction of IL2 receptors by the HTLV [9]. Still another possibility is that leukemic stem cells have a specific way of turning on the release of IL2 (or other factors) from the producer cell. We have shown that normal cells have receptors for OKT3 and Pan T2 which induce IL2 production and cell proliferation (Feldman et al. 1982, submitted). We have further demonstrated that the receptor for Pan T2 is altered in ALL cells [17]. These data strongly suggest a very specific alteration of the membrane of ALL cells.

The importance of the molecular structures present on the surface of T cell for growth regulation is also shown by our preliminary studies of sera raised against endogenous viruses. These sera directed against baboon endogenous virus, M7, and the closely related virus RD114 stimulated the proliferation of normal PBL (Table 2) and the release of IL2 (not shown). Serum raised against SSV was unable to do so. These results also indicate that endogenous virus interacts with growth regulator sites present on the T-cell surface and should be important for the understanding of leukemogenesis and autoimmune diseases.

Modulation of IL2 production and response in vivo could provide a new and powerful approach to manipulate proliferation of specific normal and neoplastic cells in vivo. Figure 4 illustrates several possibilities of manipulating IL2 physiology. Studies of both the effect of the anti-IL2 antibody as well of cytotoxic agents coupled to IL2 have been initiated.

IV. IL2 in Immunodeficiency Syndromes

It is well known that IL2 plays an important role in the development of a variety of T-cell responses. We suggest that some human disorders associated with T-cell defects might be due to defective IL2 production or response. We have recently begun to investigate the role of IL2 in primary and acquired immunodeficiency syndromes. The data obtained so far show complete or partial normalization of T-cell

proliferation by purified IL2 in vitro in the majority of patients with Kaposi sarcoma, CVI, Hodgkin's disease, hemophiliacs with acquired IDS, chemotherapy-induced immunosupression (data not shown), and burn patients (data not shown). Since these observations suggest an important in vivo role of IL2 in several congenital and acquired IDS, we have initiated a phase I clinical trial of IL2. The preliminary results support in vivo activity of subcutaneously administered IL2, both in animal models (Merluzzi et al., unpublished) and in man (Flomenberg et al., unpublished).

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