IMMUNOREGULATORY T CELL CIRCUITS IN MAN

Alloantigen-primed Inducer T Cells Activate Alloantigen-specific Suppressor T Cells in the Absence of the Initial Antigenic Stimulus*

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An allogeneic mixed leukocyte reaction (MLR)[1] offers an ideal system to analyze interactions among various phenotypically and functionally distinct subsets of T lymphocytes. Allospecific helper, inducer, suppressor, and cytotoxic functions are all activated in this reaction (1–11). Although alloantigen-specific suppressor T cells are generated in MLR, neither their mechanism of action nor the cellular and molecular signals that lead to the activation of suppressor T cells as opposed to cytotoxic T cells are understood.

In earlier studies we observed that subsequent to mitogen-induced activation, T cells of helper/inducer lineage (Leu-3+,OKT4+) stimulated fresh T cells of suppressor/cytotoxic lineage (Leu-2+,OKT8+) to proliferate. Moreover, following activation by autologous mitogen-activated Leu-3+ cells, these Leu-2+ cells were capable of inhibiting the response of fresh T cells to a variety of stimuli (12, 13). The present study was undertaken to analyze the functional consequences of exposing fresh T cells to alloactivated T cells. The results indicate that Leu-2+ T cells respond to alloactivated autologous Leu-3+ T cells and that subsequent to activation these cells specifically suppress the proliferation of fresh autologous Leu-3+ T cells to the original allogeneic stimulator cells but not to the third party allogeneic stimulators.

Materials and Methods

Separation of E-Rosette–forming and Nonrosette-forming Cells from Peripheral Blood Lymphocytes. Peripheral blood mononuclear cells from healthy human donors were obtained by Ficoll-Hypaque gradient centrifugation (14). T cells and non-T cells were separated by a single step rosetting method (15) using 2-aminoethylisothiouronium bromide hydrobromide (Sigma Chemical Co., St. Louis, MO)-treated sheep erythrocytes (AET.SRBC) and a second Ficoll-Hypaque gradient to separate the rosette-forming T cells from the nonrosetting (non-T) cells. The rosetted T cells were freed from SRBC by hypotonic lysis of the latter and an aliquot was set aside for assessment of purity as has been previously described (16). The T cell fraction contained >95% E-rosette–forming cells and <5% alpha naphthyl acetate esterase (ANAE)-positive phagocytic cells or surface Ig+ B cells. The non-T cell fraction included 40–50% SIg+ B cells, 25–40% ANAE+ phagocytic

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plastic-adherent monocytes and 5–10% nonadherent nonphagocytic SIg– non-T-non-B (null) cells.

Monoclonal Antibodies. The monoclonal antibodies used in this study were produced by the method of somatic cell hybridization (17). Those antibodies defining mutually exclusive and functionally distinct T cell subsets, anti-Leu-2a and anti-Leu-3a, were generously provided by Dr. R. L. Evans, Memorial Sloan-Kettering Cancer Center, New York and have been described previously (18–20). Antibody 9.3 reacts with 60–80% of peripheral T cells (21) and was a generous gift from Dr. J. A. Hansen, Fred Hutchinson Cancer Research Center, Seattle. Fluorescein-conjugated anti-human HLA-DR framework antibody was obtained from Becton Dickinson Corp. (Mountain View, CA).

Isolation of T Cell Subpopulations. T cell subsets were obtained by a panning technique (22) that permitted the fractionation of fresh or cultured T cells into Leu-2+/Leu-2– or Leu-3+/Leu-3– subpopulations (19, 20). The purity of both positively and negatively selected subsets was determined by indirect immunofluorescence analysis on an Ortho Cytofluorograph System 50H (Ortho Diagnostics, Westwood, MA). If anti-Leu-3 was used as the first stage incubating antibody, then <2% of the unbound cells were Leu-3+. Similar subset purity was achieved when anti-Leu-2 was used as the first stage incubating antibody. Earlier studies from this laboratory (19, 20) have shown that Leu-2+ cells and Leu-3+ cells isolated by positive selection (that is, having bound antibody) do not differ in responsiveness to a variety of stimuli from the same subset enriched by negative selection (lacking bound antibody). To assure purity, positively selected T cell subsets were used whenever possible in the experiments reported here. In some experiments Leu-3– cells (Leu-2+) were further separated into 9.3+ and 9.3– subsets by panning with the 9.3 antibody. Cytofluorographic analysis of the 9.3– subpopulation of Leu-2+ cells showed that <4% of the unbound cells were 9.3+. Isolated subsets were suspended to 1 × 10^6 cells/ml in RPMI 1640 supplemented with 25 mM Hepes, 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, 5 × 10^{-5} M 2-mercaptoethanol and 20% heat-inactivated pooled human serum, hereafter referred to as complete medium. During the entire process of separation of T cells and their subpopulations, pooled heat-inactivated normal human serum was used as a source of serum.

Cell Cultures. For activation of T cell subsets in allogeneic MLR, 5 × 10^4 Leu-2+ or Leu-3+ cells were cultured with an equal number of irradiated (3,000 rads from a ^{137}cesium unit, Shepherd JL and Assoc., Glendale, CA) allogeneic non-T cells in 0.2 ml of complete medium in 96-well round-bottom microtiter plates (Linbro, Flow Laboratories, McLean, VA). Cultures were maintained for 7 d at 37°C in humidified 10% CO_2 atmosphere. Thereafter, cells from each well were pooled, washed, and enriched for T cells by E-rosette formation as described above. In primary cultures, 5 × 10^4 responder Leu-2+ or Leu-3+ T cells were cultured with an equal number of irradiated (2,000 rads) alloactivated or nonactivated autologous Leu-2+ or Leu-3+ cells in round-bottom microtiter plates as described above. T cells obtained from the above cultures after 7 d, were treated with mitomycin C (MMC, Sigma Chemical Co., St. Louis, MO; 50 μg/ml at 37°C for 30 min) and washed extensively before their coculture with 5 × 10^4 fresh autologous Leu-3+ cells and 5 × 10^4 irradiated allogeneic non-T cells in order to assess their regulatory influence on MLR.

To measure T cell proliferation 1 μCi/well of [^{3}H]thymidine (New England Nuclear, Boston, MA; 6.7 Ci/mM) was added to triplicate cultures 18 h before harvesting cells with a Mash II apparatus (Microbiological Associates, Walkersville, MD). The incorporation of radiolabel in the cultures was measured by a Beckmann LS 7000 liquid scintillation spectrometer and the results are expressed as counts per minute (cpm) ± standard error of the mean (SEM).

The percent suppression was calculated by the following equation:

$$\% \text{ Suppression} = 100 \times \left(1 - \frac{\text{cpm in cultures with activated T cells as regulators}}{\text{cpm in cultures with control T cells as regulators}}\right).$$

Negative values of suppression represent enhancement.

Results

Proliferative Response of T Cell Subsets to Alloactivated T Cells. Leu-2+ or Leu-3+ T cells were cultured with irradiated allogeneic non-T cells for 7 d. Experiments not shown indicated that following such cultures 60–80% of Leu-3+ cells, but only 5–20% of Leu-2+ cells expressed Ia antigens based on cytofluorographic analysis with a monoclonal anti-human HLA-DR framework-specific antibody. Cells recovered from the above cultures were enriched for T cells by E-rosetting, irradiated, and tested for their ability to stimulate proliferation of fresh autologous Leu-2+ or Leu-3+ cells. As shown in Table I, fresh Leu-3+ cells responded vigorously to autologous non-T cells, but weakly to alloactivated Leu-3+ cells. In contrast, fresh Leu-2+ cells exhibited significant proliferation when cultured with alloactivated autologous Leu-3+ cells but lacked responsiveness to autologous non-T cells as has been shown previously (19, 23). Neither T cells cultured alone nor Leu-2+ cells cultured with allogeneic non-T cells induced proliferation of fresh autologous Leu-2+ or Leu-3+ cells.

Regulatory Influence of T Cell Subsets Activated with Alloantigen-primed T Cells. The overall experimental design for these experiments is shown in Fig. 1. T cell subsets were stimulated with alloantigen-primed autologous T cells, reisolated, and examined for their regulatory influence on the proliferative response of fresh autologous Leu-3+ cells in MLR. Fig. 2 shows that Leu-2+ cells subsequent to their activation with alloantigen-primed autologous Leu-3+ cells suppressed, in a dose-related manner, the proliferative response of fresh autologous Leu-3+ cells in MLR. In contrast, Leu-2+ cells isolated from cultures stimulated either with alloactivated Leu-2+ cells or with nonactivated Leu-2+ or Leu-3+ cells had no effect on the proliferation of autologous Leu-3+ cells in MLR. Leu-3+ cells isolated from cultures stimulated with either alloactivated Leu-2+ cells or nonactivated Leu-3+ cells had no effect on the proliferation of autologous Leu-3+ cells. Moreover, Leu-3+ cells recovered from cultures stimulated with alloantigen-primed autologous Leu-3+ cells induced significant enhancement of autologous Leu-3+ cell-proliferation in MLR.

The suppressive influence of Leu-2+ cells activated with alloantigen-primed autologous Leu-3+ cells was specific for the original alloantigenic stimulus, in that these cells failed to suppress the proliferative response of fresh autologous

TABLE I

Proliferative Response of T Cell Subsets to Various Autologous Stimulator Cells

Experiment	Responder T cells	³H-TdR incorporation in cultures stimulated with*				
		Non-T cells	Nonactivated‡ Leu-3+ cells	Alloactivated Leu-3+ cells	Nonactivated Leu-2+ cells	Alloactivated Leu-2+ cells
1	Leu-2+ cells	785 ± 32§	418 ± 46	7,405 ± 539	412 ± 61	506 ± 59
	Leu-3+ cells	17,806 ± 1,347	980 ± 102	1,329 ± 247	808 ± 105	616 ± 105
2	Leu-2+ cells	693 ± 25	255 ± 25	6,869 ± 450	185 ± 6	216 ± 26
	Leu-3+ cells	12,886 ± 978	259 ± 18	359 ± 60	386 ± 85	446 ± 55
3	Leu-2+ cells	971 ± 81	737 ± 85	3,290 ± 191	469 ± 45	650 ± 61
	Leu-3+ cells	7,791 ± 551	349 ± 51	268 ± 61	133 ± 6	293 ± 25
4	Leu-2+ cells	317 ± 88	339 ± 37	5,382 ± 906	268 ± 75	403 ± 75
	Leu-3+ cells	27,833 ± 205	1,028 ± 272	1,036 ± 97	1,017 ± 18	624 ± 152

* Responder/stimulator cell ratio of 1:2.
‡ Cultured alone in complete medium for 7 d.
§ Mean of triplicate cpm ± SEM. Background uptake was always <200 cpm.
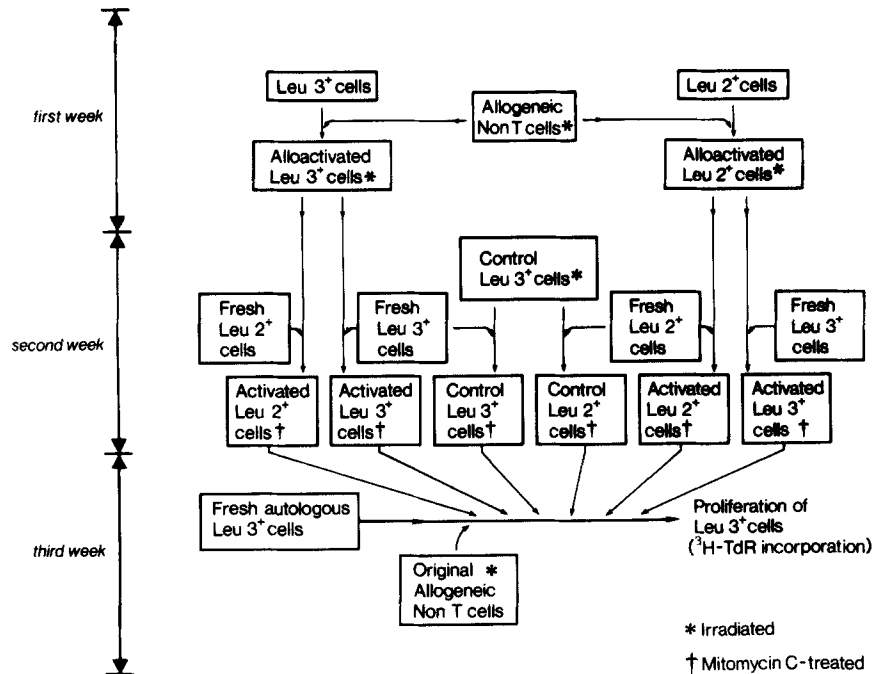
FIGURE 1.   Schematic representation of the overall experimental design for studies of the regulation of MLR.

Leu-3+ cells to third party allogeneic non-T cells (Table II). By contrast, the enhancing effect of cultured Leu-3+ cells extended to the Leu-3+ cell-response to third party allogeneic stimuli as well as the original allogeneic stimulus, although in most experiments the enhancement was greater when the original stimulator cells were used (Table II).

Subpopulations of Leu-2+ Cells Responding to Autologous Alloactivated Leu-3+ Cells.   Antibody 9.3 reacts with 60–80% of peripheral T cells which include all or nearly all of the Leu-3+ cells and also a subpopulation of Leu-2+ cells (24). Thus, the Leu-2+ subset can be divided into 9.3+ and 9.3– subpopulations. The proliferative response of 9.3+ and 9.3– subpopulations of Leu-3– (Leu-2+) cells to autologous alloactivated Leu-3+ cells was studied. Table III shows that the cells included in the Leu-2+,9.3– subpopulation proliferated to alloactivated Leu-3+ cells, whereas Leu-2+,9.3+ cells did not.

The 9.3+ and 9.3– subpopulations of Leu-2+ cells were stimulated with alloactivated Leu-3+ cells, reisolated into Leu-2+ cells and examined for their suppressive effect on fresh MLR. The data in Fig. 3 demonstrate that suppressor activity induced by alloantigen-primed Leu-3+ cells was observed in the Leu-2+,9.3– subpopulation whereas the Leu-2+,9.3+ cells recovered from these cultures had no detectable effect on the MLR response of fresh autologous Leu-3+ cells. Thus, the Leu-2+,9.3– subpopulation includes precursors of suppressor T cells which can be activated by alloantigen-primed Leu-3+ cells.

Kinetics of the Suppressive Effect of Activated Leu-2+ Cells on MLR.   In the experiments described above, the proliferative response of Leu-3+ cells in the
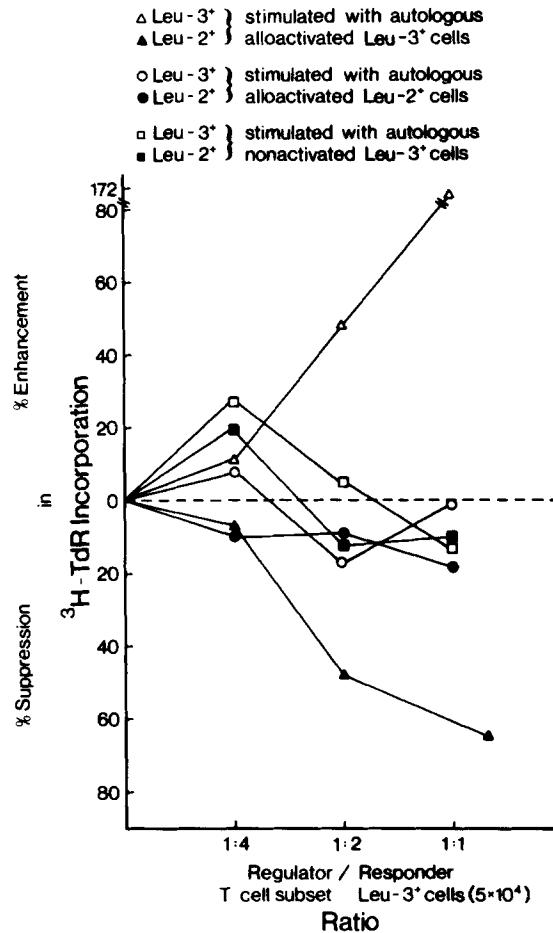
FIGURE 2.   Regulatory influence of T cell subpopulations after activation with alloantigen-primed T cell subsets. Leu-2+ or Leu-3+ cells were activated with alloactivated or naive autologous T cells, reisolated, treated with mitomycin and then cocultured in varying numbers with 5 × 10⁴ fresh autologous Leu-3+ cells and 5 × 10⁴ irradiated allogeneic non-T cells. [³H]Thymidine uptake in these cultures was measured on day 7.

final indicator MLR cultures was measured at the usual time point (day 7) of peak response in MLR (25). To assess the possibility that activated Leu-2+ cells might alter the kinetics of this response, Leu-2+ cells isolated subsequent to their activation with alloantigen-primed Leu-3+ cells were cultured with fresh autologous Leu-3+ cells and the original allogeneic stimulator non-T cells. Replicate cultures were harvested on varying days and the results are shown in Fig. 4. Suppression of the MLR response of Leu-3+ cells by activated Leu-2+ cells was observed throughout the culture period without evidence of altered kinetics. In contrast, the MLR response of Leu-3+ cells was not inhibited at any time point by Leu-2+ cells that had been exposed to nonactivated Leu-3+ cells.

We considered the possibility that the suppression of MLR by activated Leu-2+ cells was due to lysis by these cells of the stimulator non-T cells leading to apparent suppression of the MLR response. However, in standard 4–6 h

TABLE II

Specificity of Activated Regulator T Cell Subsets

Experi-ment	Regulator T cells	Stimulation of regulator cells with	^{3}H-TdR incorporation in cultures stimulated with*	
			Original allogeneic non-T cells	Third party allogeneic non-T cells
1	Leu-3+ cells	Nonactivated[‡] Leu-3+ cells	42,480 ± 1,122[§]	37,814 ± 3,490
	Leu-3+ cells	Alloactivated Leu-3+ cells	52,712 ± 2,039 (25%↑)[I]	46,192 ± 2,289 (22%↑)
	Leu-2+ cells	Nonactivated Leu-3+ cells	45,627 ± 5,395	35,076 ± 5,200
	Leu-2+ cells	Alloactivated Leu-3+ cells	22,784 ± 3,691 (50%↓)	37,000± 4,240 (6%↑)
2	Leu-3+ cells	Nonactivated Leu-3+ cells	13,598 ± 2,650	24,136 ± 3,634
	Leu-3+ cells	Alloactivated Leu-3+ cells	19,295 ± 2,244 (42%↑)	30,699 ± 2,068 (27%↑)
	Leu-2+ cells	Nonactivated Leu-3+ cells	20,607 ± 3,597	22,429 ± 2,672
	Leu-2+ cells	Alloactivated Leu-3+ cells	11,389 ± 2,257 (45%↓)	22,793 ± 4,511 (2%↑)

* Responder/regulator/stimulator cell ratio of 1:1:1.
[‡] Cultured alone in complete medium for 7 d.
[§] Mean of triplicate cpm ± SEM. Background uptake was always <200 cpm.
[I] Figures in parentheses indicate enhancement (↑) or suppression (↓).

TABLE III

Proliferative Response of Subpopulations of Leu-2+ Cells to Autologous Stimulator Cells

Experi-ment	Responder T cells	^{3}H-TdR incorporation in cultures stimulated with*		
		Non-T cells	Nonactivated[‡] Leu-3+ cells	Alloactivated Leu-3+ cells
1	Leu-2+ cells	777 ± 114[§]	181 ± 34	14,001 ± 824
	Leu-2+,9.3+ cells	326 ± 19	684 ± 72	918 ± 173
	Leu-2+,9.3− cells	392 ± 72	404 ± 121	13,711 ± 1,858
2	Leu-2+ cells	596 ± 71	373 ± 75	8,235 ± 1,258
	Leu-2+,9.3+ cells	721 ± 98	232 ± 90	669 ± 130
	Leu-2+,9.3− cells	684 ± 92	231 ± 91	14,469 ± 1,947
3	Leu-2+ cells	785 ± 75	345 ± 99	5,193 ± 492
	Leu-2+,9.3+ cells	693 ± 22	808 ± 98	839 ± 073
	Leu-2+,9.3− cells	317 ± 67	469 ± 98	9,651 ± 1,244

* Responder/stimulator ratio of 1:2.
[‡] Cultured alone in complete medium for 7 d.
[§] Mean of triplicate cpm ± SEM. Background uptake was always <200 cpm.

^{51}chromium release assays, Leu-2+ cells stimulated by alloactivated autologous Leu-3+ cells did not exhibit any detectable cytotoxic activity against autologous or allogeneic lymphoid cells even at effector to target cell ratios as high as 100:1 (data not shown). These results thus argue against lysis of stimulator non-T cells as a mechanism to explain the observed inhibition of the MLR response and suggest that this method of Leu-2+ cell-activation results in the selective induction of suppressor T cells. In additional studies, fresh Leu-3+ responder cells
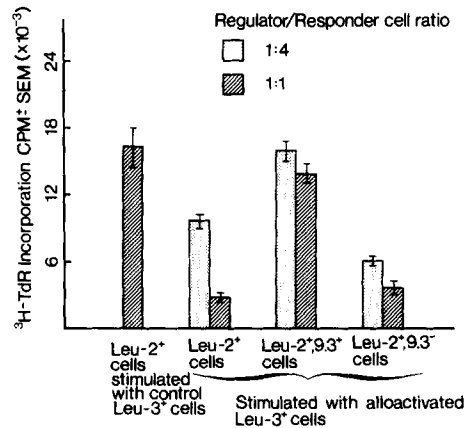
FIGURE 3.   Precursors of suppressor T cells in the Leu-2+,9.3— subpopulation. Leu-2+ cells were separated into 9.3+ and 9.3— subsets, stimulated with alloantigen-primed Leu-3+ cells and then tested for their ability to suppress a fresh MLR between 5×10^4 fresh autologous Leu-3+ cells and 5×10^4 irradiated allogeneic non-T cells. [^{3}H]Thymidine uptake in these cultures was measured on day 7.
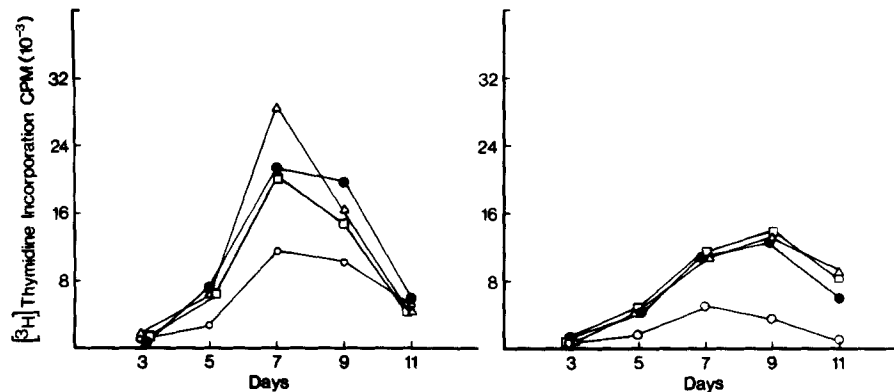

FIGURE 4.   Effect of activated Leu-2+ cells on the kinetics of MLR response. 5×10^4 fresh Leu-3+ cells were mixed with 5×10^4 irradiated allogeneic non-T cells and were cultured alone (△) or with 5×10^4 Leu-2+ cells isolated from cultures with either alloantigen-primed Leu-3+ (○) or nonactivated Leu-3+ cells (●) or with medium alone (□). On days 3, 5, 7, 9, and 11 aliquots of cells were tested for [^{3}H]thymidine uptake. Data from two independent experiments are shown.

were stimulated with an increasing number of allogeneic non-T cells in the presence of a fixed number of activated Leu-2+ cells. Fig. 5 shows that the inhibition by activated Leu-2+ cells was observed at all the concentrations of stimulator non-T cells tested, suggesting that the suppressive effect of activated Leu-2+ cells may be directed toward the responder Leu-3+ cells rather than stimulator non-T cells.

Effect of Interleukin-2 on the Suppression by Activated Leu-2+ Cells.   Absorption and depletion of interleukin-2 (IL-2) by putative suppressor T cells has been proposed as a mechanism to explain the antigen-specific suppressor effects of mitogen-activated T cells (26). To evaluate the effect of IL-2 on the suppressor
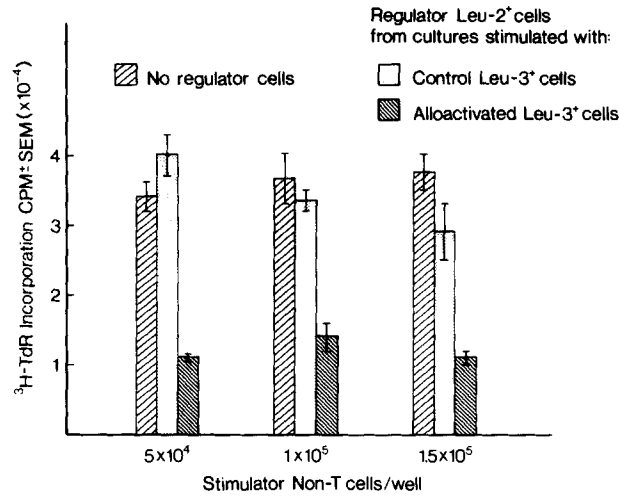
FIGURE 5. Effect of stimulator non-T cell concentration in the indicator MLR on the suppression by activated Leu-2+ cells. Leu-2+ cells isolated from cultures with either alloantigen-primed Leu-3+ cells or naive Leu-3+ cells, were treated with mitomycin and cocultured with 5 × 10⁴ fresh autologous Leu-3+ cells and varying concentrations of irradiated allogeneic non-T cells as indicated. [³H]Thymidine uptake in these cultures was measured on day 7.
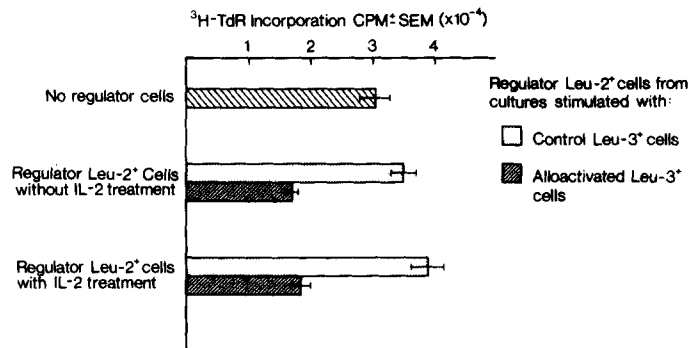

FIGURE 6. Effect of interleukin-2 (IL-2) treatment of activated Leu-2+ suppressor cells of MLR. Leu-2+ cells isolated from cultures with alloantigen-primed Leu-3+ cells or nonactivated Leu-3+ cells, were incubated for 60 min at 37°C with culture supernatant containing IL-2 activity (10 U/ml), washed and then added to MLR between 5 × 10⁴ fresh Leu-3+ cells and 5 × 10⁴ irradiated allogeneic non-T cells. [³H]-Thymidine uptake in these cultures was measured on day 7.

cells under study, Leu-2+ cells derived from cultures stimulated with alloantigen-primed Leu-3+ cells, were incubated at 37°C for 60 min with IL-2 containing (10 U/ml) culture supernatant of MLA 144, a gibbon leukemia line that constitutively produces IL-2 (27). These cells were then washed and added to the final indicator MLR cultures. Fig. 6 shows that the suppressor effect of the activated Leu-2+ cells was not reduced by incubation of these cells in the conditioned medium containing IL-2.

Effect of Activated Leu-2+ Cells Added After Initiation of MLR. In the experiments described above, activated Leu-2+ cells were added at the initiation of indicator
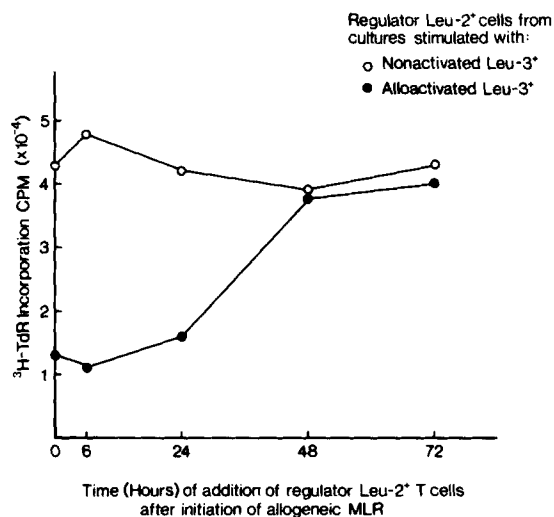
FIGURE 7. Effect of delayed addition of activated Leu-2+ cells to MLR. 5 × 10⁴ Leu-2+ cells isolated from cultures with alloantigen-primed Leu-3+ cells, were added at different times after initiation of MLR consisting of 5 × 10⁴ fresh Leu-3+ cells and 5 × 10⁴ irradiated allogeneic non-T cells. [³H]Thymidine uptake in these cultures was measured on day 7.

MLR cultures. It was of interest to determine whether suppressor T cells exert inhibitory effects at an early or late stage of T cell activation. To address this question, Leu-2+ cells activated with alloantigen-primed Leu-3+ cells were added at different time points to replicate MLR and their suppressive effects were measured on day 7. It is apparent from Fig. 7 that activated Leu-2+ cells added as late as 24 h after initiation of the MLR could still suppress the response of fresh Leu-3+ cells. However, if the addition of activated Leu-2+ cells to the MLR was further delayed, no inhibition was observed, suggesting that suppressor Leu-2+ cells affect an early stage in the activation of Leu-3+ cells in MLR. Once activated, these Leu-3+ cells cannot be suppressed by activated Leu-2+ cells in this system.

Discussion

This study was an attempt to elucidate the mechanism whereby suppressor T cells are induced in MLR. Leu-3+ cells activated with allogeneic non-T cells acquired the ability to stimulate proliferation of fresh autologous Leu-2+ cells. Following their proliferation and presumed differentiation, Leu-2+ cells suppressed the response of fresh autologous Leu-3+ cells in a dose-dependent manner to the original, but not to third party, allogeneic non-T cells in the MLR (Fig. 2 and Table II). Thus the data reported here extend previous findings in an antigen-nonspecific system (12, 13) to an antigen-specific one. Although the antigenic stimulus for the induction of suppression was not defined here, previous studies have suggested that products of HLA-DR or linked loci are major stimuli (10, 11).

The mechanism whereby activated Leu-2+ cells suppress proliferation in MLR was not completely elucidated, although several possibilities were examined. Leu-

2+ suppressor cells had not been directly exposed to either class I or class II alloantigen-bearing non-T cells that classically serve as stimulators and targets for cytotoxic T cells. Furthermore, the Leu-2+ cells that responded to alloantigen-primed autologous Leu-3+ cells lacked detectable cytolytic activity for autologous or allogeneic targets. Therefore, cytolysis of stimulator allogeneic non-T cells by activated Leu-2+ cells does not appear to be responsible for their suppressive effect. Although Leu-2+ cell-mediated lysis of alloantigen-responsive Leu-3+ cells in the final indicator MLR cannot be completely ruled out, the observation that delayed addition of activated Leu-2+ cells to MLR cultures did not result in suppression (Fig. 7) argues against this possibility. Selective absorption of IL-2 by suppressor T cells (26) also seems unlikely as the treatment of activated Leu-2+ cells with IL-2 containing medium had no effect on their ability to inhibit the MLR response of fresh autologous Leu-3+ cells. Furthermore, the fact that the suppression exerted by these cells was antigen-specific argues against growth factor depletion as an explanation for the suppression of the MLR.

In the present study, suppressor T cell activity was confined to the Leu-2+ subset. Using different assay systems, investigators have described the development of suppressor activity in the Leu-3+ (OKT4+) subset (28, 29). However, in the current study Leu-3+ cells cultured with alloantigen-primed Leu-3+ cells not only failed to suppress, but consistently augmented the MLR response of fresh Leu-3+ cells. As shown in Table II, this enhancement was greatest when the original allogeneic cells were used as stimulators in the indicator MLR, although the response to third party allogeneic stimuli was augmented to a lesser degree. One possible explanation for this partial specificity may be that some irradiated alloantigen-primed Leu-3+ cells remained viable in the culture and upon reexposure to the original stimulator cells released antigen-nonspecific growth-promoting factors. The alternative possibility that antigen-specific as well as nonspecific helper factors are released by these cells cannot be excluded. Based on the observation that alloactivated Leu-3+ cells enhanced the fresh MLR, no further studies of this subset were undertaken.

Although the activation of suppressor T cells appears to be dependent on functionally intact inducer T cells or their soluble products (30, 32), it is not known whether the signals that activate cytotoxic T cells also activate suppressor T cells. Activation of cytotoxic T cells in MLR is thought to be dependent on two types of inductive signals: an initial signal represented by class I alloantigens and a second signal delivered by alloactivated T cells of helper/inducer lineage (6, 33–36). The data presented here indicate that structures either displayed on the surface of alloactivated Leu-3+ cells or secreted by them induce precursors of Leu-2+ suppressor T cells to become suppressor-effectors in the apparent absence of allogeneic stimulator cells. In this regard, a number of studies have shown that upon activation in MLR some T cells synthesize and express Ia antigens of responder type (11, 37–41). In the present study a significant fraction of Leu-3+ but not Leu-2+ cells recovered from MLR were Ia+, presumably reflecting the differential ability of cells within the two major sublineages of T cells to recognize and respond to class II MHC determinants (19). Although Ia antigens on alloantigen-primed Leu-3+ cells might play a role in the activation of Leu-2+ suppressor T cells, it is difficult to envisage how recognition of

autologous Ia determinants could lead to alloantigen-specific suppression. On the other hand, the induction of Leu-2+ suppressor cells could be due to recognition by these cells of allogeneic Ia antigens adsorbed onto the surface of alloactivated Leu-3+ cells (40) in a manner that leads to the selective activation of suppressor but not cytotoxic or helper/inducer cells. Finally, it has been reported that alloactivated T cells express clonally specific (idiotypic) determinants that trigger a specific proliferative response of fresh autologous T cells (42, 43). An approach in which primed Leu-2+,9.3− suppressor cells are tested for their secondary proliferative response to either autologous alloactivated Leu-3+ cells or allogeneic non-T cells might help to distinguish between these two possibilities.

Recent studies indicate that precursors of Leu-2+ alloantigen-specific cytotoxic T cells are included in the Leu-2+,9.3+ subset but not in the Leu-2+,9.3− subset of T cells, whereas in a variety of systems all or nearly all Leu-2+ suppressor T cells are derived from the Leu-2+,9.3− subset (Damle, N., N. Mohagheghpour, J. A. Hansen, and E. G. Engleman, manuscript submitted for publication). As shown here, Leu-2+ cells that proliferate in response to alloantigen-primed autologous Leu-3+ cells are included in the 9.3− but not in the 9.3+ subpopulation of Leu-2+ cells. In addition, the suppressor function activated by alloantigen-primed autologous Leu-3+ cells appears to be confined to the Leu-2+,9.3− subpopulation of Leu-2+ cells. These observations further support our contention that precursors of suppressor but not cytotoxic T cells are activated by alloantigen-primed autologous Leu-3+ cells. This type of T-T interaction appears to be an important mechanism whereby suppressor T cells are preferentially activated by inducer T cells.

T cell circuits that regulate the immune response to antigens have been studied extensively in murine models and like the human system under study here, such circuits appear to be dependent on helper/inducer T cells for their initiation (44). For example, antigen-stimulated Lyt-1+ T cells, in addition to helping B cells to differentiate into antibody-secreting plasma cells, also induce nonstimulated Lyt-1+,2/3+,Qa-1+ T cells to mediate specific suppressor effects (32, 45). In the human T cell circuit, described schematically in Fig. 8, the Leu-3+ helper/inducer population plays a central role in the activation of two types of negative feedback regulator cells: Leu-2+,9.3− suppressor cells, which presumably inhibit at the level of Leu-3+ helper/inducer cells and Leu-2+ cytotoxic cells, which eliminate allogeneic stimulator cells. Although functionally distinct subsets have been described within the Leu-3+ lineage (46, 48), it is not known whether the suppressor-inducer T cells differ from the cytotoxic-inducer T cells. Additional T cell subsets, not depicted in this figure but involved in the regulation of other model systems of immunity, may also play a role in the regulation of MLR. For example, optimal suppression of immunoglobulin synthesis in vitro requires collaboration of at least two distinct subsets of Leu-2+ cells (47, 48), one of which, upon activation, amplifies the suppressor effects of fresh Leu-2+ cells (47). It is not known whether Leu-2+ cells activated with the approach described here can amplify the suppressor effect of fresh Leu-2+ cells, since fresh Leu-2+ cells were not added to indicator MLRs to assess their regulatory effect.

We have succeeded in generating alloantigen-specific suppressor T cells from
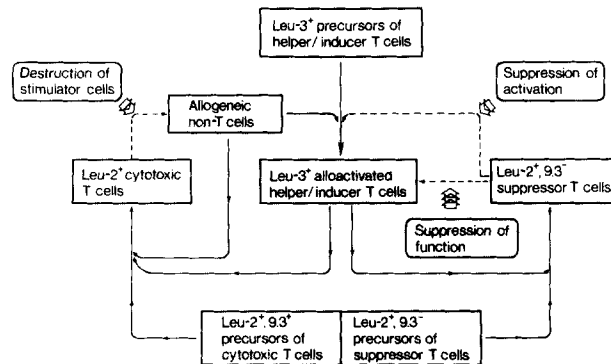
Regulation of allogeneic MLR

FIGURE 8.   Schematic representation of the cellular interactions involved in the induction of regulatory and effector mechanisms in the MLR.

every individual tested to date using the above described approach. However, it remains to be seen whether T-T interactions of the type described here apply in other antigen-specific systems and in particular to the response to soluble antigens. If so, a general scheme can be suggested for the induction of antigen-specific suppressor T cells. Conceivably, suppressor T cells with a variety of specificities can be generated with this approach and used in the analysis of the molecular events that mediate feedback regulation of immunity.

Summary

Although alloantigen-specific suppressor T cells are generated in MLR, the cellular signals that lead to activation of suppressor T cells as opposed to cytotoxic T cells are unknown. The current study was undertaken to characterize interactions among T cell subsets involved in the generation of suppressor T cells in MLR. Human peripheral blood Leu-2+ (suppressor/cytotoxic) and Leu-3+ (helper/inducer) T cell subsets were activated with allogeneic non-T cells and then examined for their inductive effects on fresh autologous T cells. Fresh Leu-2+ cells proliferated in response to alloantigen-primed Leu-3+ cells and subsequently suppressed the response of fresh autologous Leu-3+ cells to the original, but not third party, allogeneic stimulator non-T cells. Moreover, only Leu-2+ cells that lacked the 9.3 marker, an antigen present on the majority of T cells including precursors of cytotoxic T cells, differentiated into suppressor cells. The alloantigen-specific suppressive effect of Leu-2+,9.3− cells was not mediated by cytolysis of allogeneic stimulator cells, nor could it be explained by alteration of MLR kinetics. Suppression was observed only when activated Leu-2+ cells were added to fresh MLRs within 24 h of initiation of cultures, suggesting that these cells block an early phase of the activation of Leu-3+ cells in MLR. These results indicate that alloantigen-primed inducer T cells can activate alloantigen-specific suppressor T cells in the absence of allogeneic stimulator cells.

Note added in proof: Since submission of this manuscript, we have become aware of a report demonstrating the activation of influenza virus-specific suppressor T

cells by a helper T cell clone (Lamb, J. and M. Feldmann. 1982. A human suppressor T cell clone which recognizes an autologous helper T cell clone. *Nature (Lond.).* 300:456). In addition we have succeeded recently in inducing specific suppressor T cells with tuberculin PPD-primed inducer T cells.

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