RESEARCH ARTICLE

The Interleukin-2 T-Cell System: A New Cell Growth Model

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Abstract. *Synchronized interleukin-2 receptor-positive T cells, homogeneous immunoaffinity-purified interleukin-2, and a monoclonal antibody to interleukin-2 receptors were used to show that only three factors are critical for T-cell cycle progression: interleukin-2 concentration, interleukin-2 receptor density, and the duration of the interleukin-2 receptor interaction. Since the proliferative characteristics of T cells are identical to those of both prokaryotic and all other eukaryotic cells, these findings provide a new model that accounts fully for the variables that determine cell cycle progression.*

Mitosis of T lymphocytes results from the interaction of the T-lymphocytotrophic hormone interleukin-2 (IL-2) with specific membrane receptors (*1–4*). As a result of the availability of homogeneous IL-2 (*5*), the capability to examine IL-2 receptors quantitatively (*4–6*) and qualitatively (*7–11*), and the ability to synchronize IL-2 receptor-positive (receptor$^+$) T cells (*10*), it was possible to formulate new approaches to the determinants of T-cell cycle progression. Consequently, the basis for variable cell cycle transit times of individual cells among genetically homogeneous populations, a perplexing observation fundamental to the mechanisms that control cell proliferation, could finally be clarified. The results from several types of experiments indicate that T-cell cycle progression is predictable and depends on a critical threshold of interactions between IL-2 and the receptor that determines the quantal response, namely, cell division. Since IL-2 receptor density is heterogeneous and follows a log-normal distribution among T-cell populations, the cell cycle times of individual cells reflect the logarithmic-normal distribution of the IL-2 receptors.

Numerous experiments with many cell types (*12–23*) reveal that, within a cell population, the cell cycle times follow a normal distribution when examined as a function of the division rate (the rate-normal distribution) (*16, 17*). Kinetic studies of the cell cycle with either asynchronously proliferating populations or synchronized cell populations indicate that most of the variability in transit times occurs in the prereplicative phase (G_0-G_1) of the cell cycle since the replicative phases (S, G_2, M) remain relatively constant (*23*). Also, duration of the cell cycle is not genetically determined, nor is it passed on at division, since the correlations of cell cycle times for mother-daughter cells are generally poor (*23–26*). However, cycle times of sister cells do show a positive correlation, an indication that sibling cells inherit some property that contributes to similar cell cycle transit times for the next cycle, which then disappears by the subsequent cell cycle (*23–26*). Consequently, there is no selection for more rapidly growing cells, and cell populations retain the same rate-normal distribution of cell cycle times over many generations.

These and similar observations have led to the presentation of two basic models to explain the variability of cell cycle times. (i) The earlier deterministic model, proposed 20 years ago by Koch and Schaechter (*27*), is based on the assumptions that cells initiating their cycle are functionally different and that cell cycle variability arises from the cumulative effects of many small differences. (ii) The probabilistic model, originally proposed by Burns and Tannock (*28*) and extended by Smith and Martin and co-workers (*18, 20, 24–26, 28–30*), is based on the assumptions that cells initiating their cycle are functionally identical and that the transition of cells from an indeterminate resting phase to a determinant proliferative phase is regulated by a single Poissonian event, quite independent of other events or properties. While both models provide explanations for many of the observed data, in each instance there are discrepancies that point toward the presence of hidden variables operative in cell division kinetics. Accordingly, to achieve a better understanding of cell cycle control mechanisms, it appeared desirable to proceed beyond mathematical analysis of studies of cell populations and to correlate kinetic studies of cell growth with quantitative measurements of the critical factors known to promote DNA duplication and cellular division.

Recent advances in the dissection of the molecular mechanisms that regulate T-lymphocyte mitosis have permitted an analysis of the variables that determine the heterogeneity of interdivision times observed within proliferating T-cell populations. Such studies have become possible because IL-2 can now be purified to homogeneity by monoclonal antibody affinity adsorption (*5*). Furthermore, the development of a radiolabeled IL-2 binding assay made possible the quantitative measurement of IL-2 receptors (*4, 6*), and a recently described monoclonal antibody reactive with IL-2 receptors (anti-Tac) (*7–9*) made possible a qualitative assessment of IL-2 receptors within T-cell populations (*10, 11*). These reagents have been used to show that the initiation of T-cell cycle progression depends on immunostimulatory signals such as antigens, mitogenic lectins, or T-cell–specific monoclonal antibodies, whereas the transition from G_1 into the replicative phases of the cell cycle is mediated by IL-2 alone (*3, 4, 10, 11*).

The relevance of the IL-2 receptor hormone system to T-cell cycle progression is attested by experiments showing that the IL-2 receptor interaction is obligatory for DNA synthesis and mitosis; that is, monoclonal antibodies reactive with IL-2 (*5*), or the IL-2 receptor (*9*), inhibit T-cell proliferation by preventing IL-2 receptor binding. Moreover, the IL-2 membrane binding sites satisfy the criteria for authentic hormone receptors (such as high affinity, saturability, and ligand and target cell specificity), and the concentrations of IL-2 that bind to IL-2 receptors coincide precisely with those that promote T-cell proliferation (*4, 6*). Thus, as would be predicted from the interaction of a single ligand and a single class of high affinity receptors, at equilibrium the rate of T-cell proliferation is directly dependent on the concentration of IL-2 available to the cells (*4*).

A characteristic of the IL-2 hormone-receptor mechanism, which permits the

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synchronization of IL-2 receptor$^+$ cells, stems from the finding that, unlike those of all known hormone receptor systems, IL-2 receptors are not continuously expressed by T cells. Rather, receptor expression occurs only after appropriate immune stimulation and is thus transient (*10*). When cells are exposed to antigen or lectin, IL-2 receptors accumulate asynchronously among the individual cells within a population. Once a critical density of IL-2 receptors is reached, cell cycle progression ensues provided that an adequate concentration of IL-2 is present. If the initial immunostimulatory activating signal is removed, the number of IL-2 receptors progressively declines regardless of the maintenance of an adequate IL-2 concentration. In parallel, the proliferative rate of the cell population also declines, finally resulting in the reaccumulation of all of the cells within the population into the G_0-G_1 phase of the cell cycle. Of significance for our studies is that the reintroduction of the initial activating signal results in an acquisition of IL-2 receptors so accelerated that maximum receptor levels now occur within 18 to 24 hours. However, cell cycle progression resumes only when IL-2 is provided. Thus, a T-cell population can be synchronized into the G_0-G_1 phase of the cell cycle and can be stimulated to express maximal levels of IL-2 receptors. The kinetics of IL-2–dependent cell cycle progression can then be followed and analyzed in relationship to IL-2 receptor concentration and distribution among the cells within the population.

Heterogeneous Distribution of IL-2 Receptors

When the IL-2 receptor distribution of a T-cell population is analyzed by flow microfluorometry with anti-Tac, and the data are plotted on a linear scale, a skewed distribution pattern is obtained (Fig. 1A). However, if the data are plotted on a logarithmic scale, the receptor density follows a normal distribution with only a slight skewing toward a frequency of cells with a higher receptor concentration (Fig. 1B). The heterogeneous distribution of receptors within T-cell populations could not be explained by receptor variation with the recognizable phases of the cell cycle, since identical receptor distribution patterns were observed with G_0-G_1 synchronized cells. Moreover, clonal variation of receptor density could not account for these observations, as identical patterns were consistently observed with cloned murine (data not shown) and human T-cell populations (*11*).

The log-normal distribution of IL-2 receptors within a T-cell population is strikingly reminiscent of the rate-normal distribution of cell cycle times. Because of the similarity of these distributions and because the IL-2-receptor interaction is obligatory for T-cell cycle progression, the possibility was considered that cell cycle variability is determined by the intrapopulation heterogeneity of IL-2 receptor density per cell. Should this supposition be correct, IL-2 receptor$^+$ cell populations would be heterogeneous with regard to their responsiveness to IL-2. Accordingly, to examine the functional implications of heterogeneous IL-2 receptor expression, the initial experimental approach was to establish whether conditions that limit IL-2 receptor interactions modify cell cycle progression, particularly with respect to the proportion of cells triggered.

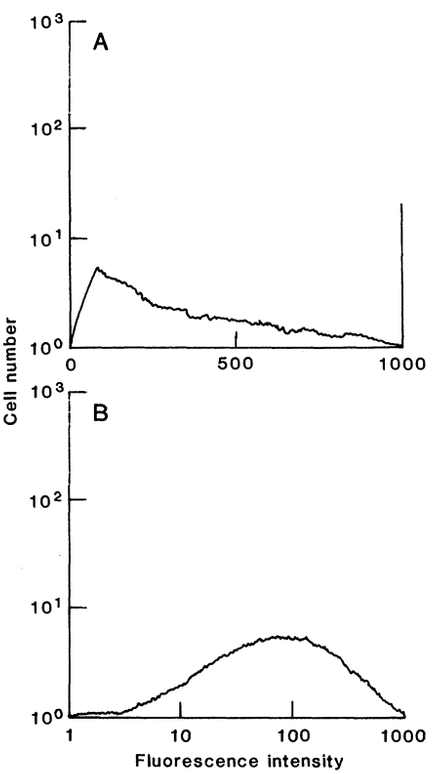

Fig. 1. Fluorescence of anti-Tac reactivity. Human peripheral blood mononuclear cells (PBM) isolated by Ficoll-Hypaque discontinuous gradient centrifugation were cultured (10^6 cells per milliliter) in RPMI 1640 medium supplemented with heat-inactivated fetal calf serum (10 percent) (RPMI 1640/10 percent fetal calf serum). Cells were stimulated with phytohemagglutinin (PHA) (1 μg/ml) for 72 hours to ensure expression of the IL-2 receptor. For analysis of anti-Tac reactivity, samples (10^6 cells) were incubated (1 hour, 4°C) with saturating concentrations of biotinylated anti-Tac (20 μg/ml) and fluorescein or Texas red–conjugated avidin (1 hour, 4°C). Anti-Tac reactivity was determined on an Orthocytofluorograph (System 50H, Orthodiagnostic System, Inc.) for analysis of linear plots of fluorescence intensity (A) and on an Epics 5 cell sorter (Coulter Electronics) for analysis of logarithmic plots of fluorescence intensity (B). The percentage anti-Tac$^+$ cells, estimated against a background of nonspecific labeling with normal mouse immunoglobulin (1 to 3 percent), was 84 percent (A) and 87 percent (B). For each plot 10,000 cells were collected.

IL-2 Concentration and Exposure Time Determine Cell Cycle Progression

Since the proportion of occupied receptors is dependent on the IL-2 concentration, and since the distribution of IL-2 receptors among the cells within a population follows a log-normal pattern, the absolute number of occupied receptors should vary according to the receptor density of each cell. Thus, if the number of occupied receptors is critical, a G_0-G_1 synchronized cell population with a heterogeneous IL-2 receptor profile would be expected to enter the proliferative phases of the cell cycle asynchronously, as a function of the IL-2 concentration. As a test for this assumption, phytohemagglutinin (PHA)-stimulated human peripheral blood mononuclear cells (PBM) were harvested from culture after 10 days of IL-2–dependent growth. The cells were again stimulated with PHA to ensure maximal receptor expression, cultured without IL-2 for 3 days to allow the accumulation of the cell population in the G_0-G_1 phase of the cell cycle, and then exposed to various concentrations of IL-2. The entrance of the cells into the S phase of the cell cycle was monitored by incorporation of tritiated thymidine ([^{3}H]TdR) and by cytofluorometric analysis of propidium iodide (PI)–DNA binding. As anticipated, the proportion of cells that entered the cell cycle varied with time, depending on the IL-2 concentration (Fig. 2A). Within the population, the asynchronous entry of cells into S phase is reflected by the gradual increase in [^{3}H]TdR incorporation at each IL-2 concentration and by the PI profile of the cells, which 24 hours after exposure to IL-2 is consistent with that of a population in asynchronous growth, comprised of cells exhibiting G_1, S, G_2, and M content of DNA. The IL-2 concentration-dependent effect is discerned by examining a single time interval after the addition of IL-2 (Fig. 2B). A typical sigmoid log-dose response curve resulted when the response of the cell population was monitored either by [^{3}H]TdR incorporation or by PI analysis of the proportion of cells in the replicative phases of the cell cycle. The coincidence

of the curves obtained by the different monitoring techniques also affirms the validity of the interpretation that [^{3}H]TdR incorporation, measured over short intervals, reflects the proportion of cells within the population that are in the replicative phase of the cell cycle.

The capability to synchronize the cell population into G_0-G_1 while simultaneously ensuring maximal IL-2 receptor expression by lectin stimulation also permitted an analysis of the effect of altering the IL-2 receptor exposure time on cell cycle progression. Although IL-2 receptor binding reaches equilibrium within 15 minutes, the results of the previous series of experiments led us to anticipate that the duration of IL-2 receptor interaction could be an important determinant of cell cycle progression. When a G_0-G_1 IL-2 receptor$^+$ population was exposed to 250 p*M* IL-2, a receptor-saturating concentration, for varying intervals, a 3-hour exposure was insufficient to trigger detectable DNA synthesis over the ensuing 26 hours (Fig. 3, inset). In four additional experiments, it was found that a minimum of 5 to 6 hours of IL-2 exposure was essential for triggering detectable cell cycle progression; moreover, exposure times in excess of 6 hours resulted in a progressively greater proportion of cells within the population entering the S phase. In the experiment shown in Fig. 3 (inset), exposure times of 6, 11, and 26 hours resulted in increasingly greater incorporation of [^{3}H]TdR at each interval studied.

If the cell cycle progression were determined by the extent of IL-2 receptor interactions, the IL-2 concentration would also be expected to influence the duration of exposure necessary to promote cell cycle progression. In fact, at 26 hours, 20 percent of the maximal [^{3}H]TdR incorporation occurred when 10 p*M* IL-2 was present for the entire 26-hour culture period, whereas 60 p*M* and 100 p*M* IL-2 were necessary to promote a similar effect when the IL-2 cellular exposure times were limited to 11 or 6 hours, respectively (Fig. 3). Therefore, both IL-2 concentration and the duration of IL-2 cellular interaction are critical determinants of cell cycle progression. There appears to be an interplay between these two variables, such that the proportion of cells responsive to suboptimal concentrations of IL-2 can be increased by lengthening the exposure period.

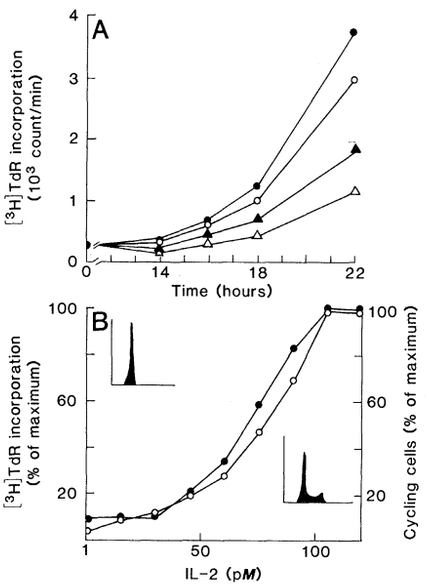

Fig. 2. IL-2 concentration-dependent T-cell cycle progression. (A) PHA-stimulated (72 hours) human PBM were harvested from IL-2–dependent proliferative growth after 10 days of culture, restimulated with PHA (1 μg/ml, 2 hours) to ensure maximal IL-2 receptor expression and cultured without IL-2 for 72 hours to allow for the reaccumulation of the cell population into G_0 or G_1. The synchronized cells were cultured (5×10^4 cells per milliliter) in a humidified atmosphere (5 percent CO_2 in air) in microtiter wells (0.2 ml per well) with the following concentrations of immunoaffinity-purified IL-2 (5); (●) 500 p*M*, (○) 100 p*M*, (▲) 50 p*M*, (△) 25 p*M*. Tritiated thymidine ([^{3}H]TdR (Schwarz/Mann; specific activity, 2.0 μCi/ml) incorporation was monitored during 1-hour intervals (as indicated) by precipitation onto glass fiber filter paper and subsequent liquid scintillation counting. Symbols represent the mean of quadruplicate cultures. The standard errors of the mean in all cultures were < 5 percent. (B) Incorporation of [^{3}H]TdR (●) and the percentage cycling cells (○) from a second experiment performed as described in (A) 24 hours after the addition of IL-2. The data are expressed as percent of maximum [^{3}H]TdR incorporation (50,526 ± 536 count/min per 10^5 cells). The percentage cycling cells was determined by labeling 10^6 cells with propidium iodide (50 μg/ml in 1.12 percent sodium citrate, 0.05 percent Nonidet P-40, and ribonuclease, 100 Kunitz unit/ml) and subsequent analysis for cellular DNA content (Orthocytofluorograph). The relative number of cells in the cycling phases of the cell cycle (S, G_2, and M) was estimated by the Quick Estimate method of cell cycle analysis provided with the Ortho 2150 Data Handler System. (Inset, upper left) Linear plot of DNA content for cells cultured without IL-2. (Inset lower right) Linear plot of DNA content for cells cultured with the maximum IL-2 concentration (250 p*M*). Identical data were obtained from three additional experiments.

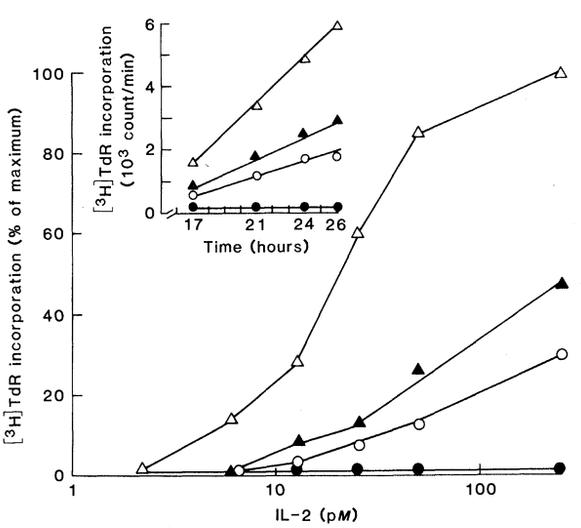

Fig. 3. The effect of varying the IL-2 exposure period on the proliferative response of G_0 or G_1 IL-2 receptor$^+$ T cells. IL-2 receptor$^+$ cells were synchronized (legend to Fig. 2) and exposed to IL-2 concentrations for different time intervals. IL-2 was then removed by washing, and the subsequent progression of the population into the S phase of the cell cycle was monitored by [^{3}H]TdR incorporation (1-hour intervals). The data represent the percent maximum [^{3}H]TdR incorporation after 26 hours of culture plotted as a function of IL-2 concentration after a 3-hour (●), 6-hour (○), 11-hour (▲), and 26-hour (△) exposure to IL-2. The inset shows the [^{3}H]TdR incorporation of each population (that is, 3-, 6-, 11-, and 26-hour exposure periods) in response to an IL-2 receptor saturating IL-2 concentration (250 p*M*) monitored at the times indicated.

IL-2 Receptor Concentration Determines Cell Cycle Progression

The above observations suggested that the absolute number of IL-2 receptor interactions occurring during G_1 was important in the initiation of the biological response. Moreover, as conditions that limit IL-2 receptor interactions modify the proportion of cells that enter the proliferative phases of the cell cycle, the observations were consistent with an underlying heterogeneity within the cell population with regard to the capacity to respond to a given concentration of IL-2. A possible explanation for this differential responsiveness is afforded by the observation that IL-2 receptor density is also heterogeneous and distributed log-normally within the cell population. Therefore it could be anticipated that, when the IL-2 concentration or exposure period is reduced, only cells with relatively high IL-2 receptor levels would be triggered to initiate DNA synthesis. Moreover, under conditions of saturating levels of IL-2, cells with a high IL-2

receptor density would exit from G_1 before cells with relatively low IL-2 receptor densities. Thus, synchronized T-cell populations that differ with respect to IL-2 receptor levels would be expected to enter the S phase of the cell cycle after varying periods of G_1.

In 16 experiments with cell populations that differed with respect to the mean IL-2 receptor density, the duration of G_1 ranged between 10 and 28 hours (mean duration = 18.5 ± 1.0 standard error of the mean) and varied inversely with the number of IL-2 receptors. Moreover, when cells from the same population were examined sequentially (Fig. 4), similar data were obtained. Human PBM cells were cultured for 3 days with lectin, washed and placed in lectin-free and IL-2–free medium. After 8 days more than 95 percent of the cells had accumulated in G_0 or G_1, although the population still expressed relatively high levels of IL-2 receptors (anti-Tac mean fluorescence intensity, 270). The remainder of the cells were harvested from culture after 11 days, at which time the IL-2 receptor density of the population had decreased approximately threefold (anti-Tac mean fluorescence intensity, 85). On addition of 500 p*M* IL-2, the cell population with the higher IL-2 receptor density entered the S phase of the cell cycle 10 hours earlier than the population with lower IL-2 receptor density (Fig. 4).

Cell sorting experiments permitted a direct approach to the issue of the relevance of IL-2 receptor density and cell cycle progression (Table 1). Synchronized IL-2-receptor$^+$ cells were separated into subsets on the basis of low or high anti-Tac fluorescence intensity, washed, and placed into culture with 500 p*M* IL-2. Entrance into the S phase of the cell cycle in each population was monitored at various intervals by [^{3}H]TdR incorporation. The data from the longest interval studied (31 to 48 hours) indicate that the subsets with a high density of IL-2 receptors contained a greater proportion of cells within the S phase of the cell cycle. In a representative experiment, unseparated (Fig. 5A) and separated (Fig. 5, B and C) cell populations were examined at intervals after exposure to a receptor-saturating concentration of IL-2 (250 p*M*) (Fig. 5D). In the high density IL-2 receptor population the proportion of cells in the S phase of the cell cycle was greater than that in the low density IL-2 receptor subset; also, the higher density population appeared to account for most of the [^{3}H]TdR incorporation of the unseparated population. In addition, as would be anticipated from the experiments with unseparated cell populations, the response of the separated cell populations was dependent on IL-2 concentration (Fig. 5E). At limiting IL-2 concentrations (10 to 20 p*M*), only the high IL-2 receptor density cells could be detected in the S phase of the cell cycle. Thus, the log-normal distribution of cellular IL-2 receptor density within a T-cell population is responsible for the normal sigmoid shape of the IL-2 log dose-response curve.

Table 1. The proliferative response of IL-2 receptor$^+$ cell subpopulations separated on the basis of IL-2 receptor density.

Experiment	Culture (hours)	[^{3}H]TdR incorporation*		
		Unseparated	IL-2 receptors	
			Low	High
1	38	10,461	6,555	12,571
2	40	30,852	13,160	39,122
3	40	38,064	4,248	51,344
4	48	19,824	5,556	36,754
5	31	26,120	10,760	59,600

*Synchronized IL-2 receptor$^+$ cells (cultured as described in the legend to Fig. 2) were labeled with anti-Tac (ascites 1:5000, 1 hour, 4°C), and fluorescein isothiocyanate (FITC)–conjugated rabbit antiserum to mouse immunoglobulin (1:20, 1 hour, 4°C). Viable cells were selected for high or low anti-Tac reactivity by means of a cell-sorting facility available with the Orthocytofluorograph. The mean fluorescence intensities of the unseparated cells, low IL-2 receptor subset and high IL-2 receptor subset were 375 ± 44, 162 ± 33, and 605 ± 34 (mean ± standard error of the mean), respectively. After the cells were sorted, unbound anti-Tac was removed from the cells by washing, and the separated and unseparated G_0 or G_1 cell populations were placed into culture in the presence of affinity-purified IL-2 (250 p*M*). At the indicated times [^{3}H]TdR incorporation was determined during a 1-hour period. Data shown represent the mean number of counts per minute per 10^5 cells of quadruplicate cultures. The standard error of the mean of the quadruplicate determinations was <5 percent of the mean.

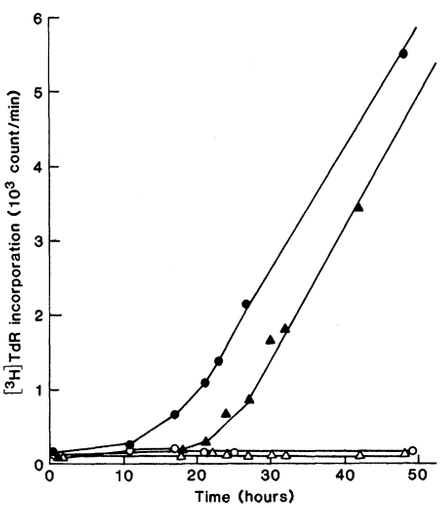

Fig. 4. The effect of IL-2 receptor density on T-cell cycle progression. Human PBM cells were cultured (1×10^6 cells per milliliter) for 72 hours with phytohemagglutinin (PHA) (1 μg/ml), washed, and placed into culture without PHA or IL-2. At day 8 (●) and day 11 (▲), the cells were washed and cultured with a receptor-saturating concentration (250 p*M*) of IL-2. Open symbols indicate cultures without IL-2 addition. Incorporation of [^{3}H]TdR was monitored as indicated at 1-hour intervals. Before the addition of IL-2, portions of each cell population were analyzed for IL-2 receptor expression with flow microfluorometry and anti-Tac (see legend to Fig. 1). A comparison of fluorescence intensity was made by means of the mean channel number for different plots (range 1 to 1000). The mean anti-Tac fluorescence intensity for the cells harvested after 8 days of culture was 270 and that for cells harvested after 11 days was 85. The mean fluorescence obtained with control immunoglobulin was 25 and 5, respectively.

Conclusions

Our study shows that (i) synchronized T cells differ with respect to IL-2 receptor density and (ii) analysis of the key elements that control T-cell growth in relation to IL-2 receptor expression provides an explanation for cell cycle asynchrony within T-cell populations. IL-2–directed T-cell mitosis is a quantal response determined by a critical threshold of signals generated by the interaction between IL-2 and the IL-2 receptor. Thus, T-cell cycle progression is predictable, provided that the variables of IL-2 concentration, IL-2 receptor density, and the duration of the IL-2 receptor interactions are known.

To arrive at this interpretation, it was first necessary to identify that the IL-2 receptor interaction is the key event that signals T-cell cycle progression. In addition to the coincidence of the IL-2 binding and biological response curves (*4, 6*), this conclusion rests on the finding that prevention of the IL-2 receptor interaction completely abrogates T-cell proliferation (*5, 9*). However, the observation that IL-2 receptor density has a log-normal distribution within asynchronous, synchronized, and cloned T-cell populations provided new insight as to a cellular characteristic essential for growth, one which could underlie variability of cell cycle times. Review of the many reports where cell cycle variability has been investigated indicates that previous workers were hampered by a lack of knowledge of the variables that determine cell cycle progression in the systems under study. Moreover, so long as these variables remained hidden, they could not be analyzed quantitatively or qualitatively.

Thus an important issue relating to cell cycle variability is now amenable to analysis. Numerous observations in both prokaryotic (*12, 17, 20, 31*) and eukaryotic systems (*14–17, 19–22, 24–26, 29, 30, 32–34*) indicate that cell populations in exponential growth retain the same

distribution of generation times over many cycles and that there is no selection for more rapidly growing cells. Consequently, there can be no strong mother-daughter correlation with respect to cell cycle transit times. In the T-cell system this leads to the prediction of a lack of a mother-daughter correlation with respect to IL-2 receptor number. Consistent with this prediction, when monoclonal antibodies to anti-Tac or IL-2 were used to detect receptor bound IL-2, cloned lines of murine and human T-cell populations showed the same heterogeneous log-normal distribution of IL-2 receptor expression as observed in polyclonal T-cell populations. These findings can be accounted for only if deterministic differences in IL-2 receptor levels are created at each cell division and modified in each cycle. Moreover, in addition to the inexact partitioning of membrane receptors between the daughter cells, the cellular components responsible for IL-2 receptor synthesis and degradation would also be expected to be distributed unequally. Thus, the importance of these findings resides not only in the logic of the explanation for the lack of selection for more rapidly growing cells over time, but also in the experimental directions provided by the knowledge that the elements in question are the IL-2 receptors and the determinants of IL-2 receptor expression.

The salient information derived from these studies relates to the probability that these concepts can be extrapolated to other cell systems. It is indeed striking that the growth characteristics found for IL-2–dependent T cells are identical to those of all other cells that have been examined, including bacteria (*12, 17, 20*), yeasts (*12, 32, 33*), protozoa (*14, 23*), and all mammalian cells (*15, 16, 19–26, 29, 30, 34*). Accordingly, it could be postulated that the mechanisms operative to signal DNA duplication and cell division are common to all living forms and that the specificity of cell cycle progression, especially in multicellular organisms, is conferred by cell-specific ligands and receptors. With the identification of the variables determining T-cell growth, it should now be possible to proceed beyond the IL-2 receptor interaction to begin to unravel the biochemical and molecular reactions that are critical for cell division. In addition, both normal (*35*) and neoplastic (*36*) IL-2 receptor$^+$ cell populations are available for study. Thus, future experiments designed to understand the intracellular reactions triggered by the IL-2 receptor interaction may yet provide the means for discriminating between the mechanisms underlying normal and neoplastic cell growth.

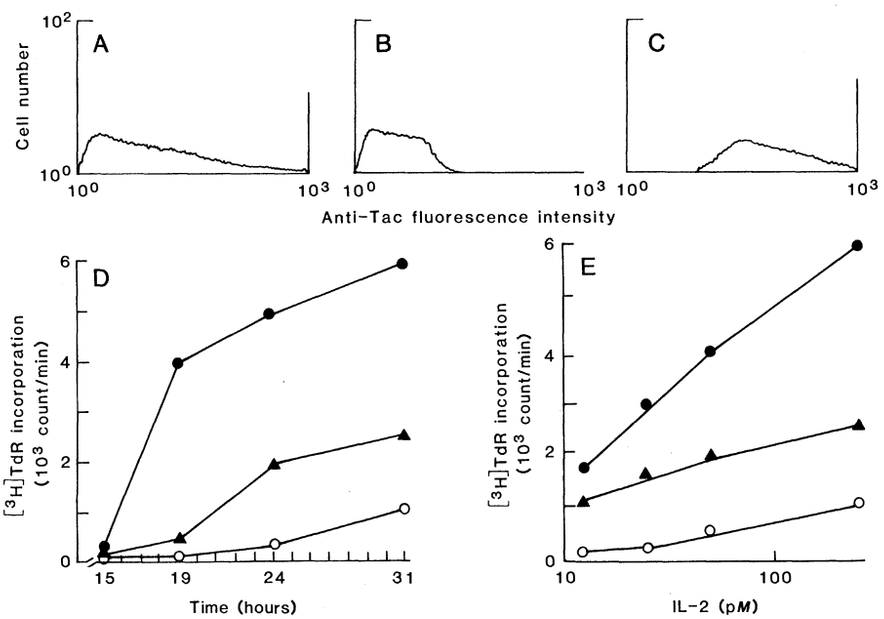

Fig. 5. The proliferative response of IL-2 receptor$^+$ cell subpopulations separated on the basis of IL-2 receptor density. Synchronized IL-2 receptor$^+$ cells (Fig. 2 legend) were labeled with anti-Tac (1:5000 dilution of ascites, 1 hour, 4°C) and rabbit FITC-conjugated antiserum to mouse immunoglobulin (1:20 dilution, 1 hour, 4°C). Viable cells were selected for low or high anti-Tac reactivity (Orthocytofluorograph). Analysis of anti-Tac reactivity of the unseparated cells (A), low anti-Tac reactive subset (B), and high anti-Tac reactive subset (C) is shown in the upper panels. The percentage anti-Tac$^+$ cells in the unseparated population was 97 percent (background labeling with control immunoglobulin, 1 to 3 percent). The mean fluorescence intensity plots shown in (A), (B), and (C) was 344, 158, and 646, respectively. After the cells were sorted, anti-Tac was removed from the cell suspensions by washing, and the separated and unseparated G_0 or G_1 cells were placed into culture in the presence of immunoaffinity-purified IL-2 (0 to 250 p*M*). Residual cell-bound anti-Tac did not prevent IL-2–mediated proliferation: although anti-Tac can block IL-2 receptor binding, antibodies must be present continuously at high concentrations (50 μg/ml; 3.3×10^{-7}*M*) to inhibit IL-2–dependent proliferation. (D) [^{3}H]TdR incorporation in response to receptor saturating concentrations of IL-2 (250 p*M*) of unseparated cells (▲), low anti-Tac reactive cells (○), and high anti-Tac reactive cells (●). (E) [^{3}H]TdR incorporation of unseparated (▲), low (○), and high (●) IL-2 receptor$^+$ cells, plotted as a function of IL-2 concentration after 31 hours of culture.

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