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Interleukin-2-induced lymphoproliferative responses*

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Summary. Interleukin-2 (IL-2) is capable of both stimulating an in vitro lymphoproliferative response and augmenting non-major-histocompatibility-complex-(MHC)-restricted cytotoxicity. However, there are conflicting reports about the phenotypes of responding cells. In the present studies, we determined phenotypes of Ficoll/Hypaque-separated peripheral blood mononuclear cells stimulated with 50, 100 or 1000 U/ml IL-2; analyses were performed after 1, 3 and 5 weeks. With all concentrations, there was a progressive increase in CD3+ cells; after 3–5 weeks more than 90% of the cells reacted with this antibody. However, the proportions of CD4+ and CD8+ cells proved to be a function of the IL-2 concentration. Cultures containing 50 U/ml or 100 U/ml favored the expansion of the CD4+ subset. By contrast, in cultures stimulated with 1000 U/ml, CD8+ cells predominated. At baseline, CD8+ cells comprised 28±2%; after 3 weeks, this value increased to 51±5%. In addition, the proportion of CD56+ (Leu19, NKH-1) cells depended on the amount of IL-2. At 50 U/ml, there was no appreciable change in CD56+ cells. However, at 1000 U/ml, CD56+ cells increased from 17±1% (day 0) to 39±4% (3 weeks). This increase was primarily due to an expansion of the CD3+ CD56+ subset (non-NMC restricted cytotoxic T cells). By contrast, natural killer (NK) cells, as measured by the CD16 antibody, steadily declined at all IL-2 concentrations.

Introduction

Interleukin-2 stimulation of lymphoid cells induces both proliferative responses [21, 22, 28, 34, 35] and activation of non-major-histocompatibility-complex(MHC)-restricted cytotoxic cells [29]. The latter results in the generation of "lymphokine-activated killer" (LAK) cells; these lymphocytes have been shown to have extensive cytolytic activities in vitro [5, 29]. They are capable of killing both natural-killer(NK)-sensitive and -resistent tumor cell targets. On the basis of these properties, LAK cells are currently being tested in several immunotherapeutic trials for neoplastic diseases [30].

Previous studies aimed at phenotypically characterizing the cells responding to this growth factor have presented divergent results. Some investigators claim that LAK activities are due primarly to a subset of natural killer cells [1, 4, 9, 11, 26, 34, 36, 38]. Others have ascribed the cytolytic properties to cells with characteristics of T lymphocytes [3, 5, 6, 21, 25, 32, 37]. In part, these disparities may reflect differences in the culturing procedure [25, 32]. There is a wide variability in both the IL-2 concentration used to stimulate lymphocytes and the duration of the in vitro incubation.

The present studies were undertaken to evaluate the effects of different concentrations of IL-2 on lymphocytes present in long-term cultures. These data indicate that low concentrations of IL-2 favor the expansion of CD4+ lymphocytes. By contrast, in cultures stimulated with high concentrations of this growth factor, responding cells preferentially express both the CD8+ and the CD56+ (Leu19, NKH-1) phenotypes.

Materials and methods

Mononuclear cells were isolated from heparinized peripheral blood by Ficoll/Hypaque gradient centrifugation. The cells were enumerated and resuspended in RPMI-1640 media containing 10% fetal calf serum, glutamine (10 µl/ml) and gentamicin (10 µl/ml). Recombinant IL-2 (Cellular Products Inc., Buffalo, N.Y.) was added to produce final concentrations ranging from 1 U/ml to 1000 U/ml. For proliferative assays, cells were cultured in 96-well flat-bottom microtiter plates. Each well contained 2×10^5 cells in 0.2 ml medium. These were incubated for either 6 or 7 days at 37° C in a 5% CO_2 humidified atmosphere. Six hours prior to harvesting, each well was pulsed with 1 µCi tritiated thymidine

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Table 1. Monoclonal antibodies

Antibody[a]	Cellular targets
CD2[b] (T11, Leu5)	Pan T cell
CD3 (T3, Leu4)	Mature T cells (T cell receptor complex)
CD4 (T4, Leu3)	Helper-inducer T cells
CD8 (T8, Leu2)	Suppressor/cytotoxic cells
CD19 (B4, Leu12)	Pan B cells
CD20 (B1, Leu16)	Pan B cells
CD57 (Leu7, HNK-1)	Subsets of natural killer and T cells
CD16 (Leu11)	Natural killer cells (reacts with IgG-Fc receptor)
CD56 (Leu19, NKH-1)	Natural killer cells and non-MHC-restricted cytotoxic T cells
CD25 (Tac)	IL-2 receptors
CD45RA (2H4)	CD4 suppressor/inducers; naive T cells
CD29 (4B4)	CD4 helper/inducers; memory T cells
CD71 (T9)	Transferrin receptor

[a] All antibodies except CD45RA and CD29 were purchased from Becton Dickinson Immunocytometry Systems, Mountainview Calif. These two were obtained from Coulter Corp., Hialeah, Fla. [b] Cluster designation. Names in parenthesis are common or trade names for each antibody

Table 2. Interleukin-2 (IL-2)-induced proliferative responses[a]

IL-2 concentration (U/ml)	Net radioactivity (cpm)
1000	23 380 ± 1848
500	23 160 ± 2046
250	23 042 ± 1957
100	22 165 ± 1920
50	22 033 ± 1850
25	21 039 ± 1447
10	14 182 ± 1183
5	13 627 ± 1586
2.5	12 038 ± 1293
1.0	7914 ± 660

[a] Mean ± SEM. All cultures contained 2×10^5 cells in 0.2 ml media. They were harvested after 7 days incubation; [^{3}H]dT was added for the final 6 h. The mean isotope incorporation in unstimulated cultures was 1786 ± 332 cpm. Each value represents the mean of at least 10 individual experiments

([^{3}H]dT, specific activity, 6.7 Ci/mmol). Plates were harvested using a PHD Cell Harvester, (Cambridge Technology, Cambridge, Mass.) and the incorporated radioactivity was measured in a Packard Tricarb Liquid Scintillation Counter (Downers Grove, Ill.). Proliferative responses were determined by averaging values from three replicate cultures; the net incorporation was determined by subtracting the isotope incorporation of cultures not stimulated with IL-2 from those incubated with this growth factor.

Long-term cultures were established in 6-well Costar plates (Cambridge, Mass.). Each well contained 10×10^6 cells in a final volume of 10 ml complete medium. Cultures were stimulated with either 50, 100 or 1000 U/ml IL-2. For these cultures, half of the medium in each well was replaced twice weekly with fresh medium containing the same concentration of IL-2 as in the original culture. During the second week of incubation, cultures were divided at a 1 : 2 dilution. Cultures were maintained for periods up to 5 weeks.

To determine the ability of various monoclonal antibodies to block cell replication, four antibodies were tested. These included anti-Tac [anti IL-2R, CD25], kindly supplied by Dr. Thomas A. Waldmann, National Cancer Institute, antibodies to the transferrin receptor (CD71), the class II histocompatibility complex (HLA-Dr), and the anti-CD38 antigen. The latter three were all commercial preparations and contained azide as the preservative. Prior to inclusion in the culture, each serum was extensively dialyzed against complete culture media. All antibodies were added at the beginning of the incubation period in quantities ranging from 25 ng/ml to 500 ng/ml. Inhibition was measured by comparing the net incorporated radioactivity in cultures containing the monoclonal antibodies with replicates lacking these potentially inhibitory substances. The lymphoproliferative response was induced with 50 U/ml IL-2.

Phenotypic studies were performed by labeling the cultured cells with a panel of fluorescein- or phycoerythrin-conjugated monoclonal antibodies. These are listed and described in Table 1. Cells were analyzed on a FACScan flow cytometer (Becton Dickinson, Mountainview, Calif.). In studies in which two-color analyses were performed, one of the monoclonal antibodies was labeled, either directly or indirectly, with fluorescein, the other with phycoerythrin. Each data point represents the average of 8 – 11 separate experiments.

Cell-mediated cytotoxicity against both ^{51}Cr-labeled K562 and Raji cells [13] and pokeweed-mitogen-induced immunoglobulin synthesis assays [41] were performed using techniques previously described.

Results

Proliferative responses

Recombinant IL-2, by itself, proved to be a potent mitogen. The magnitude of the proliferative response was a function of the concentration of this growth factor (Table 2). In our assays, maximum [^{3}H]dT incorporation occurred over a wide concentration range, 25 – 1000 U/ml. However, even at concentrations as low as 1 U/ml, lymphoproliferative responses were observed. Kinetically, peak isotope uptake occurred after 5 – 7 days incubation.

Monoclonal antibodies to the β chain of the IL-2 receptor (anti-CD25), produced a dose-dependent inhibition of cell growth. At an antibody concentration of 500 ng/ml, the [^{3}H]dT incorporation induced by 50 U/ml IL-2 was reduced by 75%. By contrast, antibodies to class II MHC determinants (anti-Dr), the CD71 antibody that binds to the transferrin receptor and an antibody to the CD38 antigen did not impair cell replication. Likewise, neither antibodies to the CD4 nor those to the CD8 epitopes altered reactivity.

In long-term cultures, proliferative responses tended to decline after the first week of incubation. At both 3 and 5 weeks, the maximum isotope uptakes were approximately 25% of that measured at the 1-week interval.

Phenotypic studies

Cells cultured in 50, 100 or 1000 U/ml IL-2 were sequentially analyzed cytofluorographically for expression of multiple cell-membrane-associated determinants. With all three concentrations, there was a progressive increase in cells reacting with the CD3 antibody (Fig. 1). At the 3-week and 5-week intervals, more than 90% of the cultured cells were CD3$^+$. This increase was paralleled by an increase in CD2$^+$ cells (data not shown). By contrast,

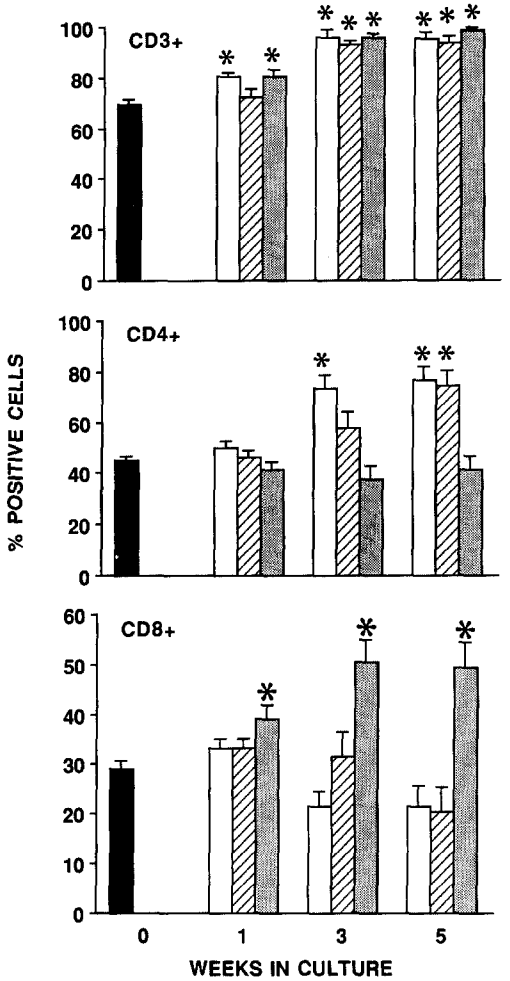

Fig. 1. Changes in the percentage of cells expressing the CD3, CD4 and CD8 antigens. The *solid black bar* represents the percentage positive cells at the start of the culture; the *open white bars* are values measured in cultures containing 50 U/ml IL-2; the *stippled bars*, 100 U/ml IL-2 and the *shaded bars*, 1000 U/ml IL-2. At all concentrations, there is a progressive increase in $CD3^+$ cells. However, the distribution of $CD4^+$ and $CD8^+$ cells is a function of the concentration of this growth factor. In particular, low concentrations (50 U/ml or 100 U/ml) favor the expansion of $CD4^+$ cells whereas $CD8^+$ cells predominate in the cultures containing 1000 U/ml. Values are means ± SEM. The *asterisk* indicates that the value is significantly different from the day-0 control (P <0.05 or less)

B cells, as assessed by reactivity with CD19 and CD20 antibodies, had virtually disappeared from the cultures by 3 weeks.

The proportions of $CD4^+$ and $CD8^+$ cells in long-term cultures proved to be a function of the IL-2 concentration (Fig. 2). In cultures containing 50 U/ml or 100 U/ml, there was a marked increase in $CD4^+$ cells at 3 and 5 weeks. The baseline value for $CD4^+$ cells was 44.9±1.8%; after 5 weeks in cultures containing 50 U/ml, this increased to 76.8±5.1%. The increase in $CD4^+$ cells was associated with a slight, but statistically insignificant, decrease in $CD8^+$ cells. As such, the ratios of $CD4^+$:$CD8^+$ cells progressively increased from 1.5 to 3.6 at 5 weeks.

By contrast, there was a preferential increase in $CD8^+$ cells in those cultures stimulated with 1000 U/ml. Compared to fresh cells, the fraction of $CD8^+$ cells had significantly increased after 1 week in culture. $CD8^+$ cells comprised 29.0±1.8% of the cells at day 0. By 3 weeks the mean $CD8^+$ value had increased to 50.5±4.6%. Correspondingly, the number of $CD4^+$ cells showed a slight decline. Thus, at 3 and 5 weeks, the CD4:CD8 ratio was consistently less than 1.

Table 3. Non-major-histocompatibility-complex-restricted cytotoxic cells after IL-2 stimulation

Antigen	IL-2 conc (U/ml)	Percentage $CD56^+$ cells in culture after			
		0	1 week	3 weeks	5 weeks
CD56	50	17.3±1.3	11.7±0.8	13.6±3.2	12.8±3.4
	100		19.0±2.1	17.4±3.0	12.6±2.8
	1000		23.2±2.1**	38.7±4.4**	33.5±4.5**
CD16	50	12.1±1.3	5.9±0.6**	3.9±1.6**	2.2±0.9**
	100		12.0±1.3	4.8±1.0**	2.0±0.6**
	1000		9.1±2.0	3.2±0.6**	1.7±0.3**
CD3/CD56	50	4.8±0.5	6.5±1.4	11.9±0.8**	11.2±1.3**
	100		5.0±0.8	11.1±3.0	9.1±2.7
	1000		12.3±2.1**	34.6±4.5	32.6±5.0**
CD57	50	6.8±0.9	8.2±0.6	3.7±0.3	2.0±0.3**
	100		7.8±0.9	3.2±1.7	2.6±1.7**
	1000		10.0±1.5	5.7±1.4	2.3±0.6**

* Mean ± SEM (Based on 9–11 experiments)
** Significantly different from day-0 controls (P <0.05 or less)

Further analysis indicated that the proportion of cells reacting with the CD56 antibody was also a function of the concentration of this lymphoid growth factor (Table 3). This monoclonal antibody identifies two types of cells, true NK cells and a subset of T cells that can effect non-MHC-restricted cytotoxicity [18, 19]. In cultures stimulated with 50 U/ml, there was a minor decrease in $CD56^+$ cells at 1 week; however, the fractions of cells reacting with this antibody at 3 and 5 weeks were not different from the baseline value. At 100 U/ml, the percentage of $CD56^+$ cells remained comparatively stable throughout the 5-week incubation. By contrast, the percentage of $CD56^+$ cells increased markedly in cultures stimulated with 1000 U/ml. After 3 weeks and at this time, $CD56^+$ cells had more than doubled when compared to the day-0 value.

To define the phenotypes of non-MHC-restricted killer cells more precisely, the fraction of $CD16^+$ cells was sequentially examined. This antibody identifies the majority of "true" natural killer cells [16, 18, 19]. As can be seen in Table 3, there was a gradual decrease in $CD16^+$ lymphoid cells. At all three concentrations of IL-2, only 3%–4% of the lymphoid cells after 3 weeks in culture were $CD16^+$. These percentages declined further by 5 weeks. The decrease in total $CD16^+$ cells was paralleled by a concomitant decrease in cells double-labeling with the CD16 and CD56 antibodies; at 5 weeks, fewer than 1.5% of the cells reacted with these two antibodies.

By contrast, the percentage of cells co-expressing both the CD3 and the CD56 antigens increased with all three

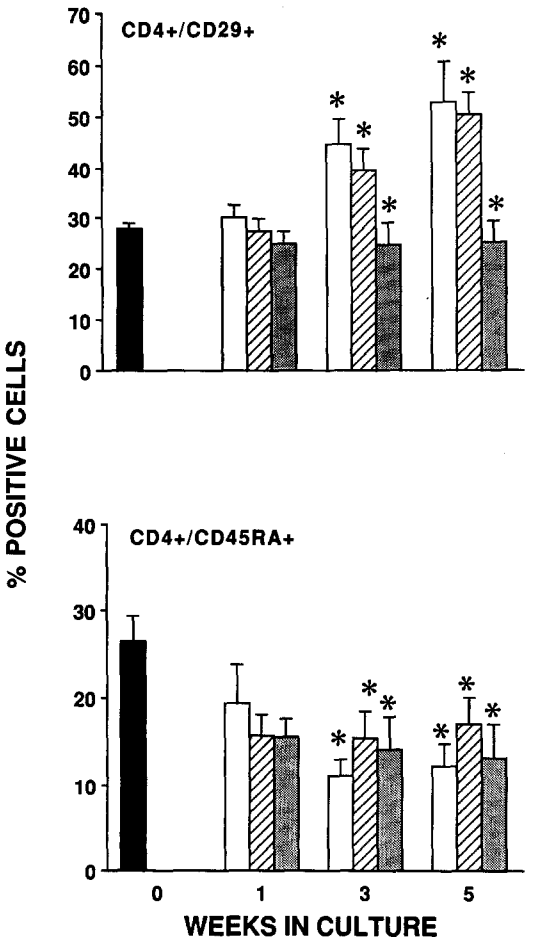

Fig. 2. Percentage of cultured cells co-expressing the CD4 and either the CD29 or CD45RA antigens. CD4+/CD29+ cells are variously considered either helper/inducers or memory (previously activated) cells. CD4+/CD45RA+ cells are functionally defined as either suppressor/inducers or naive T lymphocytes. At IL-2 concentrations of 50 U/ml and 100 U/ml, there is a significant increase in CD4+/CD29+ cells; by contrast, the number of these double-labeled cells remains comparatively constant in cultures with 1000 U. At all three concentrations of this growth factor, there is a progressive decrease in CD4+/CD45RA+ cells. As indicated by the *asterisks*, the values at 3 and 5 weeks were all significantly different ($P < 0.05$) compared to the day 0 value

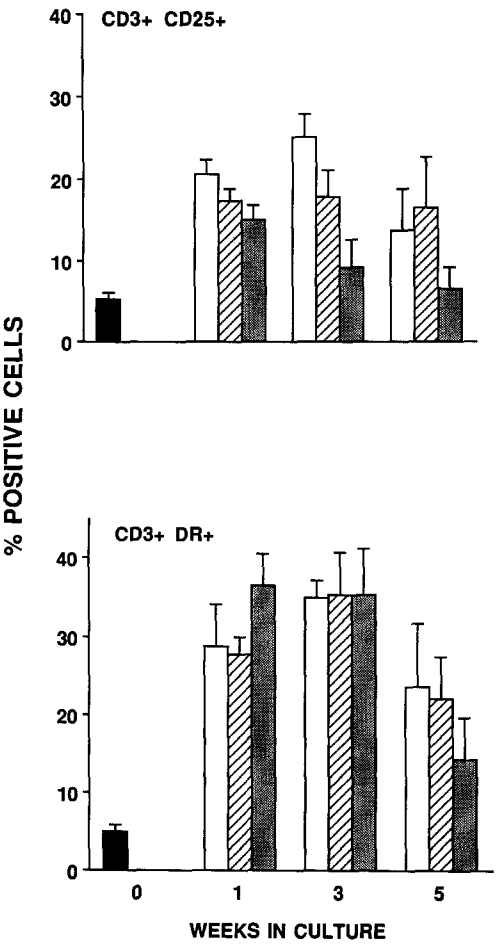

Fig. 3. Expression of T cell activation antigens, CD25 and HLA-Dr. In general, the presence of activated T cells, using either marker, significantly increases at both the 1-week and 3-week time points. However, despite the continued presence of IL-2, the number of positive cells declines from their maximum values by 5 weeks. This suggests that some stimulated T cells spontaneously revert to the resting stage. The *black bar* is the baseline (day 0) value; the *white bars*, 50 U/ml, the *stippled bars*, 100 U/ml and the *shaded bars* 1000 U/ml. Values are means ± SEM

concentrations of IL-2. However, the fraction of CD3+/CD56+ lymphocytes was significantly less in cultures containing 50–100 U/ml compared to those containing 1000 U/ml. At the low concentrations, the percentage of cells expressing both antigens approximately doubled at 3 and 5 weeks. By contrast, there was more than a sixfold increase in this subset in cultures containing 1000 U/ml. At the start of the culture, 4.8% of the lymphoid cells were CD3+/CD56+; after 3 weeks in media containing 1000 U/ml IL-2, this had risen to 34.6%.

Additional studies to define further the changes in NK cell phenotypes were performed using the CD57+ (Leu7, HNK-1) antibody. At all three concentrations of IL-2, there were no significant changes at 1 week in CD57+ cells. Thereafter, the number of these cells progressively decreased. Furthermore, there were no differences in the percentages of CD57+ cells in the three IL-2 concentrations. Parallel results were obtained using two-color analyses with CD57+/CD16+ and CD57+/CD8+ combinations.

The concentration of IL-2 also affected the distribution of CD4+ subsets. These cells can be subdivided into two groups based on their relative expressions of the CD29 and CD45RA antigens. At the start of the culture, the distribution of CD29 and CD45RA subsets in the CD4+ population was approximately equal (Fig. 2). At all three IL-2 concentrations, the proportion of CD4+/CD45RA+ cells decreased. However, there was a striking increase in the CD4+/CD29+ subset in cultures stimulated with either 50 U/ml or 100 U/ml. By contrast, there was no change in the number of these cells in cultures containing 1000 U/ml IL-2. When viewed in context of the changes in total CD4+ cells, it appears that the increase in CD4+ cells seen with low IL-2 concentrations is primarily due to an expansion of the CD29+ subset.

Table 4. Cytotoxic activities of cultured cells

E:T ratio	Cytotoxic activity (%)				
	Fresh cells	Cultured cells[a]	IL-2-cultured cells		
			1 week	3 weeks	5 weeks
K562					
50:1	23.3±5.6[b]	20.9± 9.6	47.4±11.3	51.1±4.3	16.7±5.3
25:1	14.9±2.9	23.3±10.8	43.7±13.3	34.0±6.7	11.9±3.4
12.5:1	10.5±2.2	15.9± 9.1	26.9±10.7	21.7±5.6	6.6±2.3
6.25:1	5.8±0.9	6.9± 1.3	19.1± 6.0	14.2±4.2	6.2±2.1
Raji					
50:1	9.6±2.2	11.3± 1.2	24.3± 6.1	25.9±7.8	14.6±8.3
25:1	8.4±0.8	11.3± 3.1	19.6± 3.9	21.6±6.5	10.3±3.2
12.5:1	8.4±2.2	9.9± 6.4	16.7± 3.4	15.3±5.8	4.3±1.6
6.25:1	6.6±2.7	8.7± 1.7	20.3± 5.9	13.2±6.0	9.4±2.7

[a] Cells cultured for 1 week in media alone; no added IL-2

[b] Mononuclear cells cultured with or without IL-2 (50 U/ml) for 7 days. ^{51}CR release was measured in a 4-h assay; mean ± SEM (6 experiments)

Table 5. In vitro immunoglobulin synthesis (Representative experiment[a])

Cells	IgG (ng/ml)	IgM (ng/ml)
Unseparated mononuclear cells	8209	8292
Non-T cells	36	62
IL-2-cultured cells[b]	59	0
Non-T cells + cultured cells (1 week)	19806	4644
Non-T cells + cultured cells (3 weeks)	21044	3142

[a] Similar results were obtained in three other assays

[b] Peripheral blood mononuclear cells were cultured for 1 and 3 weeks in media containing 50 U/ml IL-2. The cells were harvested, washed and recultured either alone or with autologous fresh non-T cells for 10 days in the presence of pokeweed mitogen. Supernatants were collected and assayed by an enzyme-linked immunosorbent assay procedure for concentrations of IgG and IgM

T cell activation antigens

Culturing mononuclear cells with varying concentrations of IL-2 leads to the expression of a series of determinants associated with T cell activation. In the present studies, we measured the fraction of T cells ($CD3^+$) expressing class II MHC antigens (Dr) and receptors for IL-2 (CD25 antigen) at three concentrations of this interleukin (Fig. 3). After 1 week, there was an increase in the T lymphocytes expressing these two markers of cell activation.

Furthermore, there were no differences in the responses related to the IL-2 concentration. Minor differences in expression of activation markers were observed by the 3rd week; however, by 5 weeks, the numbers of T cells expressing either CD25 or Dr declined appreciably from their maxima. These results suggest that, despite the continued presence of IL-2, a high proportion of stimulated cells spontaneously revert to a resting or inactive stage. These findings are consistent with the decrease in [^{3}H]dT incorporation noted in long-term cultures.

In vitro functional activities

In order to correlate the phenotypic changes occurring in cultures containing 50 U/ml IL-2 with functional activities, cells were analyzed both for their ability to lyse NK-sensitive and -resistant target cells and to provide help for B lymphocytes in a pokeweed-mitogen-induced immunoglobulin synthesis assay.

Mononuclear cells displayed increased cytolytic activities for both NK-sensitive (K562) and NK-resistant (Raji) tumor cell lines. Table 4 shows the activities of fresh cells, cells cultured for 1 week without IL-2 and the cytolytic activities of cells obtained after 1, 3 and 5 weeks of culture. As can be seen, there are only minor differences in the cytolytic potentials of fresh cells and cells from the same donor cultured for 1 week in the absence of IL-2. By contrast, there was an increase in the cytolytic activities for both tumor cell lines using effector lymphocytes cultured in this interleukin. These heightened cytolytic activities persisted through the 3-week time point; however, by 5 weeks, tumor cell killing did not differ from that observed with fresh lymphocytes.

In view of the increase in $CD4^+/CD29^+$ cells in cultures containing 50 U/ml IL-2, the ability of these cells to provide help for B lymphocytes in the pokeweed-mitogen-induced immunoglobulin synthesis assay was also examined. Four separate assays were performed; data from a representative experiment are shown in Table 5. Non-sheep-cell rosetting mononuclear (non-T) cells alone did not produce significant amounts of either IgG or IgM. As expected, IL-2-cultured cells also did not synthesize either immunoglobulin. However, cells cultured in IL-2 for 1 and 3 weeks were potent helpers for B cells; they induced isolated non-T cells to produce both IgG and IgM. As judged by the amounts of IgG produced, it appears that IL-2-stimulated cells may have enhanced helper activities when compared to uncultured T cells.

Discussion

Data presented in this study indicate that the phenotypes of cells responding to mitogenic IL-2 stimulation are highly dependent upon two variables, the growth factor concentration and the duration of the culture. At all three IL-2 concentrations tested, there was a time-dependent progressive increase in the number of $CD3^+$ T cells. However, in long-term cultures, the ratio of the two major T-cell subsets, CD4 and CD8, varied with the amount of growth factor. At low IL-2 concentrations, 50 U/ml and 100 U/ml, there was a preferential expansion of $CD4^+$ cells and a corresponding increase in the $CD4^+$:$CD8^+$ ratio. By contrast, T cell subset analysis of cultures incubated with 1000 U/ml showed a progressive increase in $CD8^+$ cells and a concomitant reduction in the $CD4^+$:$CD8^+$ ratio.

In addition to the differences in the $CD4^+$:$CD8^+$ cell ratio, there were appreciable changes in the fraction of cells reacting with antibodies identifying non-MHC-restricted cytotoxic lymphocytes. Lanier et al. showed that the CD56 antibody reacts with both NK cells and a subset of non-MHC-restricted cytotoxic T cells [16, 18, 19]. The "true" NK cells can be identified by their co-expression of the CD16 antigen; phenotypically, these cells lack the CD3 polypeptide complex. Conversely, the non-MHC-restricted cytotoxic T cells react with both CD56 and CD3 antibodies but not with the CD16 antigen. $CD56^+$ cells represented about 17% of the initial cell inoculum; of these, it appears that approximately two-thirds were $CD16^+/CD3^-$ and one-third $CD3^+/CD16^-$.

The differential effects of IL-2 concentrations were pronounced with respect to the subset reacting with both the CD56 and CD3 antibodies. This subset appeared to be particularly responsive to high IL-2 concentrations; at 1000 U/ml, there was an increase from 4.8% to a maximum of 35% at 3 weeks. By contrast, cultures stimulated with either 50 U/ml or 100 U/ml showed only modest increments in $CD56^+/CD3^+$ cells throughout the culture period. When viewed in the context of the progressive decrease in $CD16^+$ cells, it appears that most of the cytotoxic effectors found in the long-term cultures, including those used in various clinical trials, are probably due to expansion of the $CD56^+/CD3^+$ subset.

Previously published phenotypic studies of IL-2-responsive cells have yielded conflicting results. In most reports, cell phenotypes have been analyzed after a short incubation period. Furthermore, these studies often employed only a single concentration of IL-2. As a result, some investigators suggest that the predominant phenotypes are those of NK cells [1, 4, 9, 11, 26, 34, 36, 38] whereas others claim that a proportion of effectors are T cells [3, 5, 6, 21, 25, 32, 37].

Others report that responsive cells are heterogeneous with respect to their phenotypic expression [9, 25, 36]. In short-term culture, Ortaldo et al. [25] found that both NK cells and cytotoxic T cells respond to IL-2. However, the NK response reached its maximum after 1 day whereas the response of T cells was delayed until 2–3 days. Lanier et al. have also shown that CD56 is expressed on many IL-2-dependent cytotoxic and non-cytotoxic T cell lines. These lines were generated in a mixed lymphocyte culture system [15].

Another interesting phenomenon related to the effects of IL-2 stimulation on lymphocyte phenotypes is the progressive change in the distribution of CD4 subsets. In cultures stimulated with IL-2, there is a progressive increase in $CD4^+$ cells co-expressing the CD29 antigen. This is associated with a corresponding reduction in $CD4^+/CD45RA^+$ cells. Morimoto et al. proposed that the CD29 and CD45RA antigens separate the $CD4^+$ subset into helper-inducers and suppressor-inducers [23, 24].

Other studies, however, indicate that these antigens may not represent distinct lineages but different maturational stages [2, 31]. T cells expressing high-density CD45RA show functional characteristics of naive lymphocytes. By contrast, those with high-density CD29 appear to be memory (previously activated) cells. Furthermore, lymphocytes can convert from the CD45RA to the CD29 phenotype but the reverse has not been demonstrated. On the basis of this thesis, it would appear that $CD4^+$ cells grown in low concentrations of IL-2 undergo this conversion; this suggests that exposure to this growth factor causes a maturation of naive cells to memory lymphocytes.

In accord with other studies, lymphocytes cultured in IL-2-containing media show increased cytolytic activities. Maximum target cell lysis from cells incubated with 50 U/ml occurred during the first 3 weeks of culture. However, there was no apparent correlation between increased cytolytic activities and cells bearing any of the non-MHC-restricted killer cell phenotypes. These data suggest that this lymphocyte growth factor has stimulatory activities in addition to those related to its ability to promote cell replication. Other studies found that IL-2 can directly enhance the cytolytic capacities of individual killer cells [17, 27]. Enhancement can be seen with incubations as brief as 5 min [8] to 1 h [12]. This interval is too short to allow for lymphoid replication.

As judged by the presence of activation antigens on T cells, [10, 14] all three concentrations of IL-2 appear to possess similar lymphocyte-stimulatory activities. These phenotypic observations are consistent with the equivalent uptake of [^{3}H]dT observed with all three IL-2 concentrations. Thus, it appears that the variation in phenotypic expression of cultured cells reflects either differential sensitivities of various lymphocyte subsets or altered expression of cell-membrane-associated polypeptides.

There also appears to be a biphasic response to IL-2. Initially, there is a progressive increase in T cells bearing either receptors for IL-2 (CD25) and class II MHC (Dr) determinants (Dr). These findings are consistent with the early proliferative expansion phase as measured by [^{3}H]dT incorporation. Nevertheless, despite the addition of fresh IL-2-containing media, there is a late decline in T cells expressing these activation antigens and a corresponding reduction in isotope incorporation. Thus, a proporation of activated cells appear to revert spontaneously to their resting phase. Gullberg and Smith [7] have shown previously that, despite continued exposure to IL-2, $CD4^+$ cells become refractory to this growth factor; these observations are in accord with the decreased response observed in the current experiments. These results have clinical implications; if long-term IL-2-stimulated LAK cells are to be employed in immunotherapeutic protocols, techniques must be developed to prevent reversion to resting cell phenotypes.

The mechanisms by which IL-2 induces proliferative responses are not clearly defined. Receptors for this growth factor are not expressed on resting T cells; they appear shortly after cell activation. At the start of culture, there is a small subset of T cells expressing IL-2 receptors ($CD25^+$); these lymphocytes presumably comprise cells that have been activated in vivo and may constitute the in vitro responsive elements. Alternatively, there is evidence that IL-2 may be capable of "up-regulating" its own receptors and thus might activate a subset of resting T cells [20, 33, 39, 40]. Additional studies will be needed to determine the activating mechanisms more precisely.

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