T SUPPRESSOR CELL GROWTH FACTOR AND ANTI-CD3 ANTIBODIES STIMULATE RECIPROCAL SUBSETS OF T LYMPHOCYTES

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Lymphoid cells may be categorized with respect to regulatory functions; certain T cell subpopulations upregulate the immune response, whereas others lead preferentially to downregulation. Evidence that these T cell subsets are distinct is based on in vitro function (1), hormonal products (2-4), associations with MHC antigens used in antigen recognition (5-7), drug sensitivity (8), and expression of specific arrays of cell-surface antigens (9-19).

Two mAbs have been useful in distinguishing CD8⁺ cells with suppressor and cytotoxic activity. One mAb, termed 9.3, recognizes the CD28 (Tp44) cell-surface molecule found on 90-95% of CD4⁺ cells and 50-60% of CD8⁺ cells (9). Among CD8⁺ cells, the CD8⁺,CD28⁺ subset appears to include all CTL (10) and precursor CTL (11). CD28 is not detected on the precursors of alloantigen-specific Ts cells (11), nor on cells that suppress PWM-induced B cell differentiation (12). Another mAb, Leu15, recognizes the CD11 antigen (C3b, receptor) present on ~50% of CD8⁺ cells, but only 5-10% of CD4⁺ cells (13). Activated CD8⁺,CD11⁺ cells suppress Ig synthesis and T cell proliferative responses (13-15). The CD28 and CD11 antigens appear to be reciprocal; very few, if any, cells express both (16).

Discrimination between CD4⁺ subsets involved in regulation of the immune response has been provided by the mAbs designated 2H4 (anti-CD45R) and 4B4 (anti-CDw29). CD4⁺,CD45R⁻,CDw29⁺ cells function as helpers for Ig production; they proliferate poorly to Con A and in autologous MLRs, but do respond to soluble antigens (17, 18). In contrast, CD4⁺,CD45R⁺,CDw29⁻ cells induce CD8⁺ Ts function and have the opposite proliferative reactivities (18, 19). The cell-surface antigens CD45R and CDw29 are also present on subpopulations of CD8⁺ cells, but no association has been made between expression of either marker and CD8⁺ functional activity.

We postulated that regulation of T cell subset proliferation might be achieved, at least in part, by distinct requirements for induction of IL-2 responsiveness. In previous experiments (20), we used the polyclonal B cell and T cell activator PWM to study the hormonal products of CD4⁺ cells that support Ts proliferation.

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Purified CD8+ cells did not proliferate in response to PWM, even with the addition of exogenous IL-2. In the presence of CD4+ cells, however, PWM-stimulated CD8+ cells proliferated and differentiated into activated Ts (21) with the cell-surface phenotype CD8+, CD28−, HLA-DR*, Leu7−, Leu8+ (22). Supernatants of 48-h PWM cultures of PBMC depleted of CD8+ cells contained both IL-2 and Ts cell growth factor (TsGF), a protein of ~8,000 M, that induces IL-2-R expression on CD8+ Ts precursors (20); CD8+ cells cultured in such supernatants suppressed the response of fresh autologous cells to PWM. To determine the specificity of this hormonal signal, we compared the activity of TsGF on cells of suppressor and nonsuppressor lineages. We were surprised to find that these experiments not only demonstrated subset specificity of TsGF for IL-2-R induction, but also discriminated reciprocal subpopulations of both CD4+ and CD8+ T cells with respect to responsiveness to a T cell receptor mAb.

Materials and Methods

Purification of CD4+ and CD8+ Cells. PBMC were obtained from buffy coat isolations of units of peripheral blood from healthy volunteers. After passage over Isosymph gradients (Gallard-Schlessinger Chemical Mfg. Corp., Carle Place, NY), the cells were depleted of monocytes by plastic adherence on 100 × 15 mm Petri dishes (Fisher Scientific Co., Pittsburgh, PA). CD4+ and CD8+ cells were then isolated by a panning procedure adapted from Wysocki et al. (23). In short, 150 × 15 mm Petri dishes (Falcon Labware, Oxnard, CA) were coated with an affinity-purified preparation of goat anti-mouse Ig (Pel-Freeze Biologicals, Rogers, AR) at 4°C overnight. Cells were incubated in pretitered amounts of either OKT4 or OKT8 mAb (Ortho Pharmaceutical, Raritan, NJ) for 1 h at 4°C. After addition of 10⁶ cells per plate in 7.5 ml RPMI 1640 medium containing 5% heat-inactivated pooled AB+ serum, plates were incubated at 4°C for 70 min. Nonadherent cells were removed by thorough washing with RPMI 1640 containing 2% AB+ serum, and adherent cells were recovered by gentle scraping with a rubber policeman. The purified populations were >95% CD4+ or CD8+, and were <5% positive for the reciprocal T cell marker. Cells were stored in liquid nitrogen in 7.5% DMSO until used.

Anti-CD3-Sepharose preparation. Concentrated OKT3 mAb was dialyzed against the coupling buffer (0.1 M NaHCO₃, pH 8.3, containing 0.5 M NaCl) overnight. CNBr-activated Sepharose 4B was conjugated with anti-CD3 mAb (anti-CD3-Sep) according to the protocol outlined by the manufacturer (Pharmacia Fine Chemicals, Piscataway, NJ). In short, 1 g of powder was swollen and washed in 1 ml HCl. 5 ml of the protein preparation containing anti-CD3 mAb was added at 1 mg/ml to the swelled beads in a Falcon Labware 2095 tube and gently rotated for 1 h at 4°C. The beads were then washed in coupling buffer, followed by incubation of the beads in 0.1 M Tris-Cl buffer, pH 8, for 1 h at 4°C to block any remaining active groups. The coupled beads were washed with three cycles of alternating pH using 0.1 M acetate (pH 4) containing 0.5 M NaCl, followed by 0.1 M Tris (pH 8) containing 0.5 M NaCl. The anti-CD3-Sep was stored at 4°C in HBSS containing 1% AB+ serum, and was used at a dilution causing maximal IL-2-dependent proliferation of unseparated PBMC.

Recombinant IL-2 (rIL-2). An aliquot of rIL-2 (Lot 380, Amgen Biologicals, Thousand Oaks, CA) was compared with the NIH Jurkat-IL-2 standard (24) using the IL-2-responsive HT-2 cell line, and was found to contain 5 NIH units of activity per Amgen unit. rIL-2 was then used at 0.5 NIH U/ml in a total volume of 200 µl within microculture assays.

TsGF Preparation. A supernatant containing TsGF was prepared by depleting PBMC of CD8+ cells with mAb and complement and incubating the remaining cells in either a 1:80 dilution of PWM (Grand Island Biological Co., Grand Island, NY) or, where designated in Table II, a pretitered amount of anti-CD3-Sep plus 2.0 U rIL-2 per

1 Abbreviations used in this paper: Anti-CD3-Seph, anti-CD3 mAb conjugated to Sepharose 4B beads; PE, phycoerythrin; TsGF, T suppressor cell growth factor.
Table I
Description of Antibodies Used

| Antigen designation | mAb used | Distribution of T lymphocytes | Comments | Reference |
|---------------------|----------|-------------------------------|----------|-----------|
| CD3                 | OKT3     | Virtually all peripheral      | Associated with T cell antigen receptor | 26        |
| CD4                 | OKT4     | 55-70% of peripheral          | Associated with HLA class II-restricted responses | 6         |
| CD8                 | OKT8     | 25-40% of peripheral          | Reciprocal with CD4 on PBMC; associated with HLA class I-restricted responses | 6         |
| CD11                | Leu15    | 5-10% of CD4+ cells           | Receptor for C3b; associated with Ts function within CD8+ subset | 13-15     |
| CD28                | 9.3      | 90-95% of CD4+ cells          | Reciprocal with CD11 on T cells; associated with CTL function within CD8+ subset | 9-12      |
| CDw29               | 4B4      | 40-45% of both CD4+ and CD8+ cells | Associated with helper-inducer activity within CD4+ subset | 17-18     |
| CD45R               | 2H4      | ~40% of CD4+ cells, ~50% of CD8+ cells | Largely reciprocal with CDw29 on CD4+ subset; associated with suppressor-inducer activity within CD4+ subset | 18-19     |

CD8-depleted cells were incubated at 5 x 10^6 cells/ml in Falcon Labware 2051 tubes at a volume of 2 ml per tube. Medium (RPMI 1640 with 2.0 g/liter NaHCO₃, 4.8 g/liter Hepes, and 2 mM glutamine) containing 1% AB' serum was used for incubations at 37°C with 5% CO₂ for a total of 48 h. Culture supernatants were harvested by centrifugation and passed through a 0.45-μm filter to remove any residual cellular debris. 50 ml of each supernatant was concentrated using an ultrafiltration system with a 1,000 mol wt cut-off membrane (Amicon Corp., Danvers, MA) and lyophilized. After reconstitution in 0.1 M NH₄HCO₃, HPLC size fractionation on an SW Spherogel-TSK column (No. 3000, Altex Scientific Inc., Berkeley, CA) using a Beckman Instruments, Inc. (Fullerton, CA) HPLC system was performed by collecting fractions corresponding to an M₅ of 5-10,000. The column was run in 0.1 M NH₄HCO₃ at a flow rate of 1 ml/min. The pooled fractions containing TsGF activity were lyophilized and reconstituted in 10 ml in assay medium (including 10% AB' serum) and were sterilized by passage through a 0.22 μm filter. Aliquots of the TsGF were then stored at -70°C and used at 50 μl per microculture well. Fractions with TsGF activity lacked detectable IL-2 activity, as shown by assay on the IL-2-dependent T cell line HT-2 (data not shown).

Isolation of CD4+ and CD8+ Subpopulations by Flow Cytometric Sorting. Purified CD4+ and CD8+ cells were thawed and incubated in mAbs (Table I) on ice with occasional shaking for 30 min. For indirect immunofluorescence using anti-CD28 mAb (9.3; kindly supplied by Dr. John Hansen, Puget Sound Blood Center, Seattle, WA), excess mAb was removed by washing, and FITC-conjugated goat anti-mouse IgG was added on ice for 30 min at 5 μl/10^6 cells. For direct immunofluorescence, phycoerythrin (PE)-conjugated anti-CD11 (Leu15; Becton-Dickinson Monoclonal Center Inc., Mountain View, CA), and anti-CD45R and anti-CDw29 (2H4 and 4B4, respectively; Coulter Monoclonal Antibodies, Inc., Hialeah, FL) were used at 20 μl/10^6 cells. After thorough washing, the cells were analyzed by flow cytometry (EPICS V; Coulter Electronics Inc., Hialeah, FL), and fluorescence was determined using logarithmic amplification. Lower and upper sorting windows were set for each sorted population, and purity of each population was tested by flow cytometric reanalysis.

Cell Proliferation. To measure the incorporation of [³H]thymidine, 1.0 μCi (2 Ci/mmol,
Proliferative responses of purified CD8+ and CD4+ cells. 5 x 10⁴ CD8+ cells (A) or CD4+ cells (B) were incubated in various culture conditions, including medium, 1:80 PWM, and pretitered amounts of anti-CD3–Seph or TsGF. All cultures were in the presence or absence of 0.1 U/well rIL-2. 1.0 µCi/well [³H]thymidine was added for the final 24 h of a 5-d assay. Mean cpm of triplicate wells ± SEM are shown.

Amersham Corp., Amersham, United Kingdom) was added to three replicate microtiter wells on 96-well round-bottomed plates (Costar, Cambridge, MA) for the final 24 h of the assay period. The nucleic acid was then isolated on filtermats (Skatron, Inc., Sterling VA) and counts per minute were determined.

Results

Proliferative Responses of CD4+ and CD8+ Cells. Puck and Rich have previously shown (21) that a CD8-depleted culture exhibits CD4+ cell proliferation in response to PWM, whereas purified CD8+ cells do not grow in a PWM culture of CD4-depleted cells. We wished to investigate the proliferative responses of isolated CD4+ and CD8+ cells to PWM and other stimuli. Purified CD4+ and CD8+ cells both proliferated when incubated in anti-CD3–Seph or HPLC-fractionated TsGF, but only in the presence of added IL-2 (Fig. 1). Therefore, either stimulus was sufficient to cause IL-2 responsiveness within an unfractonated population of CD4+ or CD8+ cells. Neither population proliferated to IL-2 alone at the dose used, although responses to rIL-2 alone were observed at doses exceeding 5.0 U/ml (data not shown). The PWM response of purified CD4+ cells was presumably reduced due to the lack of monocytes in the cellular preparation, reflecting the monocyte dependence of PWM-induced T cell proliferation (25). CD8+ cells, as expected, did not proliferate in PWM, even in the presence of exogenous IL-2.

Proliferative Responses of Sorted CD8+ Subpopulations. To determine whether T cells displaying subset-associated surface molecules differed with respect to requirements for IL-2 responsiveness, fresh CD8+ cells were separated by virtue
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of their cell-surface phenotypes (Fig. 2, A and B). Sorted populations were then cultured in exogenous IL-2 alone, or with IL-2 and either anti-CD3–Seph or TsGF (Fig. 2C). The proliferative responses of the sorted subsets to anti-CD3–Seph or TsGF were completely reciprocal; the subsets responded to one stimulus or the other, but not both. CD11+,CD28+ cells, associated with CTL activity (10, 11), proliferated when cultured with anti-CD3–Seph plus IL-2. In contrast, CD11−,CD28− cells, associated with suppressor effector activity (11–15), did not mount a proliferative response to a stimulus (anti-CD3–Seph) that crosslinks T cell receptors (26).

Proliferative Responses of Sorted CD4+ Subpopulations. The differential response of CD8+ cells to the stimuli tested suggested reciprocal specificities for TsGF and anti-CD3–Seph. To investigate whether the CD4+ population could be subdivided in the same manner, CD4+ cells were also sorted by flow cytometry, using the reciprocal cell-surface antigens CD45R and CDw29 (Fig. 3, A and B). CD4+,CD45R−,CDw29+ cells did not respond to TsGF and IL-2, but proliferated when incubated in anti-CD3–Seph and IL-2 (Fig. 3C). On the other hand, subpopulations with a CD4+,CD45R+,CDw29− phenotype responded to TsGF and IL-2, but surprisingly did not proliferate after antigen receptor crosslinking.

Kinetics of the Proliferative Responses of Cell Subsets. To insure that the lack of responsiveness of cell subsets measured 5 d after initiation of culture was not due to accelerated or delayed kinetics, sorted CD4+ and CD8+ cells were incubated under similar conditions for times ranging from 2–8 d. No culture unresponsive at 5 d had a significant response at any other time point tested.
Expression of CD3 on T Cell Subsets. A possible explanation for the lack of reactivity to anti-CD3–Seph by certain T cell subpopulations was that the level of CD3 expression on the unresponsive cells was substantially less. By using the same mAb (OKT3) used for conjugation to Sepharose beads to quantify the amount of CD3 antigen expressed on the sorted subpopulations, it was seen that 95% of both CD4+,CD45R+ and CD4+,CD45R− cells were CD3+ (Fig. 5A). The mean fluorescence was also comparable, indicating that CD3 was expressed in relatively equal quantities on the two populations.

After sorting CD8+ cells into CD11+ and CD11− populations, both were stained with anti-CD3 mAb (Fig. 5B). Whereas 90% of CD8+,CD11− cells were CD3+, only 65% of the CD8+,CD11+ population expressed measurable amounts of CD3. However, CD3+,CD8+,CD11+ cells had a peak fluorescence level equal to CD3+,CD8+,CD11− cells, indicating similar levels of expression on the positive cells within both subsets.

TsGF Production Induced by Anti-CD3–Seph. Having ascertained that cells within the suppressor lineage did not proliferate in response to anti-CD3–Seph and rIL-2, it was of considerable interest to investigate whether these signals were capable of stimulating TsGF production from a population of CD4+ cells.
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Figure 4. Kinetics of subpopulation responses. Cell sorting was performed as described in Figs. 2 and 3. CD8⁺ cells were sorted into (A) CD28⁺ and (B) CD28⁻ subpopulations. CD4⁺ cells were sorted into (C) CD45R⁺ and (D) CD45R⁻ subpopulations. Separated subpopulations were cultured at 2.5 × 10⁴ cells per microculture well for 2–8 d in rIL-2 alone (0.1 U/well) (●), TsGF plus rIL-2 (---), and anti-CD3-Sep-h plus rIL-2 (○). 1.0 μCi [³H]thymidine was added to the cultures 24 h before time of harvesting. Mean cpm of triplicate wells ± SEM are shown.

Figure 5. CD3 expression on sorted T cell subsets. (A) Purified CD4⁺ cells were sorted into CD45R⁺ (---) and CD45R⁻ (-- --) populations with PE-conjugated anti-CD45R mAb. The sorted populations were then stained with FITC-conjugated anti-CD3 mAb and analyzed by flow cytometry. Sorted populations were also stained with FITC-conjugated goat anti-mouse antibody to quantify nonspecific staining. The fluorescence patterns of the control populations were identical, and 95% of the control populations were to the left of the vertical line. Both the CD45R⁺ and CD45R⁻ populations were 95% CD3⁺. (B) Purified CD8⁺ cells were sorted into CD11⁺ (---) and CD11⁻ (----) populations using PE-conjugated anti-CD11. The cells were then stained with FITC-conjugated anti-CD3 mAb and similarly analyzed. The CD11⁺ population was 65% CD3⁺, whereas the CD11⁻ population was 90% CD11⁺.
Thus, CD8-depleted cells were divided into separate aliquots and incubated in either PWM or anti-CD3–Seph plus 2.0 U/ml rIL-2. After concentration of the supernatants, TsGF activity was eluted from HPLC in identical fractions. The TsGF preparations were then used to investigate the subset specificity of responsiveness. Sorted subpopulations of both CD4+ and CD8+ cells responded equally to both TsGF preparations (Table II), indicating that TsGF activity was not specific to supernatants of CD4+ cells stimulated by PWM.

**Anti-CD3–Seph Inhibits the Response of CD8+ Cells to TsGF.** The lack of a proliferative response to either anti-CD3–Seph or TsGF does not necessarily imply that the stimulus has no effect whatsoever on the cellular population. To investigate the possibility that the stimulus not leading to IL-2 responsiveness might affect a positive response, sorted CD8+,CD11+ and CD8+,CD11− populations were incubated in a combination of the two signals and IL-2 responsiveness was tested (Fig. 6). CD8+,CD11− cell proliferation caused by antigen receptor crosslinking was not affected by the addition of TsGF to the culture. In contrast, the proliferation of CD8+ cells in TsGF and IL-2 was abrogated by the addition of anti-CD3–Seph at the initiation of culture.

**Discussion**

A central role for IL-2 in proliferation of both CD4+ and CD8+ T cells is generally acknowledged (27). Moreover, cell-surface events stimulating expression of IL-2-R on naive T cells are required as a preliminary to IL-2-driven cell

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**Table II**  
**TsGF Production Induced by Anti-CD3-Sepharose.**

| Exp | Sorted population* | [3H]Thymidine incorporation (cpm × 10^3) induced by: | Anti-CD3-Sepharose + rIL-2 | TsGF + rIL-2 | Anti-CD3-Sepharose + rIL-2 |
|-----|---------------------|---------------------------------|-----------------|-------------|---------------------|
| 1   | Unssorted CD4+      | 0.15                            | 10.58           | 18.38       | 23.25               |
|     | CD4+,CD45R−         | 0.26                            | 11.82           | 0.30        | 0.18                |
|     | CD4+,CD45R+         | 0.14                            | 0.21            | 41.91       | 48.08               |
| 2   | Unssorted CD4+      | 0.19                            | 3.73            | 5.71        | 6.03                |
|     | CD4+,CDw29−         | 0.25                            | 1.82            | 35.61       | 30.03               |
|     | CD4+,CDw29+         | 0.19                            | 6.10            | 1.03        | 0.13                |
| 3   | Unssorted CD8+      | 0.37                            | ND              | 9.69        | 6.25                |
|     | CD8+,CD11−          | 0.18                            | ND              | 0.52        | 0.67                |
|     | CD8+,CD11+          | 0.20                            | ND              | 11.63       | 10.23               |
| 4   | Unssorted CD8+      | 0.91                            | 21.77           | 18.15       | 16.07               |
|     | CD8+,CD28−          | 5.17                            | 2.70            | 34.68       | 30.89               |
|     | CD8+,CD28+          | 1.71                            | 22.65           | 1.19        | 0.40                |

* Purified CD4+ or CD8+ cells were sorted as described in Figs. 2 and 3. Cells were cultured at 5.0 × 10^5 per microculture well in 5-d proliferative assays.

† TsGF prepared from 48-h supernatants of CD8-depleted cells in a 1:80 dilution of PWM.

§ TsGF prepared from 48-h supernatants of CD8-depleted cells in anti-CD3–Sepharose plus 2.0 U/ml rIL-2.

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division (28). By using flow cytometry, we have sorted T cell subsets and have analyzed these subpopulations for unique patterns of responsiveness to two signals shown to cause IL-2-R induction, anti-CD3–Seph and TsGF. Our results indicate that within both the CD4+ and CD8+ populations, heterogeneity exists with regard to stimuli required for induction of responsiveness to IL-2.

Studies using T cell clones have described the reactivity of cells to crosslinked anti-CD3 antibodies. CD8+ clones with cytolytic activity have been demonstrated to proliferate in response to anti-CD3–Seph (26, 29). However, Bensussan et al. (30) have reported that treatment of CD8+ Ts clones with anti-CD3 antibodies reduced responsiveness to exogenous IL-2. The present experiments substantially expand this concept by defining a subset of fresh CD8+ lymphocytes that was unable to proliferate in an IL-2-dependent manner after crosslinking cell-surface antigen receptors, but did become responsive to IL-2 in the presence of TsGF.

Surprisingly, differential responsiveness was also seen within the CD4+ population. CD4+ cell subsets that were isolated based on the expression of CD45R or CDw29 antigens displayed different patterns of responsiveness to TsGF and anti-CD3–Seph. Strong expression of the CD45R antigen correlated with TsGF responsiveness, whereas CDw29 expression was associated with responsiveness to anti-CD3–Seph. It should be noted that subset isolation by flow cytometry selected against cells with ambiguous phenotypes; only cells with the dimmest and brightest fluorescence were sorted. The reactivities of unselected cells (ranging from 11 to 33% of the unseparated populations) might not be as clearly delineated as the more definitively phenotyped cells. Furthermore, we have used preterited amounts of TsGF, anti-CD3–Seph, and rIL-2 for these studies, and have not tested cellular responses to a range of dilutions for each stimulus. Nevertheless, a clear association was seen between cell-surface phenotype and cellular reactivity.

An implication of our findings is that independent regulation of inducer populations within the CD4+ subset may occur in a manner similar to that seen with the CD8+ cytotoxic and suppressor subsets. Congruence of the responses of CD4+ and CD8+ cells is particularly significant; both subsets associated with downregulation of immune responses required a proliferative stimulus that differed markedly from that of subsets characterized by increased reactivity. Proliferation of cells within opposing limbs of the immune system might therefore...

**Figure 6.** Anti-CD3–Seph inhibits the response of CD8+ cells to TsGF. CD8+ cells were sorted into CD11+ (■) and CD11- (□) subpopulations as described in Fig. 2. Cells were cultured at 1.0 μCi [3H]thymidine was added to the cultures 24 h before time of harvesting. Mean cpm of triplicate wells are shown.
be independently regulated by the signals each subset requires for induction of IL-2 responsiveness.

Binding of antibodies to cell surfaces has been shown to stimulate certain functional effects. For instance, anti-CD28 mAb binding has been implicated in an alternate activation pathway for T cell proliferation (31, 32). Results of studies using mAbs for subset separations may thus be affected by antibody-stimulated functional alterations. The experimental design used, however, in which both positive and negative selection were used for isolation of T cell subsets, essentially obviates this potential problem; any effect mediated by a specific mAb on a stained population would not also be expected to occur with the identical population of unstained cells sorted by using the reciprocal mAb.

Studies investigating the relative expression of CD3 on the nonresponder compared with the responder subpopulations showed either no difference (CD4+,CD45R+ cells compared with CD4+,CD45R- cells) or a moderate decrease in the percentage of CD3+ cells without a change in the peak fluorescence of the positive populations (CD8+,CD11+ cells compared with CD8+,CD11- cells). The number of CD8+,CD11+ cells capable of being affected by anti-CD3 binding was probably reduced due to the presence of CD3-,CD8+,CD11+ NK cells (33). It is highly unlikely that the difference in reactivity to the conjugated mAb was simply due to lack of CD3 expression on a subpopulation of CD8+,CD11+ cells; the majority of such cells expressed CD3, yet the population as a whole was unresponsive to anti-CD3-Sep and rIL-2.

Clearly, the apparent failure of suppressor pathway cells to proliferate in response to anti-CD3-Sep and IL-2 does not imply that cells involved in induction or expression of suppressor activity are intrinsically unresponsive to antigen. Incubation of CD8+ cells in anti-CD3 antibodies has been reported to cause polyclonal Ts activation (34). Furthermore, our finding that a TsGF response is possibly inhibited by anti-CD3-Sep indicates that rather than having no effect, binding of antigen by CD8+,CD11+ cells may lead to downregulation of a proliferative response. Thus, crosslinking cell-surface antigen receptors may have a functional role in the induction of suppression, but mediate a negative signal for proliferation by an unknown mechanism. A possible implication of these findings is that Ts precursors exhibit polyclonal growth, but functional activation results in at least temporary unresponsiveness to the same soluble signals. Further experiments will be required to differentiate possible transduction of a negative signal by anti-CD3-Sep from other cell-surface events with similar consequences, such as blocking or modulation of TsGF binding sites.

Although the CD8+ subset of Ts precursors proliferated in response to TsGF plus IL-2, these signals are not sufficient for induction of differentiated Ts activity. Indeed, recent experiments from our laboratory (35) have shown that two additional factors, prostaglandin E2 and IFN-γ, that are present in a culture supernatant of CD4+ cells plus monocytes, cause Ts differentiation independent of proliferation. Thus, our studies have implicated four distinct signals involved in the induction of CD8+,CD11+ Ts: TsGF and IL-2 for proliferation and a prostaglandin and IFN-γ for differentiation of TsGF activity. The precise role of antigen recognition by CD8+,CD11+ cells stimulated by these soluble signals remains unclear, however.
Recognition of the CD3 molecule by OKT3 mAb on all subsets analyzed does not necessarily imply that the nature of the antigen receptor is the same in responder versus nonresponder populations. Cells incapable of proliferating in response to anti-CD3–Seph may express antigen receptors not conforming to the conventional α/β T cell receptor complex. For instance, the murine γ gene is frequently rearranged and transcribed in CTL clones, but is uncommonly transcribed in Th cell lines (36). Two genes, homologous to the mouse γ gene, that show multiple rearrangement patterns have also been found in the human genome (37). Alternate components of the T cell antigen receptor might therefore be used by certain T cell populations, not affecting anti-CD3 binding to the antigen receptor complex, but altering the resultant internal signal.

IL-4 (B cell stimulatory factor 1), a 20-kD murine glycoprotein, has recently been shown (38) to cause T cell stimulation in an IL-2-independent manner. IL-4 is a product of Th cells and is secreted by a number of T cell lines, including ones that also produce IL-2. Those T cell lines producing IL-4 are also responsive to it in an autocrine manner (39). As a human analog has been found with similar activities (40), it is of interest to compare the activities and properties of this molecule and TsGF. In contrast to TsGF, IL-4 does not act as a cofactor with IL-2, but as an independent stimulator of T cell proliferation. The response of B cells to TsGF has not been tested, but it is notable that B cell function is downregulated by activated cells within the T cell populations responsive to TsGF, whereas, the cell lines responsive to IL-4 are described as B cell helper lines (41). The specificity of these two signals may therefore be reciprocal; further study of IL-4 activity within human T cell subpopulations will resolve this question.

Only a portion of CD4+ cells respond to anti-CD3–Seph in a proliferative assay. However, our results suggest that treatment of CD4+ cells with anti-CD3–Seph and IL-2 stimulates TsGF production. The phenotype of TsGF-producing cells with regard to expression of the CD45R and CDw29 antigens is therefore of considerable interest. The CD4+, Leu8+ subset, described previously as containing the population necessary for TsGF production (20), contains both CD45R+ and CDw29+ cells (19). CD4+, Leu8+, CD45R+ cells did not proliferate in response to stimulation with anti-CD3–Seph, but may be capable of TsGF production in response to antigen-receptor binding. This population would then be expected to respond to TsGF in an autocrine manner by expressing IL-2-R. However, anti-CD3–Seph plus exogenous IL-2 was insufficient for CD4+, CD45R+ cell proliferation; consequently, the CD45R+ population is unlikely to produce sufficient TsGF for autocrine stimulation of this subset. A more likely possibility is that CD4+, Leu8+, CDw29+ cells, responsive to either PWM (19) or anti-CD3–Seph and IL-2, produce TsGF after appropriate stimulation. CD4+, CD45R+ cells have been described by Morimoto et al. (19) as suppressor-inducers, but the role of this population may be solely in stimulating differentiation of CD8+ Ts and not in the induction of proliferation. For example, the population characterized as suppressor-inducers may actually be IFN-γ producers, whereas at least one of the two necessary signals for suppressor-effector proliferation would be delivered by a CDw29+ population. Definition of the exact roles these molecules have in the stimulation of IL-2 responsiveness and
the induction of effector function should provide significant insight into the processes whereby immune function is governed.

Summary

Because of the central role of IL-2 in clonal expansion of T cells, we have postulated that lymphocyte subpopulations with opposing regulatory functions might be independently regulated by differential requirements for expression of cell-surface IL-2-R. Purified CD4+ and CD8+ cells proliferated in an IL-2-dependent manner to crosslinked anti-T cell receptor antibodies (anti-CD3–Seph). Similarly, both CD4+ and CD8+ cells became IL-2 responsive after incubation in T suppressor cell growth factor (TsGF), a newly described ~8,000 M_r product of activated CD4+ cells. In support of our hypothesis, however, we observed that subpopulations of CD4+ and CD8+ cells, possessing distinct cell-surface antigens, showed differential responses to these stimuli. Those cells of suppressor-inducer or suppressor-effector phenotype failed to proliferate when cultured in anti-CD3–Seph plus IL-2, but did proliferate in an IL-2-dependent manner to TsGF. Furthermore, the suppressor-effector population was unresponsive to TsGF plus IL-2 when cocultured in anti-CD3–Seph, suggesting that functionally induced Ts may be refractory to growth stimuli. Conversely, cells with helper-inducer or cytolytic phenotype proliferated when incubated in anti-CD3–Seph and IL-2, while remaining essentially unresponsive to TsGF and IL-2. The results could not be explained by differences in the level of CD3 expression by the T cell subsets. Thus, cells within the helper and suppressor lineages appear to have distinct and reciprocal patterns for the induction of IL-2 responsiveness.

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