REGULATION OF T CELL AUTOCRINE GROWTH.

T4+ Cells Become Refractory to Interleukin 2

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IL-2 is responsible for T cell proliferation after activation of the T cell antigen receptor complex (1-4). Detailed studies using freshly isolated lymphocytes and functional T cell clones have led to the realization that T cell clonal expansion is a complex phenomenon, based on both autocrine and paracrine mechanisms involving the IL-2-R system (5-7). All T cells respond to antigen activation by the expression of high-affinity, IL-2-specific membrane receptors (8-10), whereas only a proportion of antigen-stimulated T cells are triggered to produce detectable amounts of IL-2. Most T helper cells expressing the T4 surface glycoprotein (11, 12) are capable of proliferating in an autocrine fashion in response to the IL-2 that they themselves produce (3, 8-10). In contrast, the great majority of T cells of the cytolytic subclass, which express the T8 surface glycoprotein (11, 12), proliferate in a paracrine fashion, in response to the IL-2 produced by T4+ helper T cells (3, 8-10).

In view of the understanding that autocrine and paracrine IL-2-R systems are responsible for T cell clonal expansion, feedback regulatory controls surely are operative for limiting IL-2 responsiveness, thereby ensuring that autonomous cell proliferation does not persist after antigen stimulation, and result in uncontrolled neoplastic cell growth. One facet of the IL-2-R operational mechanism that serves to restrict uncontrolled autonomous growth, and which may be unique to the immune system, relates to the antigen-dependency of the response: IL-2-R expression is transient, and quite dependent on antigen, so that upon its removal, IL-2-R decline gradually and eventually disappear (5). As a consequence, the proliferative rate of the cell population diminishes, and eventually all of the cells reaccumulate in the resting (G0) phase of the cell cycle. Another mechanism that facilitates the disappearance of IL-2-R was recently revealed via IL-2-R turnover studies. In effect, IL-2 itself regulates its growth-promoting effects by a novel mechanism; one that leads to an IL-2-promoted accelerated disappearance of biologically relevant, high-affinity IL-2-R, and their replacement by very low-affinity IL-2 binding sites that do not mediate T cell cycle progression (13). Consequently, the removal of antigen and the IL-2-promoted
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switch from high- to low-affinity IL-2-R both operate to restrain T cell clonal expansion via the regulation of IL-2-R expression.

Soon after the discovery that lymphocyte-conditioned medium supports the long-term growth of human T cells (1), it was found that antigen-specific cytolytic T cells could be selected (2) and cloned (14) using similar methods. A high proportion of clones (80%) proved to be of the cytolytic subclass (14). Although cytolytic T cells could have been selected by the conditions used for their growth and clonal isolation, the mechanism responsible for this phenomenon and the immunologic or biologic significance attributable to the observation remained obscure. However, in the course of recent experiments exploring the regulation of IL-2-R expression by human peripheral T cells, we found that T8⁺ cells also predominate after only a short-term (14-d) IL-2-dependent expansion culture. This observation was reminiscent of the high frequency of cytolytic T cell clones isolated after long-term culture in lymphocyte-conditioned medium. As we did not expect that T4⁺ cells could differ from T8⁺ cells with respect to the IL-2-R system, we designed several experimental approaches to characterize the phenomenon.

Using freshly isolated human peripheral mononuclear cells stimulated polyclonally with anti-T3 and cultured using homogeneous immunoaffinity-purified IL-2, we found that T4⁺ cells became refractory to the growth-promoting effects of IL-2 even though T8⁺ cells continued to proliferate. Since the loss of T4⁺ cell IL-2 responsiveness was not attributable to suppression by T8⁺ cells, nor to the number, affinity, or recognizable functional capacities of IL-2-R expressed, it appears likely that a unique mechanism renders T4⁺ cells unresponsive to IL-2, thereby curtailing IL-2-dependent clonal expansion of T4⁺ cells. Accordingly, three mechanisms are now identifiable that inevitably make for the cessation of T4⁺ cell autocrine growth: two are mediated via the control of IL-2-R expression, whereas the third level of regulation is IL-2-R-independent and restricted to T4⁺ cells.

Materials and Methods

**Antibodies and Reagents.** Anti-T4a (19 Thy 507 [IgG2]) and anti-T8c (7Pt 3F9 [IgG₂]) (15) were kindly provided by Dr. Ellis Reinherz (Dana-Farber Cancer Institute, Boston, MA). OKT-3 was purchased from Ortho Pharmaceutical Corp., Raritan, NJ. Phorbol-12,13-dibutyrate (Pdbu) (Consolidated Midland Corp., Brewster, NY) was used in some experiments to reactivate IL-2-R expression.

**Cell Cultures.** Human peripheral mononuclear cells (PMC), isolated by Ficoll-IvPaque discontinuous gradient centrifugation, were cultured in a humidified atmosphere of 5% CO₂ in air in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated (56°C, 30 min) FCS (Sterile Systems, Inc., Logan, UT), 50 U/ml penicillin, 50 μg/ml gentamicin, 200 μg/ml L-glutamine, 5 μg/ml insulin, 6 μg/ml transferrin, and 5 ng/ml selenious acid (ITS; Collaborative Research, Inc., Lexington, MA).

The murine IL-2-dependent cytolytic T lymphocyte line CTLL-2 (subclone 15G) was maintained in Iscove's modified DMEM as described previously (14).

**Selective Depletion of T Cell Subpopulations by mAb plus Complement.** To separate PMC into individual subpopulations, cells were resuspended (10⁷ cells/ml) in anti-T4a or anti-

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**Abbreviations used in this paper:** ATL, adult T cell leukemia; HTLV, human T lymphotrophic virus; Pdbu, phorbol-12,13-dibutyrate; PI, propidium iodide; PMC, peripheral mononuclear cell.
T8c at a 1:800 dilution of ascites fluid, and incubated, with constant rotation, at room temperature for 1.5 h. Subsequently, the cells were sedimented by centrifugation. As a source of complement, Low-Tox-M rabbit complement (1 ml, diluted 1:3; Cedarlane Laboratories, Ltd., Hornby, Ontario, Canada) was added, and cells were incubated for 1 h at 37°C. Viable cells were isolated by Ficoll-Hypaque discontinuous gradient centrifugation, and purity was determined by cytofluorographic analysis. The viable T4* cell populations contained <5% T8* cells, and T8* cell populations manifested <4% T4* cells. After 72 h of culture, the cells were routinely reexposed to antibody and complement as described here; after this second treatment, contaminating cells of the respective subpopulations were no longer evident.

Production and Preparation of Affinity-purified IL-2. Jurkat subclone 6.8 was induced to produce IL-2 as described previously (16, 17). Unlabeled and biosynthetically radiolabeled [3H]leu,lys-IL-2 were purified from culture supernatants by a one-step purification protocol using an immunoaffinity column constructed with an IgG2a mAb reactive with IL-2 (designated DMS-3), described in detail elsewhere (16, 17). The eluted material was comprised of a single protein (M, 15,500), as determined by SDS-PAGE, reverse-phase liquid chromatography, and amino-terminal amino acid sequence analysis (17).

The specific activity of radiolabeled IL-2 was determined by correlating radioactivity with biologic activity (18). Biologic activity and protein mass were determined on homogeneous, IL-2, kindly supplied by Dr. K. Kato, Takeda Chemical Industries, Ltd., Osaka, Japan (19). Using this preparation as a standard, previous calculations (16) of protein concentration and biologic activity were found to overestimated fourfold. The specific activity of [3H]leu,lys-IL-2 was calculated as 377,600 dpm/pmol.

IL-2 Biological Activity. IL-2 activity was determined as previously described (18), by the IL-2 concentration-dependent stimulation of proliferation of a cloned CTLL (14). CTLL proliferation, monitored by [3H]TdR incorporation (2.0 #Ci/ml; sp act, 1.9 Ci/mM; Schwartz/Mann Div., Becton-Dickinson and Co., Orangeburg, NY), was determined during the last 4 h of a 24-h culture period in the presence of serial twofold dilutions of the standard r IL-2 preparation (19) and the experimental sample. The dilutions that yielded 50% of maximal CTLL [3H]TdR incorporation were determined by probit analysis; dilution values of the samples were divided by that of the standard to provide the concentration of IL-2 in picomoles per liter. The standard IL-2 preparation routinely yielded 50% of maximal [3H]TdR incorporation at 6.25 pM.

Radiolabeled IL-2 Binding Assay. Radiolabeled IL-2 binding to whole cells was performed as described in detail previously (16). All cells were prepared for the assay by centrifugation, followed by incubation at 37°C in IL-2-free RPMI 1640 medium for two 1-h intervals, to facilitate dissociation and/or degradation of endogenously bound IL-2. These conditions were based upon the dissociation rate constant determined previously for intact cells and isolated plasma membranes (t0 for dissociation is 25 min) (16, 20). Serial dilutions of [3H]leu,lys-IL-2 were incubated with cells (10⁶ cells in 0.2 ml) in RPMI 1640 medium supplemented with 10% FCS at 37°C. After a 20-min incubation, cold (0°C) RPMI 1640–FCS (1 ml) was added, and the cells were centrifuged. The supernatant containing the unbound fraction was removed and counted via liquid scintillation. The cell pellet was resuspended in 0.1 ml cold RPMI and centrifuged through a 0.2-ml layer of a mixture of 85% silicone oil (Dexter Hysol 550 fluid; Dexter Corp., Orlean, NY) and 15% paraffin oil (0-119; Fisher Scientific Co., Fair Lawn, NJ). The tips of the tubes containing the cell pellet were cut off and counted by liquid scintillation to determine the level of bound radioactivity. The calculated values of the number of binding sites per cell were obtained by Scatchard analysis of equilibrium binding data, after subtraction of the nonspecific binding determined in the presence of 150-fold molar excess of unlabeled IL-2. The lower limit of detection of receptor sites per cell was 50.

Flow Cytfluorometry. Expression of T8 and T4 cell surface antigens was analyzed by flow cytfluorometry. Cells (10⁶ cells/ml) were incubated (1 h, 4°C) with saturating concentrations of mAb, washed three times, and then incubated with a 1:40 dilution of fluorescein-conjugated rabbit anti–mouse Ig (Dakopatts; Accurate Chemical and Scientific Corp., Westbury, NY). Samples were passed on an Ortho cytfluorograph (system 50H;
Ortho Diagnostic Systems, Inc., Westwood, MA) using an argon ion laser (Spectra-Physics, Inc., Mountain View, CA), at 488 nm excitation wavelength. Green fluorescence was observed using a 630-nm long-pass filter. A minimum of 10,000 cells, gated to exclude nonviable cells, was accumulated for each histogram. All data were analyzed using the Ortho 2150 Data Handler System (Ortho Diagnostic Systems, Inc.), and the percentage of positive cells was calculated against a background of nonspecific labeling obtained using normal mouse IgG (1–3%).

For analysis of the cellular DNA content of cells, 10⁶ cells were labeled with the DNA-binding dye, propidium iodide (PI) (50 μg/ml; Calbiochem-Boehringer Corp., La Jolla, CA) in the presence of 100 Kunitz U/ml RNase (Sigma Chemical Co.), 1.12% sodium citrate, and 0.05% Nonidet P-40. The percentage of cells in the G₀/G₁ phase of the cell cycle was estimated using the quick-estimate method of cell cycle analysis provided with the 2150 Data Handler software.

[^3H]TdR Incorporation. The proportion of S-phase cells in various cell populations was estimated by monitoring[^3H]TdR incorporation during 3-h culture intervals. Cultures were terminated by precipitation onto glass fiber filters, and were counted by liquid scintillation. Data are expressed as cpm[^3H]TdR incorporated by 10⁴ cells placed in culture.

IL-2 Metabolism. Cell-mediated degradation of IL-2 was determined by allowing[^3H]-leu,lys-IL-2 (75 pM) to bind to cells (8 x 10⁵), and TCA (10%)-soluble and -precipitable radiolabel was determined at the intervals indicated. TCA-soluble radiolabel present at the initiation of the assays (2–5%) was subtracted from those values obtained thereafter.

Results

Growth Characteristics of T⁴⁺ and T⁸⁺ T Cell Subsets. IL-2-dependent growth of human PMC was promoted during a 72-h culture period with anti-T₃, thereby initiating IL-2 production and IL-2-R expression in situ. The cells were then washed and subcultured in 125 pM IL-2, a concentration in fivefold excess of that required to saturate high-affinity IL-2-R. Growth was maintained for 10–12 d by replenishment of IL-2 at 2-d intervals, when the cell concentration was reduced 10-fold to prevent the IL-2 concentration from becoming growth-limiting. Periodically, cell aliquots were removed and examined for expression of T⁴ and T⁸ membrane molecules by immunocytofluorescence using T cell subset-specific mAb. As illustrated in Fig. 1, there is a striking reversal of the

![Figure 1. T⁴/T⁸ Phenotypic analysis during IL-2-dependent growth. PMC activated with anti-T₃ (0.5 ng/ml) for 72 h were cultured at 2-10 x 10⁵ cells/ml in the presence of a saturating concentration of affinity-purified IL-2 (125 pM; replaced where indicated by arrows). The percentage of T⁴⁺ (■) and T⁸⁺ (▲) cells was determined by cytofluorographic analysis. Data shown represent the mean ± SEM of three separate experiments.](image-url)
T4+/T8+ ratio during a 12-d culture period, such that between day 5 and day 12, T8+ cells become predominant, and eventually comprise >80% of the cultured cells.

To explore the possibility that T8+ cells actively suppress the growth of T4+ cells, the two subsets were cultured separately, using identical culture conditions. Homogeneous T4+ and T8+ subpopulations were selected by antibody-dependent complement-mediated lysis using anti-T8 and anti-T4 at the start of culture, and again after 3 d. The cells were then maintained as before in purified IL-2, and the viable-cell numbers and cell surface phenotypes were monitored at regular intervals. A cumulative growth curve of unseparated cells and separated T4+ and T8+ subpopulations (Fig. 2) is especially illuminating, since it shows that isolated T4+ cells cease proliferation after 8 d of culture, whereas T8+ cells continue to expand exponentially for at least 3 d more. Consequently, after 13 d of culture, 4.0 × 10^6 T8+ cells accumulated, while T4+ cells had expanded only to about 6.5 × 10^7. The actual increases in cell numbers that occurred between day 3 and day 13 were 125-fold (unseparated cells), 106-fold (T8+ cells), and 27-fold (T4+ cells). Consistent with these data, quantitative determination of cellular DNA content, using PI and cytofluorometric analysis (Fig. 3) revealed that by day 9, 90% of T4+ cells had already reaccumulated in the G0/G1 phase of the cell cycle, whereas a significant number of T8+ cells continued to cycle until day 13 of culture. Moreover, the earlier cessation of proliferation among the T4+ cells compared to T8+ cells was reflected by a more rapid decline in [3H]TdT incorporation, also evident in Fig. 3. Accordingly, the premature cessation of T4+ cell proliferation compared to T8+ cell proliferation cannot be attributed to any suppressive influence emanating from T8+ cells.

**IL-2-R Expression by T4+ vs. T8+ Cells.** Results from our previous studies (6) using unseparated T cells indicated that three variables determine the rate of T cell cycle progression; IL-2 concentration, IL-2-R density, and the duration of the IL-2-IL-2-R interaction. Since the IL-2 concentration and the duration of exposure were identical in the experiments described on the isolated subsets, it

![Figure 2](image-url)
appeared likely that the premature cessation of T4+ cell growth was likely to be attributable either to quantitative or qualitative differences in the IL-2-R expressed by T4+ vs. T8+ cells. However, upon direct examination throughout the 12-d culture period, no differences were found in the number of high-affinity IL-2-R expressed by T4+ and T8+ cells (Fig. 4). Both subsets express IL-2 binding sites asynchronously and transiently after polyclonal anti-T3 activation, so that the kinetics of IL-2-R appearance and disappearance for each subset were identical to those observed previously (5) for unseparated T cells activated with PHA.

To examine the cell subpopulations for differences in the affinity of the receptors expressed and for the receptors' functional capacity to mediate a proliferative response, cells were harvested from IL-2-dependent culture after 5 d just before the cessation of T4+ cell growth. Essentially identical radiolabeled IL-2 equilibrium binding curves were obtained, yielding superimposable Scatchard plots (Fig. 5A). Moreover, the IL-2-R expressed by the separated subsets conveyed the IL-2 proliferative signal with equal efficiency, as evidenced by the symmetrical sigmoid log-dose/response to varying concentrations of IL-2 (Fig. 5B). Thus, just before the time when T4+ cells cease to proliferate in response
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Figure 5. IL-2-R expression and IL-2 biologic response of T4/T8 subsets determined after 5 d of culture. (A) Equilibrium [3H]leu,lys–IL-2 binding to T4+ cells (●) and T8+ cells (▲). Inset: Scatchard plot. (B) Proliferative response of unseparated cells (●), T4+ cells (■), and T8+ cells (▲) 24 h after culture (10^5 cells/ml) with varying concentrations of immunoaffinity-purified IL-2.

to IL-2, the number, affinity, and functional response attributed to IL-2-R are identical to those of T8+ cells, which continue to proliferate.

IL-2 Metabolism. In previous experiments, we (21) and others (22, 23) had shown that cells expressing biologically active IL-2-R functioned to remove IL-2 from culture medium in a time-, temperature-, and cell concentration–dependent fashion. Subsequent findings (Cantrell, D. A., and K. A. Smith, manuscript in preparation) have shown that the depletion of IL-2 activity from the culture medium is due to a receptor-mediated internalization and degradation of IL-2 molecules that bind to the cells. Since T4+ cells ceased to respond to IL-2 before T8+ cells, the rate of degradation of radiolabeled IL-2 by the separated subpopulations was assessed. The cell-mediated conversion of [3H]leu,lys–IL-2 (75 pM) into a TCA-soluble form was analyzed with both subsets in exponential growth (day 4), and when the two subpopulations differed in their proliferative response to IL-2 (day 8 and day 10 of culture). The results, depicted in Fig. 6, attest to an absence of any difference in the kinetics of IL-2 degradation mediated by T4+ vs. T8+ cells, although IL-2 degradation diminished as the culture intervals lengthened, and the mean density of high-affinity IL-2 binding sites decreased.

The rate of radiolabeled IL-2 degradation was calculated from the data shown in Fig. 6, and is given in Table I together with the number of IL-2-R/cell and the IL-2-dependent proliferative response of the separated cell populations for each time point monitored. Despite equivalent numbers of IL-2-R and equivalent
IL-2 degradation rates for the two separate subsets at each time point, by day 8 of culture, T4+ cell $[^3H]$TdR incorporation had decreased to 50% that of the T8+ cells, and by day 10, T4+ cell proliferation was 40% of that of the T8+ population.

$T4^+$ Cells Are Refractory to IL-2 Despite Adequate IL-2-R. The premature loss of IL-2 responsiveness by T4+ cells compared with T8+ cells after the primary activation with anti-T3 suggested that T4+ cells became refractory to continued IL-2 effects, and that this state was unrelated to IL-2-R interaction or receptor-mediated IL-2 internalization and metabolism. To substantiate this interpretation, and to investigate whether this refractory state could be overcome by reactivation of the T cell antigen receptor complex, T4+ and T8+ cell subpopulations were activated with anti-T3 and cultured with IL-2 for 14 d, as before. Subsequently, stimulation of IL-2-R reexpression was accomplished by a 6-h exposure to Pdbu in the absence of accessory cells. As shown in Fig. 7 A, 20 h after Pdbu exposure, T4+ cells and T8+ cells reexpressed equivalent levels of high-affinity IL-2-R. However, as is evident in Fig. 7 B, only T8+ cells proliferated in response to IL-2. Despite continued viability throughout the succeeding 8
Discussion

Our results show that T4+ human T cells differ substantially from T8+ cells with respect to their IL-2 responsiveness. T4+ cells cease to proliferate well before T8+ cells during a primary response; moreover, upon restimulation of IL-2-R, T4+ cells are still refractory to growth-promotion by IL-2. As no differences have been discerned between T4+ and T8+ cells in the rate, extent, or affinity of the IL-2-R expressed after a primary or secondary in vitro stimulation, the results suggest that T4+ cells become refractory to IL-2 at some point after IL-2–IL-2-R interaction.

The various experimental approaches used here focused on the expression of high-affinity (Kₐ, 5 pM), biologically relevant IL-2-R. We found previously (13) that IL-2 induces the expression of low-affinity (Kₐ, 5 nM) IL-2 binding sites via interaction with high-affinity IL-2-R. However, the functional relevance of the IL-2-induced low-affinity binding sites still remains obscure, since saturation of these sites by raising the IL-2 concentration 1,000-fold neither enhances nor retards the magnitude of the proliferative response observed when only the high-affinity IL-2-R are saturated (13, and other unpublished observations).

To compare the IL-2-promoted proliferative response of T4+ cells with T8+ cells, unfractionated peripheral mononuclear cells were chosen deliberately so as to avoid removing accessory cells required for optimal activation of the T cell antigen receptor complex. Thus, the in vitro cell population should have reflected, as closely as possible, the cells that are likely to encounter antigen in vivo. Moreover, anti-T3 was selected as the initial activating ligand since it is now known (24, 25) that antigens activate T cells via interaction with a 90 kD
disulfide-linked heterodimer noncovalently associated with three smaller proteins on the cell surface, the Ti-T3 complex. By using mAb reactive specifically with T3, we hoped to activate all mature T4+ and T8+ cells and at the same time avoid stimulation of other surface structures that might affect the resulting IL-2-promoted response. Consequently, it is especially noteworthy that both T4+ and T8+ cells express high-affinity IL-2-R at an equal rate, and, initially, proliferate in an identical fashion in response to IL-2 in situ, whether the two subsets have been cultured together or separately.

Upon removal of anti-T3 and maintenance of the cells in homogeneous IL-2, the levels of high-affinity IL-2-R declined progressively, as reported previously (5) for cells activated initially with PHA. However, despite identical rates of IL-2-R decay and equivalent rates of IL-2 degradation mediated by T4+ vs. T8+ cell populations, after 6 d in culture, T4+ cell proliferation decelerated more rapidly than did T8+ cell proliferation. These results are particularly important because the IL-2-R levels at days 5 and 6 had decreased only to ~50% of the maximum levels detected on day 3 of culture. Thus, the decline of IL-2 responsiveness of T8+ cells follows, and is actually attributable to the disappearance of IL-2-R, whereas T4+ cells cease proliferation well before the IL-2-R decline to low levels.

Although the mechanism of the T4+ cells' IL-2 refractoriness remains uncertain, it is clear that T4+ cells do not respond to IL-2 for some time after their initial response. Indeed, they remain refractory to IL-2 even after a secondary restimulation of IL-2-R expression. It bears emphasis that adherent non-T cells are lost progressively during the initial 14-d culture period while T cells proliferate exponentially and the cells are passed to new culture flasks. Consequently, by day 14, >99% of the cells are T cells. Although the progressive diminution of adherent non-T cells does not account for the observed differences in IL-2 responsiveness of T4+ cells compared with T8+ cells, it is conceivable that T4+ cells do require accessory cells for optimal IL-2 responsiveness, whereas T8+ cells do not. In previous studies we (26) and others (27, 28) have noted that immune T helper cells require accessory cells for optimal IL-2-dependent proliferative expansion and clonal isolation. In this respect, it may be significant that IL-2-R reexpression could be achieved even in the absence of accessory cells, especially in view of reports (29) suggesting that accessory cells or their products (IL-1) are necessary for IL-2-R expression, and for acquisition of the IL-2-responsive state (29). Rather, from our present findings, it seems likely that accessory cells contribute to IL-2 responsiveness of T4+ cells not by facilitating expression of IL-2-R, but instead by promoting a cellular change necessary for activated IL-2-R to transmit signals required for progression through the cell cycle to DNA replication and mitosis. In that event, during an in vivo, antigen-initiated, IL-2-dependent T cell proliferative response, the presence of a fixed number of accessory cells would indeed limit the extent of T4+ cell clonal expansion in a manner consistent with our observations in vitro.

In considering further alternative mechanisms to account for T4+ cell IL-2 unresponsiveness, it is conceivable that physiologic inhibitory feedback signals are generated via the IL-2-IL-2-R interaction itself. At present, we can only speculate on the molecular pathways that could function to extinguish the signals
that promote DNA replication. However, we can categorize inhibitory mechanisms as those which operate through preexisting biochemical pathways, or those requiring specific gene expression. Regarding the latter, we have already shown that high-affinity IL-2-R induce the expression of specific genes. For example, the mechanism whereby IL-2 downregulates the expression of high-affinity IL-2-R involves the induction of mRNA encoding low-affinity IL-2 binding proteins (13, 30–32). Moreover, our recent studies (33) have shown that IL-2-promoted T cell cycle progression is preceded by the transient expression of the cellular protooncogene, c-myb. It will be interesting to compare to IL-2-induced gene expression in T4+ vs. T8+ cells, as this would allow us to determine whether an expressed gene promotes or inhibits subsequent DNA replication.

The difference between T4+ cells and T8+ cells revealed herein must be interpreted in the context of its significance for the development of an effective T cell immune response. Within T cell populations, T4+ cells are the primary source of IL-2 after antigen activation (11). Consequently, the finding that T4+ cell clonal expansion is only ~25% of the proliferative expansion achieved by T8+ cells indicates that IL-2 itself is the major parameter retarding clonal expansion within the total cell population; especially because the rate of T cell proliferation is directly dependent on the amount of IL-2 available to the cells. Thus, the immunologic relevance of the T4+ cell IL-2 refractory state resides in its potential as a feedback regulatory control retarding the extent and duration of IL-2-dependent T4+ and T8+ cell clonal expansion, ensuring that IL-2-producing cells ultimately will become limiting. This interpretation is entirely consistent with our previous observations (18, 21), which indicate that IL-2 rapidly disappears from the culture medium of stimulated T cells. Actually, the premature development of T4+ cell IL-2-unresponsiveness would have gone undetected had we not ensured experimentally that IL-2 could not become depleted in our culture system. Thus, two mechanisms contribute to the depletion of IL-2 from the medium of rapidly proliferating T cells: IL-2 internalization and degradation by responding cells, and cessation of T4+ (IL-2 producer) cell proliferative expansion.

These results may have still further significance in view of the T4+ cell tropism of the human T lymphotrophic viruses (HTLV). Although both HTLV-I/II (34, 35) and HTLV-III (36, 37) can enter non-T4+ cells and integrate as proviral DNA, the pathologic effects of both types of viruses result primarily from their infection of T4+ cells. HTLV-I, the etiologic agent of adult T cell leukemia (ATL) (38, 39), transforms T4+ cells preferentially. Cell lines derived from ATL patients proliferate independently of added IL-2 and continuously express an abnormally high density of IL-2-R (40). It is possible that the neoplastic characteristics of ATL cells result from the abnormally high constitutive expression of IL-2-R. In addition, ATL cell lines may have lost the normal regulatory controls that ensure transient, antigen-dependent expression of IL-2-R, as well as the IL-2 refractory state of T4+ cells described herein.

The dichotomy between T4+ cells and T8+ cells manifested by an IL-2 refractory state may also be involved in the mechanism of the cytopathic effects of HTLV-III, the etiologic agent of the acquired immunodeficiency syndrome (AIDS). Since the growth characteristics of T4+ cells are clearly different from
T8+ cells, additional studies directed at the mechanism of the IL-2 refractory state are surely warranted. Then too, since accessory cells could also be important determinants of the duration of T4+ cell IL-2-responsiveness, the finding that macrophages are easily infected by HTLV-III (Z. Salahuddin and R. Gallo, personal communication) enhances the significance of our observations. Accordingly, it may be of considerable relevance that upon secondary stimulation T4+ cells express IL-2-R, yet remain refractory to IL-2. IL-2-R+, IL-2-unresponsive T4+ cells provide a unique target cell system for studying the mechanisms responsible for the IL-2 refractory state and its immunobiologic consequences.

Summary
During the course of investigating the regulation of IL-2-dependent T cell proliferation, we found that the subset of human T cells expressing the T4 surface glycoprotein become refractory to IL-2 growth promotion earlier than T8+ cells. Since T4+ cells proliferate in an autocrine fashion to endogenous IL-2, whereas most T8+ cells respond in a paracrine fashion to IL-2 derived from T4+ cells, we thought it likely that a unique mechanism was operative to restrict T4+ cell IL-2-dependent autocrine proliferation. Moreover, we anticipated that the T4+ cell IL-2-refractory state related either to suppression by T8+ cells, or to expression of T4+ cell IL-2-R. However, several experimental approaches did not support either of these mechanisms as being responsible for the loss of T4+ cell IL-2 responsiveness. Isolated T4+ cells ceased to respond to IL-2 well before T8+ cells, and before the disappearance of adequate levels of IL-2-R. Moreover, a detailed comparison of IL-2-R expression by T4+ vs. T8+ cells revealed no differences in the number, affinity, rate of expression, or functional activity of high-affinity IL-2-R expressed by the two subsets. Accordingly, T4+ cell autocrine IL-2 responsiveness is restricted by a mechanism that is independent of IL-2-R, and which ultimately results in cessation of both T4+ and T8+ cell IL-2-dependent clonal expansion.

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