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Triggering of the T3–Ti antigen–receptor complex results in clonal T-cell proliferation through an interleukin 2-dependent autocrine pathway

(T-cell clones/monoclonal antibodies/interleukin 2 receptor/growth regulation)

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ABSTRACT **Human T-cell clones and anti-T-cell-receptor antibodies (clonotypic) directed at surface receptors for antigen (T3–Ti molecular complex) as well as anti-interleukin 2 (IL-2) and anti-IL-2-receptor antibodies were utilized to investigate the mechanism by which alloantigens or antigen plus self-major histocompatibility complex (MHC) (i.e., physiologic ligand) trigger specific clonal proliferation. Soluble or Sepharose-bound anti-Ti monoclonal antibodies, like physiologic ligand, enhanced proliferative responses to purified IL-2 by inducing a 6-fold increase in surface IL-2 receptor expression. In contrast, only Sepharose-bound anti-Ti or physiologic ligand triggered endogenous clonal IL-2 production and resulted in subsequent proliferation. The latter was blocked by antibodies directed at either the IL-2 receptor or IL-2 itself. These results suggest that induction of IL-2 receptor expression but not IL-2 release occurs in the absence of T3–Ti receptor cross-linking. Perhaps more importantly, the findings demonstrate that antigen-induced proliferation is mediated through an autocrine pathway involving endogenous IL-2 production, release, and subsequent binding to IL-2 receptors.**

Recent studies using cloned antigen-specific T lymphocytes and monoclonal antibodies directed at their various surface glycoprotein components have led to identification of the human T-cell-antigen receptor as a surface complex comprised of a clonotypic 90-kilodalton (kDa) Ti heterodimer and the monomorphic 20/25-kDa T3 molecule (1, 2). Upon binding to the surface of a clone, monoclonal antibodies to either T3 or its associated unique Ti heterodimer induced rapid loss of the T3–Ti complex, a process termed modulation, and inhibited all antigen-specific functions of a given clone. However, at the same time, these antibodies produced a markedly enhanced clonal proliferative response to interleukin 2 (IL-2)-containing media (1–3). Moreover, when coupled to the surface of a solid support such as Sepharose, the appropriate surface-linked anti-Ti and anti-T3 antibodies were able to induce IL-2 secretion and clonal proliferation, analogous to physiologic ligand (i.e., antigen) itself (4). These findings suggested that clonal proliferation resulting from triggering of the T3–Ti antigen receptor might be mediated through the growth factor IL-2. The latter is a 15-kDa sialoglycoprotein that interacts with activated T cells through specific membrane receptors distinct from the T3–Ti complex and is known to induce T-cell growth upon surface binding (5–12). The present study was performed to examine the relationship between the T-cell-antigen receptor and IL-2-mediated T-cell growth.

MATERIALS AND METHODS

Human T-Cell Clones. The human T-cell clones $CT8_{III}$, $CT4_{II}$, and RW17C were obtained from a single donor's peripheral blood mononuclear cells, cloned, and maintained in culture as described (13, 14). Briefly, the cytotoxic clones $CT8_{III}$ and $CT4_{II}$ have a target specificity for class I and class II alloantigens, respectively, both of which are present on the Epstein–Barr virus-transformed human lymphoblastoid B cell line Laz 156 (1, 2, 14, 15). In contrast, RW17C recognizes ragweed antigen E (RWAGE) in the context of an autologous class II major histocompatibility complex (MHC) gene product. Moreover, RW17C provides potent help for B-cell IgG secretion (16).

Derivation of Monoclonal Antibodies and Surface Expression of T-Cell Antigens. The clone-specific murine monoclonal antibodies anti-Ti_1, anti-Ti_2, and anti-Ti_4 and the anti-T3 monoclonal antibodies were obtained from immunizations with individual T-cell clones as described (1, 2, 16, 17). The antigens defined by the various anti-Ti antibodies appear as clonally unique 90-kDa disulfide heterodimers consisting of 41–43 β and 49–53 α chains (anti-Ti_1, clone $CT8_{III}$; anti-Ti_2, clone $CT4_{II}$; and anti-Ti_4, clone RW17C) that are associated with the monomorphic T3 structure. These monoclonal antibodies are not crossreactive. Anti-T3, -T4, -T11, and -Ia monoclonal antibodies have been described (18–23). Monoclonal antibodies to the IL-2 receptor, termed anti-Tac, and to human IL-2, termed DMS-1, were produced as described (9, 10, 12).

The reactivity of clonal populations with individual monoclonal antibodies was determined by means of indirect immunofluorescence on an Epics V cell sorter (Coulter) (23), and the number of binding sites was quantitated by flow cytometry as described (2, 24). Modulation studies were performed as described (1).

Purification and Surface Coupling of Monoclonal Antibodies. Anti-Ti_1, anti-Ti_2, and anti-Ti_4 were individually purified from malignant ascites by using protein A-Sepharose (Pharmacia) (25). In each case, 5 mg of purified antibody was coupled to 1 ml of swollen CNBr-activated Sepharose 4B beads. Functional integrity of the surface-bound monoclonal antibodies was confirmed by immunoprecipitations from ^{125}I-surface-labeled T-cell clones.

Proliferative Assays. Proliferative *in vitro* assays were performed in triplicate in a standard culture system with 96-well round-bottom microtiter plates (Costar, Cambridge, MA). In the case of $CT4_{II}$ and $CT8_{III}$, 3×10^4 cloned cells were incubated with 3×10^4 irradiated Laz 156 cells, medium, or 0.5

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Abbreviations: MHC, major histocompatibility complex; IL-2, interleukin 2; kDa, kilodalton; RWAGE, ragweed antigen E; APC, antigen-presenting cells.

units of affinity-purified human IL-2 per ml. The homogeneity of the latter preparation was demonstrated by the fact that it appeared as a single protein on $NaDodSO_4$/PAGE and reverse-phase liquid chromatography (12). RW17C was incubated at 3×10^4 cells per well together with autologous antigen-presenting cells (APC; 5×10^3 per well) and RWAGE (Worthington) (10 μg/ml) or medium or human IL-2 (0.5 unit/ml). After 24 hr, the various cultures were individually pulsed with 1 μCi (1 Ci = 37 GBq) of [^{3}H]Thd (Schwartz/Mann), harvested 18 hr later on a MASH II apparatus (M. A. Bioproducts, Walkersville, MD), and subsequently measured in a Packard scintillation spectrometer. Standard deviations in these experiments were <18%.

Determination of IL-2 Secretion by T-Cell Clones. To quantitate the amount of IL-2 secreted by individual clones after stimulation, a murine T-cell proliferative assay was used. Specifically, 5000-rad-irradiated cloned human T lymphocytes (5×10^4 per well) and one or another stimulus were added to 5×10^4 murine T lymphoblasts previously induced with phytohemagglutinin. After 18 hr of culture, individual wells were pulsed for 24 hr with 1 μCi of [^{3}H]Thd, mashed, and assayed for radioactivity as above. A standard curve obtained with serial dilutions of purified human IL-2 was utilized to quantitate the amounts of lymphokine produced by the various T-cell clones.

RESULTS

To investigate the molecular basis of enhanced proliferation to IL-2 after T3–Ti modulation, we used three antigen-specific human T-cell clones and a series of anti-clonotypic monoclonal antibodies in conjunction with a homogeneous IL-2 preparation and anti-Tac (9, 10) and DMS-1 (12) monoclonal antibodies. The two human cytotoxic T-cell clones $CT4_{II}$ and $CT8_{III}$ (derived from the $T4^+$ and $T8^+$ T-cell subpopulations, respectively) and the noncytotoxic RWAGE-specific $T4^+$-inducer T-cell clone RW17C proliferated *in vitro* to purified IL-2 (0.5 unit/ml; unmodulated) (Table 1). After modulation with their specific anti-clonotypic monoclonal antibodies, anti-Ti_1 ($CT8_{III}$), anti-Ti_2 ($CT4_{II}$), or anti-Ti_4 (RW17C), each of the three clones demonstrated a markedly enhanced [^{3}H]Thd uptake (200–300% enhanced), consistent with earlier findings where stimulation was obtained by IL-2-containing T cell-conditioned media (1–3). Moreover, the homogeneous IL-2 preparation utilized here ruled out the possibility that the enhanced proliferation to conditioned media observed previously was due to a mitogenic stimulus other than IL-2. Note that while much of the increase in [^{3}H]Thd incorporation in response to IL-2 after anti-Ti modulation can be accounted for on the basis of a larger fraction of cells in the S phase of cell cycle, it is also possible that the generation time of the cells is shortened.

The IL-2-driven proliferative responses of these clones in either an unmodulated or anti-Ti-modulated state could be abrogated by anti-Tac antibodies (≥90% inhibition). In contrast, monoclonal antibodies to a constant portion of the human Ia antigen that, like Tac, appears on activated T cells did not diminish IL-2-mediated clonal proliferation. Both the failure to block this response with anti-Ia and the fact that anti-Tac did not influence the clonal cytotoxic effector function (data not shown) made it unlikely that abrogation of clonal proliferation was simply due to a general inhibition of clonal activity. Table 1 also shows that IL-2-induced proliferation could be substantially diminished (>60%) by the anti-DMS-1 antibody previously shown to be directed at human IL-2 (12).

The above results unequivocally demonstrated that IL-2 mediated the enhanced proliferation seen subsequent to antigen receptor modulation. However, the basis for this increased clonal responsiveness was not known. Because individual clones could not be induced to proliferate by anti-Ti modulation alone, it seemed unlikely that the increase in proliferation of the anti-Ti modulated clone was due to secretion of endogenous lymphokine. Rather, it was more probable that anti-Ti modulation enhanced the number of surface IL-2 receptors.

To test this notion, the various clonal populations were incubated with one or another anti-clonotypic monoclonal antibody for 18 hr at 37°C; subsequently, IL-2 receptors were quantitated by means of immunofluorescence on an Epics V cell sorter (Fig. 1). Given the known characteristics of the log amplifier of the Epics V and the existing calibration curve relating linear and log fluorescence (based on mean channel fluorescence of fluorescent peaks) (24), it was possible to quantitate the number of binding sites per cell for the various anti-T-cell antibodies. In addition to Tac, surface expression of T3, T4, and T11 antigens was evaluated.

Untreated (media preincubated) RW17C cells expressed 45,000, 105,000, 187,000, and 10,500 binding sites per cell of T3, T4, T11, and Tac antigens, respectively (Table 2; Fig. 1, row A). The numbers of binding sites for T3 and T4 were similar to those obtained previously on other clones when determined either by means of quantitative fluorescence or by using ^{125}I-labeled monoclonal antibodies (2). Note that the number of binding sites for anti-Tac on RW17C was considerably lower than the other surface glycoproteins and remained at this level or less as long as the cells were cultured in IL-2-containing media without further antigenic stimulation (see below). In fact, other studies (26) indicate that in the absence of mitogenic or antigenic stimulation, IL-2 receptor expression diminishes on IL-2-dependent T-cell clones over time.

After modulation (Fig. 1, row B) with anti-Ti_4 (or anti-T3 by soluble antibody or antibody coupled to Sepharose beads; data not shown), however, there was a 6-fold increase in the number of IL-2 receptor binding sites as measured by anti-Tac (62,000). In contrast, anti-Ti_4 modulation was associated with a 75% reduction in the number of T3 binding sites (11,500), consistent with previous data indicating that the T3 and Ti surface structures are linked to one another in the membrane of human T cells (1, 2). Unlike T3, the number of

Table 1. *In vitro* proliferative responses of various clones to IL-2 after modulation with clone-specific monoclonal antibodies (Anti-$Ti_{1,2,4}$)

	$CT8_{III}$		$CT4_{II}$		RW17C	
	Unmodulated	Anti-Ti_1-modulated	Unmodulated	Anti-Ti_2-modulated	Unmodulated	Anti-Ti_4-modulated
Medium	124	562	653	355	266	497
IL-2	3393	7359	4940	12,737	8154	25,718
IL-2 + anti-Tac	128	384	325	140	815	870
IL-2 + anti-Ia	2785	7006	4563	12,541	7937	25,133
IL-2 + DMS-1	NT	2941	1700	4,383	2906	8,851

$CT8_{III}$, $CT4_{II}$, and RW17C were individually incubated with one or another clone-specific anti-Ti monoclonal antibody or medium. Subsequently, proliferative responses to purified human IL-2 (0.5 unit/ml) were determined. Final dilutions or concentrations of monoclonal antibodies were: anti-Tac, 1:750 (ascites in medium); anti-Ia 1:500 (ascites in medium); DMS-1, 200 μg of purified antibody per ml. Values are given as cpm of [^{3}H]Thd uptake and represent the means of triplicate cultures. Standard deviations were <18%. NT, not tested.

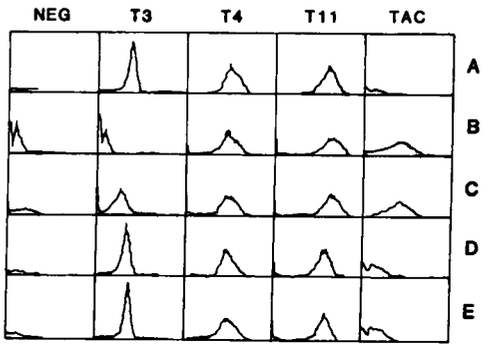

FIG. 1. Changes in cell surface expression of T-cell membrane antigens after triggering of the antigen receptor. RW17C cells were incubated for 18 hr with the following stimuli: medium (row A), anti-Ti_4 (final dilution, 1:250) (row B), autologous APC with RWAGE (10 μg/ml) (row C), autologous APC with tetanus toxoid (20 μg/ml) (row D), and allogeneic APC with RWAGE (10 μg/ml) (row E). Subsequently, cells were washed and analyzed by means of indirect immunofluorescence with saturating concentrations of monoclonal antibodies and fluorescein isothiocyanate-conjugated $F(ab')_2$ fragment of goat anti-mouse Ig on an Epics V cell sorter (final dilutions of monoclonal antibodies: anti-$T3_A$, 1:500; anti-$T4_A$, 1:1000; anti-T11, 1:1000; anti-Tac, 1:750). The negative control consisted of incubating the various cell populations with J5, 1:500 dilution, a monoclonal antibody (IgG2 isotype) specific for CALLA antigen and fluorescein isothiocyanate-conjugated goat anti-mouse $F(ab')_2$. Note that the threshold for detection in this fluorescence system is ≥4000 sites per cell.

T4 molecules remained unaffected by this process, and the T11 surface expression increased by <50%. Given these findings and the above functional data, it would appear that the enhanced *in vitro* proliferative response to IL-2 by anti-Ti or anti-T3 modulated T-cell clones is a consequence of the increased density of IL-2 receptors.

Because anti-clonotypic monoclonal antibodies appear to recognize the same antigen binding site as the natural ligand for Ti (4, 16), it was of interest to determine whether the latter would have similar effects. There was a strong increase in anti-Tac binding sites (61,000 vs. 11,000) and a reduction in T3 surface expression (24,000 vs. 45,000) when RW17C was preincubated with RWAGE and autologous APC (Fig. 1, row C; Table 2). Thus, it appears that this cloned population is fully activated vis-à-vis IL-2 receptor expression only after antigenic receptor triggering. Note that the number of T3 binding sites (i.e., 24,500) is overestimated by this analysis because the discrete anti-T3 unreactive ($T3^-$) T-cell population (Fig. 1, row C) was excluded from the calculation. Although the reason for the bimodal reactivity of anti-T3 on the MHC/antigen-triggered RW17C is not known, it is most likely a consequence of ligand–T3–Ti–receptor binding and cell proliferation.

Perhaps more importantly, Fig. 1 demonstrates that IL-2 receptor expression and T3 modulation were critically dependent on a unique combination of MHC-restricting element and antigen. Thus, a mixture of autologous APC with the irrelevant antigen tetanus toxoid (Fig. 1, row D) or, alternatively, allogeneic APC with the relevant antigen RWAGE (Fig. 1, row E) did not induce these membrane changes. The present data confirm previous results indicating that RW17C recognized RWAGE only in the context of autologous class II MHC gene products (16) and that the anti-clonotypic monoclonal antibody anti-Ti_4 reacted with the binding structure for antigen plus MHC because it induced identical phenotypic membrane changes.

In addition to induction of IL-2 receptors, other studies indicated that cell-bound antigen (e.g., alloantigen in the case of $CT4_{II}$ and $CT8_{III}$) triggered clonal proliferation and lymphokine production (4). The same effects also were observed with anti-clonotypic antibodies when the latter were coupled to a solid support but were not induced by anti-clonotypic monoclonal antibodies in soluble form (4). Taken together, these findings supported the notion that Ti receptor crosslinking, although not required for IL-2 receptor expression, was an essential prerequisite for induction of clonal proliferation.

Table 2. Changes in surface expression of T-cell differentiation antigens on clone RW17C as a consequence of T-cell-receptor triggering

	T3	T4	T11	Tac
Medium	45,000	105,000	187,000	10,500
Anti-Ti	11,500	105,000	270,000	62,000
Ag/MHC	24,500	112,000	255,000	61,000

RW17C cells were incubated with medium, monoclonal antibody anti-Ti_4 (1:250), or RWAGE with autologous APC [antigen (Ag)/MHC] for 18 hr prior to the determination of T3, T4, T11, and Tac binding sites by means of quantitative indirect immunofluorescence.

That triggering of the Ti structure should result in endogenous IL-2 production, enhanced IL-2 receptor expression, and clonal proliferation appeared more than coincidental and suggested that stimulation of clonal proliferation through antigen receptor triggering was due to release and subsequent binding of endogenous IL-2—i.e., an autocrine mechanism. If this were the case, then one would anticipate that clonal proliferation after antigen receptor triggering and crosslinking could be inhibited by anti-IL-2 or anti-IL-2-receptor antibodies. To test that possibility, $CT8_{III}$ and RW17C were incubated with the appropriate physiologic ligands (Laz 156 or RWAGE in the context of autologous APC, respectively), and the anti-clonotypic antibodies anti-Ti_1 and anti-Ti_4 coupled to Sepharose beads, or the same antibodies in soluble form. RW17C and $CT8_{III}$ produced IL-2 (0.7–1.0 and 0.25–0.4 unit/ml, respectively) and mounted strong proliferative responses upon incubation with either of the former two stimuli in the absence of exogenous lymphokines (29,659/18,509 cpm and 9,211/6,985 cpm, respectively) (Table 3). In contrast and as expected, soluble anti-clonotypic antibodies had no mitogenic effect. Importantly, anti-Tac but not antibodies to other T-cell activation structures (anti-Ia) inhibited the [^{3}H]Thd uptake in all cases (inhibition ≥50%). In addition, DMS-1 antibody diminished *in vitro* proliferation as well, although the magnitude of the effect was not as pronounced as that with anti-Tac (25–50%). The less marked inhibitory effect of DMS-1 antibody may relate to the greater affinity of surface IL-2 receptors for the IL-2 ligand than DMS-1. Nevertheless, these findings make it clear that clonal proliferation is due to release and subsequent binding of IL-2 to its surface receptor. It should be noted that, in contrast to its inhibitory effect on proliferation, anti-Tac did not influence the IL-2 release mechanism (data not shown). Thus, IL-2 production in the presence of anti-Tac was not diminished after Ti triggering. This result further supported the view that the anti-Tac antibody specifically blocks the binding of IL-2 to its receptor (10, 11).

DISCUSSION

To investigate some of the molecular mechanisms underlying T-cell proliferation after triggering of antigen receptors, we utilized three antigen-specific human T-cell clones, $CT8_{III}$, $CT4_{II}$, and RW17C (1, 2, 14, 16), in conjunction with anti-clonotypic, anti-Tac, and DMS-1 monoclonal antibodies. The results clearly demonstrated that there was a functional linkage between antigen receptor and IL-2 receptor on all three of these clones. Specifically, soluble anti-T3 or anti-Ti antibodies, like physiologic ligand, induced a 6-fold increase in IL-2 receptors. Moreover, when these antibodies were bound to Sepharose beads, they induced IL-2 secretion

Table 3. IL-2 secretion and blocking of proliferation by monoclonal antibodies after antigen-receptor triggering

	Stimuli of clone $CT8_{III}$			Stimuli of clone RW17C		
Monoclonal Ab	Laz 156	Anti-Ti_1-Sepharose	Anti-Ti_1 (soluble)	Ag/MHC	Anti-Ti_4-Sepharose	Anti-Ti_4 (soluble)
			IL-2 produced, units/ml			
None (medium)	1.0	0.7	<0.05	0.4	0.25	<0.05
			Proliferation, cpm			
None (medium)	29,659	18,509	515	9211	6985	232
Anti-Tac	2,623	3,969	458	4219	2026	NT
DMS-1	14,333	14,382	479	7829	5308	NT
Anti-Ia	28,281	18,740	468	9303	6426	NT

Cloned T cells (3×10^4) were incubated with the following stimuli in the presence or absence of monoclonal antibodies. Stimuli of clone $CT8_{III}$: 3×10^4 Laz 156 cells (irradiated with 5000 rad); anti-Ti_1-Sepharose (predetermined optimal concentration); and anti-Ti_1 (soluble), final dilution of 1:500 ascites in medium. Stimuli for clone RW17C: 3×10^3 autologous APC with RWAGE (10 μg/ml); anti-Ti_4-Sepharose (predetermined optimal concentration); and anti-Ti_4 (soluble), final dilution of 1:500 ascites in medium. Final dilutions or concentrations of antibodies: anti-Tac, 1:750 (ascites in medium); DMS-1, 200 μg of purified antibody per ml; and anti-Ia, 1:500 (ascites in medium). Values represent means of triplicate cultures and are given as cpm of [^{3}H]Thd uptake. Standard deviations were <18%. NT, not tested.

and clonal proliferation analogous to antigen. That the latter response was mediated by IL-2 was evident from the finding that either monoclonal anti-Tac or DMS-1 antibodies blocked clonal proliferation following T3–Ti triggering.

The induction of IL-2 receptors occurred as a discrete event in and of itself and did not appear to require Ti crosslinking (or more precisely, crosslinking of more than two receptors, if one assumes that the antigen receptor is univalent) because it could be mediated by binding of soluble anti-Ti monoclonal antibody to the clone's surface. In contrast, production and release of IL-2 and subsequent clonal proliferation did not occur in the absence of apparent receptor crosslinking initiated by surface-bound antigen on the stimulator cells or anti-Ti coupled to Sepharose. Note that we cannot formally rule out the possibility that Sepharose-bound antibodies may be acting on these cloned T cells independently of any crosslinking effect, for instance, either by increasing the avidity of the antibody for the receptor or interacting with other surface molecules unrelated to T3–Ti. Such possibilities seem remote because three antibodies to the Ti structure of $CT4_{II}$, regardless of their isotype, have identical functional effect when coupled to Sepharose 4B, and yet none of these soluble anti-Ti antibodies in combination with an uncoupled bead triggers IL-2 release or clonal proliferation (ref. 4; data not shown). The identity of the phenotypic changes induced by anti-Ti anti-receptor antibodies and antigen/MHC on RW17C provides further support for the notion that the Ti complex is the receptor for both antigen and MHC.

Therefore, it appears that T-cell proliferation occurs as a series of complex and precisely orchestrated events as outlined in Fig. 2. Resting T-cell clones or T cells express few or no IL-2 receptors but display a maximal number of surface antigen receptors (stage 1). However, T3–Ti receptor triggering by antigen plus MHC gene product of the appropriate haplotype or Sepharose-bound anti-clonotypic monoclonal antibodies results in modulation of the T3–Ti complex (stage 2), thus diminishing the number of surface antigen receptors and rapidly inducing surface IL-2 receptor expression (stage 3). Furthermore, such activation also leads to endogenous

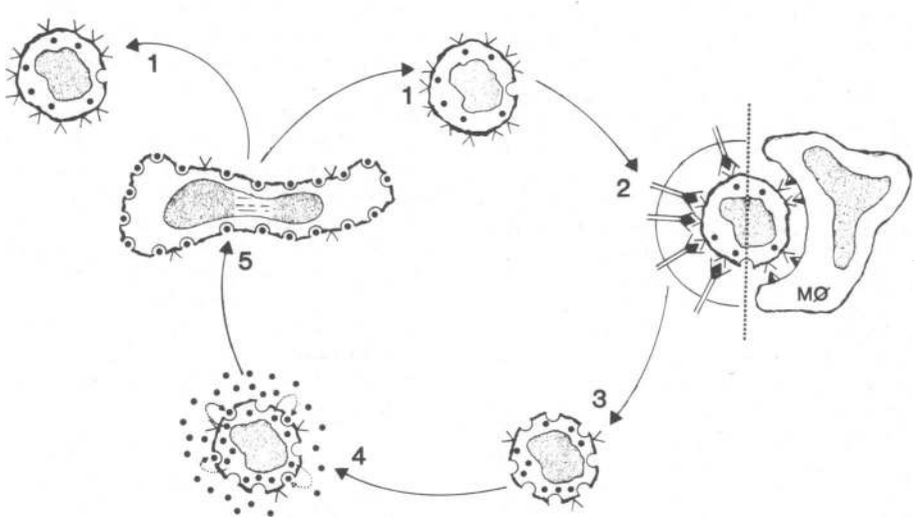

FIG. 2. Schematic model of T-cell proliferation mediated by an IL-2-dependent autocrine mechanism. Stage 1: resting T cells or T-cell clones express few or no IL-2 receptors (◡) while reciprocally displaying a maximal number of antigen receptors (∨). Stage 2: T3–Ti triggering by antigen/MHC-restricting element (▲) or surface-bound anti-T-cell-receptor antibodies (▼) results in T3–Ti modulation, thus reducing the number of surface antigen receptors and rapidly inducing surface IL-2 receptor expression. Stage 3: this event appears to occur prior to IL-2 secretion itself because IL-2 receptor expression can be observed 2–4 hr after Ti–T3 triggering, whereas IL-2 is not detectable in culture supernatants until at least 10–12 hr later. Stage 3 also can be achieved with anti-Ti or anti-T3 monoclonal antibodies in soluble form. Stage 4: activation via Ti–T3 leads to production and secretion of endogenous IL-2 (●) and subsequent binding to its own IL-2 receptors. Stage 5: once a critical density of occupied IL-2 receptors is achieved, DNA synthesis and mitosis occur. Finally, in the absence of additional antigenic stimulation, there is reexpression of the surface T3–Ti antigen receptor complex (stage 1).

IL-2 production, secretion, and subsequent binding to IL-2 receptors on the same clones (stage 4). Once a critical number of IL-2 receptors have bound IL-2, DNA synthesis and cell mitosis occur (stage 5). Finally, in the absence of continued antigenic stimulation, there is reexpression of the surface T3–Ti antigen–receptor complex and, reciprocally, a reduction in the number of IL-2 receptors (stage 1).

The present set of findings is in agreement with recent studies with lectin-stimulated peripheral blood T lymphocytes in which it was shown that IL-2 receptor expression occurred during the G_1 phase of cell cycle and preceded DNA synthesis (26). Moreover, the correlation of IL-2 receptor levels with the proportion of cells in active cycle emphasized the importance of antigen receptor triggering on IL-2 responsiveness, especially when IL-2 concentrations become limiting. In addition, induction of IL-2 receptors by antigenic stimulation as shown here provides a rational basis for the empiric observations that IL-2-mediated clonal expansion *in vitro* is facilitated by repetitive antigenic stimulation. In this regard, recent studies have demonstrated that loss of IL-2 responsiveness is not secondary to down regulation of IL-2 receptors *per se* but rather secondary to lack of repetitive antigenic challenge (26).

The view that is emerging regarding the relationship of the T3–Ti antigen receptor to the IL-2 receptor points to a mechanism whereby external stimuli—i.e., antigen—direct the magnitude and extent of T-cell clonal proliferation by means of the IL-2 hormone–receptor system. Immunologic specificity is ensured by the antigen dependence of the IL-2 receptor expression, and yet the reciprocal appearance of T3–Ti and IL-2 receptors presumably leaves the cell in a state of responsiveness to either the hormone or antigen ligand. However, the transient expression of the IL-2 receptors themselves serves as a fail-safe system to eliminate any possibility of uncontrolled growth through an IL-2-dependent mechanism. Whether alteration of this mechanism results in tumor growth *in vivo* as suggested by studies on continuously growing, IL-2-producing primate tumor lines of T-cell lineage remains to be determined but appears likely (27).

The present construct clearly implies that T-cell proliferation is mediated through an autocrine network in which antigen-receptor triggering leads to IL-2 receptor expression, IL-2 production, IL-2 release, and subsequent IL-2 receptor occupancy, which ultimately promotes cell division. Note that it does not necessarily follow that all T cells produce and respond to their own IL-2. In fact, one would predict that the failure of certain cells to proliferate to antigen occurs as a consequence of their inability to produce sufficient amounts of endogenous IL-2, although such cells would, in all likelihood, be triggered to express IL-2 receptors. Furthermore, even for IL-2-producing T cells, exogenous sources of IL-2 would amplify clonal proliferation. However, the present results are clearly different than anticipated from the conventional endocrine notion that suggests that one cell type produces IL-2 while a different one responds to it.

The above findings stress the central linkage of the T-cell receptor to the IL-2 hormonal system and the critical role of the latter in T-cell proliferation. The present reagents provide an excellent set of probes with which to evaluate the mechanisms that regulate and maintain physiologic T-cell growth. In addition, they should provide important insights into aberrations of these control mechanisms that could underlie malignant transformation within the T-cell lineage.

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