Functional Heterogeneities among Concanavalin A-activated OKT4⁺ and OKT8⁺ Cells by Using Autologous Erythrocyte Rosette Technique

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ABSTRACT Normal human peripheral blood T lymphocytes activated by concanavalin A (Con A) were fractionated into OKT4⁺ and OKT8⁺ populations by complement-dependent cell lysis using OKT8 and OKT4 antibodies, respectively. By using the preferential ability of some, but not all, Con A-activated T cells to form rosettes with autologous erythrocytes, each population was further divided into autorosetting cells and nonautorosetting cells, and thus Con A-activated OKT4⁺ autorosetting, OKT4⁺ nonautorosetting, OKT8⁺ autorosetting, and OKT8⁺ nonautorosetting cells were obtained. The immune regulatory function of these populations was then investigated using a pokeweed mitogen-driven B cell plaque-forming cell system. These studies demonstrated that (a) autorosetting cells can exert potent suppressor activity regardless of their phenotypes of OKT4⁺ and OKT8⁺ antigens, and fail to help B cell differentiation; suppressor function mediated by these cells is radiosensitive; moreover, receptors for autologous erythrocytes may constitute either the interleukin 2 (IL2) receptors themselves or a component of an IL2 receptor-effector complex involved in modulating the growth signal that IL2 transmits to T cells; (b) OKT4⁺ nonrosetting cells serve adequately as radioresistant helper cells, but are devoid of suppressor cells; and (c) OKT8⁺ nonrosetting cells are found to lack either suppressor or helper activity, suggesting that they may belong to a T lymphocyte subset distinct from the subsets related to immune regulation. The results lead us, therefore, to the conclusion that there may exist functional heterogeneities among both the OKT4⁺ and OKT8⁺ populations; these het-

erogeneities can be dissected by virtue of the autologous erythrocyte rosette technique.

INTRODUCTION

Human peripheral T lymphocytes can be subclassified on the basis of differential reactivity to monoclonal antibodies, OKT4 (inducer/helper) and OKT8 (cytotoxic/suppressor). Functional studies indicate that OKT4⁺ T cells can induce B cell differentiation in a pokeweed mitogen (PWM)¹- or antigen-driven system, but do not provide suppressor activity, whereas the reverse is true of OKT8⁺ T cells (1-3).

In our previous studies (4), we found that concanavalin A (Con A)-induced suppressor cells can be identified and separated from Con A-induced helper cells by the autologous erythrocyte rosette technique; suppressor and helper cells are confined to the autorosetting and nonrosetting T cell populations, respectively. Moreover, OKT4⁺ and OKT8⁺ cell types were equally distributed among either autorosetting or nonrosetting T cells activated by Con A (4). These experiments raised the possibility that suppressor cell populations among the autorosetting cells are limited to OKT8⁺ cells, or alternatively, that the autorosetting OKT4⁺ cells would also act as mediators of suppression induced by Con A. It also remained uncertain whether among the nonrosetting cell population, only the cells bearing OKT4⁺ antigen can function as helper cells, or the OKT8⁺ cells may also mediate helper function.

In the present study, Con A-activated T cells were divided into OKT4⁺ autorosetting, OKT4⁺ nonrosetting,

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¹ Abbreviations used in this paper: C, complement; Con A, concanavalin A; IL2, interleukin 2; PFC, plaque-forming cell; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SLE, systemic lupus erythematosus; SRBC, sheep erythrocyte.

OKT8⁺ autorosetting and OKT8⁺ nonrosetting cell populations by means of monoclonal antibodies and autorosette technique, and the regulatory effects of each separate population were investigated on the B cell immunoglobulin production in the system driven by PWM. We observed that Con A-induced suppressor cells can belong to the autorosetting populations, regardless of the phenotype of either OKT4⁺ or OKT8⁺ antigens. In contrast to the function of autorosetting cells, activated nonrosetting OKT4⁺ cells are specific for the function of help. Of interest is that nonrosetting OKT8⁺ cells are related to neither helper function nor suppressor function. Furthermore, relationship between Con-A activated T cell subpopulations and population bearing Tac antigen will be discussed.

METHODS

Isolation of T cells, non-T cells, and monocytes. Peripheral blood mononuclear cells of normal individuals were isolated, and T and non-T cells separated by the sheep erythrocyte (SRBC) rosette technique as described previously (5, 6). Double-purified rosetting T cells were recovered after lysis of SRBC by hypotonic shock. These T cell preparations consisted of >95% T cells as determined by rosetting. Double-purified nonrosetting cells were incubated in a 60 × 55-mm petri dish (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, CA) at 37°C in a 5% CO₂/95% air humidified environment for 2 h, and the cells that did not adhere to the dish and those that adhered firmly were collected separately. The latter cells are referred to as monocytes. Monocyte preparations consisted of 95% cells that were identified as monocyte after Giemsa staining (5, 6). The nonrosetting cells that did not adhere were further treated with OKT3 monoclonal antibody (Ortho Pharmaceutical Corp., Raritan, NJ) and rabbit complement (C; Behringwerke AG, Marburg, FRG). This C-dependent lysis was performed twice. The resultant population was <0.2% SRBC rosette positive and <0.5% cells reactive with OKT3 antibody. This population is designated non-T cells. The reappearance of the OKT3-defined antigen in cells of the non-T cell population (7) did not occur (<1%) when the cells were incubated for 24 h in vitro in the culture medium; moreover, this population did not respond at all to phytohemagglutinin (PHA; Wellcome Research Laboratories, Beckenham, England).

Activation of T cells with Con A (first culture). Con A-activated cultures were established in 17 × 100-mm plastic tubes (Falcon Labware) and consisted of 4 ml of culture medium, RPMI 1640 (Flow Laboratories Inc., Rockville, MD) supplemented with 10% heat-inactivated fetal bovine serum (Flow Laboratories Inc.) containing 4 × 10⁶ purified T cells and 3 × 10⁵ mitomycin (Sigma Chemical Co., St. Louis, MO)-treated monocytes. These cultures were incubated at 37°C in a 5% CO₂/95% air humidified environment in the presence or absence of 40 μg of Con A (Pharmacia Fine Chemicals, Div. Pharmacia, Inc., Piscataway, NJ). 48 h later, the cells were harvested, treated with 0.1 M α-methyl-D-mannoside (Sigma Chemical Co.) in Hanks' balanced salt solution (HBSS) for 30 min at room temperature and were followed by successive washes in 0.1 M α-methyl-D-mannoside.

C-dependent lysis of T cells with the monoclonal antibodies OKT8 and OKT4. 1 × 10⁷ T cells (fresh T cells, T cells from the first culture) were incubated with 100 μl of

either OKT8 or OKT4 monoclonal antibody (Ortho Pharmaceutical Corp.) on ice for 1 h. Thereafter 25 μl of rabbit C was added and further incubation carried out for 1 h at 37°C. To obtain highly purified T cell subpopulations, this entire procedure was repeated twice (8). After washing, the numbers of the viable cells were determined by dye exclusion. Analysis of T cell subsets obtained by the OKT8-treated population contained >92% OKT4⁺ cells and <2% OKT8⁺ cells, whereas the OKT4-treated population contained <3% OKT4⁺ cells and >90% OKT8⁺ cells. In addition, the surface phenotypes of these depleted T cell subsets held in culture for 24 h did not change. These OKT8- and OKT4-depleted cell populations will be referred to as OKT4⁺ and OKT8⁺ cell populations, respectively, because there is evidence so far that OKT4 and OKT8 antigens can be expressed independently of one another (1, 2, 9).

Rosette formation of Con A-activated T cells with autologous erythrocytes and separation of autorosetting cells from nonrosetting cells. To assess the ability of Con A-activated T cell subpopulations to form rosettes with autologous erythrocytes, 2 × 10⁶ Con A-activated OKT4⁺ and OKT8⁺ cells in 200 μl HBSS were placed in 200 μl of fetal bovine serum and 500 μl of 2.5% autologous erythrocytes in HBSS in 12 × 75-mm tubes (Falcon Labware), where they were incubated at 37°C for 10 min. After the incubation, the mixtures were centrifuged at 150 g for 5 min and further allowed to react on ice for 2 h. The cells were gently resuspended for either counting the autorosettes or for their separation on Ficoll-Hypaque gradients from nonrosetting cells (4). For counting, at least 300 lymphocytes were enumerated and T cells binding three or more autologous erythrocytes were regarded as autorosette-forming cells. For separation, the autologous erythrocyte-T cell suspension was layered over Ficoll-Hypaque, centrifuged at 400 g for 20 min at 4°C, and rosetting cells and nonrosetting cells were collected separately. The pelleted rosette-forming cells were resuspended in cold HBSS and further purified on another Ficoll-Hypaque gradient. The rosetting fraction obtained had ≥95% autorosettes. Autologous erythrocytes in this fraction were lysed by hypotonic shock. The nonrosetting population was rosetted with autologous erythrocytes exactly as described above and recentrifuged over Ficoll-Hypaque. This purification procedure gave a fraction containing only 1% autorosettes. Thus, Con A-activated OKT4⁺ autorosetting, OKT4⁺ nonrosetting, OKT8⁺ autorosetting, and OKT8⁺ nonrosetting cells were obtained.

Assay cultures for regulatory activities of Con A-activated T cell subpopulations (second culture). Responder non-T cells to be used in the second helper and suppressor assay cultures, and helper OKT4⁺ cells to be used in the second suppressor assay cultures were obtained 2 d later from the same normal individuals who originally provided the Con A-activated regulatory T cells (10). The ability of activated T cell subpopulations to exert helper activity was determined by adding graded numbers of each cell type to 2 × 10⁵ fresh autologous responder non-T cells in a 1-ml volume in 12 × 75-mm plastic tubes. Polyclonal immunoglobulin production was stimulated by the addition of 1 μg of PWM (Gibco Laboratories, Grand Island, NY) to these cultures, which were incubated at 37°C in an atmosphere of 5% CO₂ in air for 5 d. Thereafter the cells were harvested, washed extensively, and assayed for plaque-forming cell (PFC) activity by a reverse hemolytic plaque assay (see below). Activated T cell populations from the first culture were also tested for their suppressor activity in the second assay culture system. Graded numbers of the activated T cell subpopulations were added to the second autologous cultures containing 2 × 10⁵

fresh responder non-T cells and 1×10^5 fresh OKT4⁺ cells as helper cells, stimulated for 5 d with PWM, and assayed for PFC activity. In some experiments, activated T cell sub-

populations were irradiated with 1,300 rad before the addition to the second culture.

The percent suppression was calculated with the following formula:

$$\% \text{ suppression} = \left(1 - \frac{(\text{PFC activity of non-T cells + fresh OKT4}^+ \text{ cells + regulatory T cell subsets + PWM}) - (\text{PFC activity of non-T cells + fresh OKT4}^+ \text{ cells + regulatory T cell subsets})}{(\text{PFC activity of non-T cells + fresh OKT4}^+ \text{ cells + PWM}) - (\text{PFC activity of non-T cells + fresh OKT4}^+ \text{ cells})} \right) \times 100.$$

Cell counts and viability (by dye exclusion) were also performed on all the second cultures at the time of assay. The yields of viable cells at the end of the culture period were similar in all the second assay cultures.

Reverse hemolytic plaque assay for the enumeration of antibody-secreting cells. The assay for the measurement of immunoglobulin M-producing hemolytic PFC was performed by using a modification of the procedure described by Gronowicz et al. (11). Briefly, on the day of assay, PWM-stimulated cells were thoroughly washed in HBSS and resuspended. 100- μ l aliquots ($5\text{--}10 \times 10^4$ PWM-stimulated cells) were added to 300 μ l of 0.5% agarose in HBSS, 20 μ l of protein A (Pharmacia Fine Chemicals)-coupled SRBC (25% vol/vol), and 20 μ l of a 1:50 dilution of the IgG fraction of a rabbit anti-human IgM (Miles Laboratories, Inc., Miles Research Products, Elkhart, IN). This mixture was poured onto a 60 $\times$ 15-mm petri dish and allowed to gel. The dishes were incubated for 8 h at 37°C in a humidified atmosphere containing 5% CO₂/95% air. They were then covered with a 1:20 dilution of absorbed guinea pig C (Toshiba Chemical Co., Tokyo) and incubated for additional 2–3 h at 37°C. Hemolytic plaques were enumerated in triplicate and the results were expressed as the mean PFC/10⁶ non-T cells in the original culture (12). The standard error of the mean was always <20%.

RESULTS

Autorosette levels of Con A-activated T cell subpopulations. After Con A stimulation, normal T cells were fractionated into OKT4⁺ and OKT8⁺ cells, and then each cell population was further rosetted with autologous erythrocytes. Both the OKT4⁺ and OKT8⁺ cell populations formed rosettes with autologous erythrocytes to roughly the same extent (mean percentages of autorosettes with SEM in the activated OKT4⁺ and OKT8⁺ cell populations were 39.2 ± 0.8 and $35.8 \pm 1.8\%$, respectively). In contrast, $\sim 1\%$ of control unactivated OKT4⁺ or OKT8⁺ cells cultured in medium alone formed rosettes with autologous erythrocytes.

Suppressor activity of Con A-activated T cell subpopulations. Con A-activated OKT4⁺ and OKT8⁺ cell populations were further fractionated into autorosetting and nonrosetting cells. To evaluate the ability of T cell subsets to mediate suppression of B cell PFC activity, graded numbers of each subtype of Con A-activated cells were added to second autologous cultures containing 2×10^5 non-T cells plus 1×10^5 fresh OKT4⁺ cells (source of helper cells) in the presence of PWM. The results of a representative experiment are depicted

in Fig. 1 A, and clearly show that the addition of small numbers of activated autorosetting cells suppressed B cell immunoglobulin production, regardless of their phenotype of OKT4⁺ or OKT8⁺ antigen. In marked contrast, both OKT4⁺ and OKT8⁺ nonrosetting cells had only minimal inhibitory activity. Thus, these data suggest that suppressor cells within the Con A-activated cells are restricted to a distinct subset of either OKT4⁺ or OKT8⁺ cells; this subset is capable of forming rosettes with autologous erythrocytes after Con A activation.

To determine whether the above experiment was unique to lymphocytes for this single normal individual, lymphocytes from additional 11 normal individuals were studied and the results summarized in Fig. 2. In this experiment, 1×10^5 cells of Con A-activated or unactivated T cell subsets were introduced into the second autologous cultures. Consistent with Fig. 1, activated autorosetting cells, either OKT4⁺ or OKT8⁺, led to potent suppression of PFC activity. In contrast, activated nonrosetting OKT4⁺ as well as OKT8⁺ cells gave no demonstrable suppression. The suppressor T cell activity mediated by Con A-activated autorosetting cells seemed to be Con A dependent. As can be seen in Fig. 2, when fresh OKT4⁺ cells or unactivated control OKT4⁺ cells cultured in medium alone were added to the second cultures, the suppression of immunoglobulin production failed to occur. The addition of fresh or unactivated OKT8⁺ cells to the second cultures resulted in significant suppression of the PFC response; these OKT8⁺ cell populations were, however, far less efficient in suppressing B cell differentiation than activated autorosetting OKT8⁺ cells. The suppression observed in the fresh and unactivated OKT8⁺ cells would occur due to the activation of these cells by PWM-activated OKT4⁺ cells present in the second cultures.

It should be noted that the emergence of suppressor function within the activated OKT4⁺ population was not secondary to Con A-induced expansion of cells that lost OKT8⁺ antigens and acquired new antigens, OKT4⁺ antigens during the culture period; when OKT4⁺ cells were first isolated and then activated for 2 d with Con A, the potent suppression could be also generated by Con A activation of OKT4⁺ populations (data not shown); in addition, the surface phenotype of these activated OKT4⁺ cells (which had been first isolated

FIGURE 1 Suppressor and helper activities of Con A-activated T cell subpopulations: a representative experiment. T cells activated with Con A for 48 h (first culture) were fractionated into OKT4⁺ and OKT8⁺ cells, followed by further fractionation by using autologous erythrocyte rosette technique. These fractionated T cell subsets were used as regulatory cells in the second culture; graded numbers of cells from Con A-activated T cell subsets were added to the second autologous cultures containing PWM-driven non-T cells (2×10^5) plus fresh OKT4⁺ cells (1×10^5) for assay on suppressor activity (A), or those containing PWM-driven non-T cells (2×10^5) for assay on helper activity (B). 5 d later, cultures were harvested and assayed for PFC activity.

and activated by Con A) with respect to OKT3, OKT4, and OKT8 did not change (>95% OKT3⁺ cells, >90% OKT4⁺ cells, and <3% OKT8⁺ cells). It is also unlikely that such suppression was mediated by contaminated OKT8⁺ cells in the non-T cell preparations through their activation by Con A-activated OKT4⁺ populations that would function as suppressor-inducer cells (13, 14); because in the present study, only highly purified non-T cell populations were used, OKT8⁺ cells that might act as suppressor-effector cells would not have been present in sufficient numbers to mediate suppression (Methods). Moreover, it is very unlikely that such suppression mediated by OKT4⁺ cells represents merely a shift in time at which the maximal PFC response occurs; when activated autorosetting OKT4⁺ cells were added to the second autologous cultures of 2×10^5 non-T cells and 1×10^5 OKT4⁺ cells, the results of IgM-PFC generation showed profound suppression throughout the second culture period with no evidence of altered kinetics of response (Fig. 3 A). Moreover, any PFC activity of second assay cultures was not in-

duced by activated OKT4⁺ autorosetting cells, regardless of the time of harvest (Fig. 3 B). Finally, the suppression is not due to cytotoxic effect by the activated autorosetting cells, because both OKT4⁺ and OKT8⁺ autorosetting cells did not produce a cytotoxic effect at all on the non-T cells (responder cells of the second helper and suppressor assay cultures), or on the fresh OKT4⁺ cells (helper cells of the second suppressor assay cultures) as judged by ⁵¹Cr-release assay system (data not shown). Thus, Con A-activated autorosetting OKT4⁺ cells could mediate suppressor function observed.

Helper activity of Con A-activated T cell subpopulations. In earlier studies (4), we found that activated nonrosetting cells contain helper cells. We next investigated whether the autologous erythrocyte rosette technique could dissect the helper and suppressor cell functions within the activated OKT4⁺ or OKT8⁺ cells. To address this question, graded numbers of Con A-activated T cell subpopulations from the first culture were added to the second cultures containing PWM-

FIGURE 2 Differential suppressor activity of Con A-activated T cell subpopulations: summary of the results obtained from 12 normal individuals. The standard culture contained 1×10^5 fresh OKT4⁺ cells and 2×10^5 non-T cells in addition to $1 \mu\text{g}$ of PWM. To this system were added 1×10^5 cells from either Con A-activated or unactivated T cell subpopulations. After 5 d, cultures were harvested and assayed for PFC activity. The mean percent suppression and the range encompassed by ± 1 SEM are shown.

driven autologous non-T cells. As shown in Fig. 1 B (a representative experiment) and in Fig. 4 (summary of such experiments), only nonrosetting OKT4⁺ cell pop-

ulation that had been activated by Con A could function in the role of helper cells for B cell differentiation; the degree of helper activity by these cells was as great as

FIGURE 3 Kinetics of suppressor and helper activities of Con A-activated autorosetting OKT4⁺ or OKT8⁺ cells. (A) 1×10^5 of either activated autorosetting OKT4⁺ (—), OKT8⁺ (···) cells or fresh OKT4⁺ (- - -) cells were added to the second autologous culture containing 2×10^5 non-T cells and 1×10^5 fresh OKT4⁺ cells in the presence of $1 \mu\text{g}$ of PWM. (B) 1×10^5 of either activated autorosetting OKT4⁺ (—), OKT8⁺ (···) cells, or fresh OKT4⁺ (- - -) cells were added to the second culture containing PWM-driven autologous non-T cells (2×10^5). At daily intervals, cultures were harvested and assayed for PFC activity. Additional two experiments were performed and similar results were obtained.

FIGURE 4 Differential helper activity of Con A-activated T cell subpopulations: summary of the results from 12 normal individuals. The standard culture contained 2×10^5 non-T cells in addition to $1 \mu\text{g}$ of PWM. To this system were added 1×10^5 cells from Con A-activated or unactivated T cell subpopulations. After 5 d, cultures were harvested and assayed for PFC activity. The mean PFC response ± 1 SEM is shown.

the activity by either fresh OKT4⁺ cells (Figs. 1 B and 4) or by control unactivated OKT4⁺ cells (Fig. 4). In contrast, activated autorosetting OKT4⁺ and OKT8⁺ cells (that exerted potent suppressor activity) and activated nonrosetting OKT8⁺ cells (which did not exhibit any suppressor activity) failed to induce B cell differentiation at all (Figs. 1 B and 4). As expected, there was no effect of fresh OKT8⁺ cells and control unactivated OKT8⁺ cells on inducing PFC activity.

Radiosensitivity of the regulatory functions mediated by Con A-activated T cell subpopulations. We asked whether the regulatory activities induced by Con A-activated T cell subpopulations are radiosensitive. To this end, the activated T cell subpopulations were first irradiated (1,300 rad) before addition to the second cultures. 1×10^5 cells of nonirradiated or irradiated activated T cell subpopulations were then added to a constant number of fresh autologous OKT4⁺ cells and non-T cells (for assay on suppressor activity) or fresh autologous non-T cells (for assay on helper activity) in the presence of PWM (Fig. 5). The addition of non-irradiated autorosetting cells regardless of their OKT4⁺ or OKT8⁺ phenotype, suppressed the PFC response (Fig. 5 A). The activated autorosetting cells that had been irradiated, however, no longer suppressed (Fig. 5 A). In addition, these irradiated cells failed to promote the PFC response of B cells (Fig. 5 B). Thus, the suppressor function mediated by activated OKT4⁺ and OKT8⁺ cells is radiosensitive, and helper cells (at least radioresistant ones) are not included in these cell populations.

Contrary to the activated autorosetting cells, the helper activity of activated OKT4⁺ nonrosetting cells was radioresistant (Fig. 5 B). These irradiated cells exhibited a helper function similar to that of the non-irradiated OKT4⁺ nonrosetting cells (Fig. 5 B).

Response of Con A-activated suppressor T cell subpopulations to interleukin 2 (IL2). We have next examined whether Con A-activated T cell subpopulations proliferate in response to IL2 stimulation. As shown in Table I, Con A-activated autorosetting T cells regardless of their phenotypes, responded well to standard IL2 (Bethesda Research Laboratories, Bethesda, MD), whereas minimal response was observed in the activated nonrosetting OKT4⁺ and OKT8⁺ cell populations. These results indicate that Con A-activated suppressor cells (that are capable of forming autorosettes) are susceptible to further stimulation by this factor, probably by expressing receptors for IL2 during the course of Con A stimulation (15, 16). The results further suggest that the suppressor activity of Con A-activated autorosetting cells observed would occur due to the following processes: (a) Con A-activated autorosetting cells may absorb IL2 produced by the fresh OKT4⁺ cells present in the second assay cultures; (b) this process would deprive the OKT4⁺ helper cells of the necessary growth factor, thereby not causing the PWM-induced expansion of OKT4⁺ helper cells (16); and (c) thus, a decreased PFC response of B cells induced by PWM would occur.

To test this hypothesis, standard IL2 was added at a final concentration of 20% to the second suppressor

FIGURE 5 Effects of irradiation to Con A-activated T cell subpopulations on their regulatory activities. (A) Con A-activated T cell subpopulations were irradiated (1,300 rad) before the addition to the second culture. 1×10^5 cells from the irradiated or nonirradiated T cell subpopulations were added to standard autologous cultures containing 2×10^5 non-T cells and 1×10^5 fresh OKT4⁺ cells in addition to PWM. (B) 1×10^5 cells from the irradiated or nonirradiated Con A-activated T cell subpopulations were added to the standard cultures containing 2×10^5 non-T cells in the presence of PWM. Additional three experiments were performed and similar results were obtained.

assay cultures containing graded numbers of the autorosetting cells. After 5 d of incubation, the cells were harvested, and the suppressor activity evaluated by PWM-induced PFC activity. If the hypothesis is valid, then, addition of exogenous IL2 to the second culture should result in saturation of IL2 receptors expressed on the surface of Con A-activated cells, thereby causing them to lose their suppressive capacity. The results of such experiments are shown in Fig. 6. When the sufficient numbers (1×10^5 cells/culture) of OKT4⁺ or OKT8⁺ autorosetting cells were used as suppressor cells, a potent suppressive effect was observed, regardless of the addition of IL2. However, when the number of autorosetting cells to be added was reduced to 2×10^4 cells/culture, the suppressor activity was enhanced evidently in the presence of IL2, both with OKT4⁺ and with OKT8⁺ cells. This means that when the suppressor cells sufficient to inhibit the induction of B cells are present in the second culture, IL2-induced expansion of the suppressor cells is not necessary for the emergence of their suppressor function. In contrast, when the number of suppressor cells added to the second cultures is small, it is necessary that IL2 interacts with the activated autorosetting cells to promote their proliferation

and differentiation; their number then reaches an amount sufficient to maximally inhibit the assay cultures. Therefore, the suppressor activity by activated autorosetting cells does not occur because of their absorption of, and the decreased available level of IL2 produced in the second cultures; the Con A-induced autorosetting cells are presumed to contain "genuine" suppressor cells, which have a suppressive effect on the immune response.

Relationship between Con A-induced autorosetting cells and cells with Tac antigen. Tac antigen has been demonstrated to be expressed in conjunction with acquisition of IL2 receptors on the activated T cells (17, 18); the Tac antigen is associated with ~20% of Con A-activated T cells; these Con A-activated T cells bearing the Tac antigen act as suppressor cells (19, 20). Con A-activated cells bearing receptors for autologous erythrocytes also respond vigorously to IL2, indicating that expression of receptors for autologous erythrocytes may be related with expression of Tac antigen. To test this possibility, the procedure for autologous erythrocyte rosette formation was performed in the presence of anti-Tac antibody (kindly provided by Dr. Takashi Uchiyama, Kyoto University School of Medicine, Kyoto,

TABLE I
Response of Cells from Con A-activated T Cell
Subpopulations to IL2*

Cells	IL2 added	[³ H]Thymidine incorporated
		cpm†
Fresh T		
OKT4 ⁺	—	120±50
	+	1,298±466
OKT8 ⁺	—	85±26
	+	1,800±517
Unactivated T		
OKT4 ⁺	—	154±85
	+	1,969±256
OKT8 ⁺	—	223±129
	+	2,119±104
Con A-activated T		
OKT4 ⁺ autorosetting	—	93±26
	+	15,037±1,015
OKT4 ⁺ nonrosetting	—	72±15
	+	3,845±239
OKT8 ⁺ autorosetting	—	93±19
	+	16,107±2,195
OKT8 ⁺ nonrosetting	—	63±15
	+	4,545±983

* Standard IL2 was added at a final concentration of 20% to 1×10^4 cells/well of Con A-activated T cell subpopulations and incubated for 72 h at 37°C. Proliferation was determined by measuring [³H]thymidine incorporation during the last 6 h of culture.

† Mean±SEM cpm of four separate experiments, each in triplicate.

Japan). Table II illustrates the effects of varying concentrations of anti-Tac antibody (1:500 to 1:10,000 dilution) on inhibition of autologous erythrocyte binding to Con A-activated T cells. Anti-Tac antibody had no significant effect on autologous erythrocyte binding to Con A-activated OKT4⁺ cells, and similarly, this antibody had no significant effect on the binding to Con A-activated OKT8⁺ cells. Thus, it is unlikely that the anti-Tac antibody may interact directly with the receptor for autologous erythrocytes.

If anti-Tac antibody and autologous erythrocytes both preferentially bound to the same cell subclass, then the percentage of cells reactive with anti-Tac antibody should be increased or decreased in the populations enriched for or depleted of autorosetting cells, respectively. To investigate this point, cells reactive with anti-Tac antibody were determined in the Con A-activated autorosetting or nonrosetting populations by indirect immunofluorescence under a fluorescence microscope. In this experiment, anti-Tac antibody was used at a dilution of 1:5,000, because our preliminary titration studies demonstrated that this concentration was optimal to visualize positive cells. The results of such experiment are shown in Table III. Tac⁺ cells were mostly distributed in autorosetting cell populations regardless of the surface phenotype of OKT4⁺ or OKT8⁺ antigens. By contrast, there was small overlap between nonrosetting cells and cells with Tac antigen. Taken together, the data indicate that substantial overlap exists between cells bearing receptors for autologous erythrocytes and those bearing Tac antigen, although au-

FIGURE 6 Effect of IL2 on the suppressor function mediated by Con A-activated autorosetting T cells. The standard culture contained 1×10^5 fresh OKT4⁺ cells and 2×10^5 non-T cells in the presence of PWM. To this system were added graded numbers of either activated autorosetting OKT4⁺ or OKT8⁺ cells and incubated for 5 d with or without IL2 at a final concentration of 20%. Additional three experiments were performed and similar results were obtained.

TABLE II
Effect of Anti-Tac Antibody on Ability of Con A-activated T Cell Subsets to Form Autorosettes*

Dilution of anti-Tac antibody	Con A-activated T cell subsets	
	OKT4 ⁺	OKT8 ⁺
	% autorosettes†	
0	33.3±2.9	31.0±4.2
1:500	31.3±1.8	33.3±3.2
1:1,000	31.7±3.8	30.0±4.5
1:5,000	33.7±4.3	33.3±4.2
1:10,000	30.0±4.0	31.0±3.8

* Con A-activated OKT4⁺ or OKT8⁺ cells were rosetted with autologous erythrocytes in the presence of anti-Tac antibody at various concentrations.

† Mean percentage (±SEM) of three separate experiments.

tologous erythrocytes and anti-Tac antibody do not bind to the same antigenic determinants on the same cells.

DISCUSSION

The experiments reported above demonstrate functional heterogeneities within both the OKT4⁺ and OKT8⁺ subsets of human T cells. We found that although in vitro Con A-activated OKT4⁺ cells can function as helper cells, these activated OKT4⁺ cells can also inhibit PWM-induced B cell differentiation; the helper function is relatively radioresistant and the suppressor function is radiosensitive. Interestingly, the suppressor OKT4⁺ cells are separable on the basis of their preferential ability to form rosettes with autologous erythrocytes from the helper OKT4⁺ cells; Con A-activated OKT4⁺ autorosetting cells are specific for the function of suppression, and do not include cells manifesting helper activity. The reverse is true of the activated OKT4⁺ nonrosetting cell population.

TABLE III
Percentage of Tac⁺ Cells in Con A-activated T Cell Subsets*

Con A-activated T cell subsets	Tac ⁺ cells
	% †
OKT4 ⁺ autorosetting	62.0±4.0
OKT4 ⁺ nonrosetting	16.3±1.2
OKT8 ⁺ autorosetting	56.7±3.0
OKT8 ⁺ nonrosetting	12.3±3.8

* Con A-activated T cell subsets were analyzed by the use of anti-Tac monoclonal antibody (1:5,000) and indirect immunofluorescence with the fluorescence microscope.

† Mean percentage of positive cells±SEM in the Con A-activated T cell subsets from three normal individuals.

Con A-activated OKT8⁺ cells also contain radiosensitive cells important in the suppression of B cell differentiation. Moreover, these suppressor cells are also restricted to the population forming autorosettes; the autorosetting population is depleted of helper function. With regard to OKT8⁺ nonrosetting cells, neither helper nor suppressor activity can be observed in this population, so that these cells are likely a cell population with other immune functions rather than immunoregulatory T cells.

With regard to the heterogeneity of OKT4⁺ cells, it has been already reported that helper cells with the phenotype of OKT4⁺17⁺, those with the phenotype of OKT4⁺17⁻, and suppressor cells with the phenotype of OKT4⁺17⁺ are derived from PWM-stimulated OKT4⁺ cells (21). They reported that the OKT4⁺17⁺ suppressor cells are radiosensitive, but the OKT4⁺17⁻ and OKT4⁺17⁺ helper cells are radioresistant and radiosensitive, respectively (21). We also demonstrate in the present study that Con A-activated suppressor cells reside in both OKT4⁺ and OKT8⁺ cells, and both these suppressor populations are radiosensitive and capable of forming autorosettes. Damle and Gupta (25) also found that Con A-induced suppressor T cells reside in both OKT4⁺ and OKT8⁺ cell populations. With regard to helper activity mediated by OKT4⁺ cells, radiosensitive helper activity could not be detected among these cells in the present study. However, even if radiosensitive helper cells would exist in the activated autorosetting cell population, helper activity could not be detected in this population regardless of irradiation, because suppressor cells would dominate overwhelmingly in numbers in the autorosetting T cells. Similarly, the nonrosetting OKT4⁺ population would also contain radiosensitive helper cells. Thus, we can not rule out the possibility that these populations may contain radiosensitive helper cells induced by Con A. More recently, OKT4⁺ cells have also been shown to contain cytotoxic T lymphocytes specific for class II HLA antigens (22-24).

It is important to emphasize that the functional heterogeneity dissected by virtue of the autologous erythrocyte rosette technique is possible only after activation, when a subset of OKT4⁺ cells and that of OKT8⁺ cells acquire or express much more of the receptors for autologous erythrocytes. Regarding this point, Palacios et al. (26) found that the proportion of autorosetting cells is ~30% in a fresh T cell population obtained from normal peripheral blood; we and other investigators (4, 27) could observe, however, considerable proportions of the autorosetting T cells only in the activated, but not resting, T cells. In the resting T cell population, only 3% of the cells formed rosettes with autologous erythrocytes (4, 27). These differences between our studies and those of Palacios et al. may be

explained by technical difference in the method of autorosette formation; their procedure entailed incubating T cells with autologous erythrocytes in the presence of autologous serum (26); our autorosette procedure was carried out in the presence of fetal bovine, but not human, serum. Distinct T cell populations might form autorosettes under specific conditions.

There is accumulating evidence for the idea that T cells can express different surface molecules or receptors in relation to a particular state of activation (19–21, 28–30). Furthermore, various studies have described several monoclonal antibodies directed at determinants expressed on or lost from activated, but not resting, T lymphocytes (19–21). For example, Uchiyama et al. (19, 20) have described the expression of Tac antigen on most of activated T cells; this antigen is expressed in connection with the functional activities. More recently, the Tac antigen has been demonstrated to be expressed in conjunction with acquisition of IL2 receptors on the activated T cells (17, 18). Thomas et al. (18), on the other hand, describe that the defined subgroup of T cells loses OKT17⁺ antigen during activation with PWM. In this study, we have demonstrated that most autorosetting cells bear IL2 receptors, and thus cell populations capable of expressing receptors for autologous erythrocytes and cells bearing Tac antigen mostly overlap each other. However, Tac antigen and receptors for autologous erythrocytes are not identical, rather present indifferently on the same cell surface of activated T cells, because anti-Tac antibody cannot inhibit autorosette formation at all. Furthermore, Tac⁺ subsets after the PWM stimulation contain T cells with a radioresistant helper activity, while autorosetting cells do not include radioresistant helper cells as far as we have investigated.

Recently, Palacios et al. (16) have demonstrated that the suppressor activity of the Con A-activated T cells is abrogated by the addition of exogenous IL2 to the assay cultures. They concluded therefore that after Con A activation, the resting T cells have been rendered sensitive to IL2, and thus Con A-activated cells absorb IL2 activity produced by responder cells in the assay cultures, thereby leading to their apparent suppressive effect. In our present study, however, when 1×10^5 autorosetting cells were present in the second cultures, addition of exogenous IL2 did not affect the suppressor activity. When the number of autorosetting cells was reduced to 2×10^4 cells/culture, the suppressor activity was rather enhanced by the addition of exogenous IL2 (Fig. 6). These different results between our studies and those of Palacios et al. could be explained in part by differences in technique. For example, Palacios et al. used unfractionated Con A-activated T cells, and thus the OKT4⁺ nonrosetting cells (helper cells) that could have been included in them would further pro-

duce IL2 as well as both B cell growth factors and B cell differentiation factors (31) in the assay cultures. Further addition of exogenous IL2 (which would also contain both B cell growth factors and B cell differentiation factors) to the assay cultures could cause relative predominance of helper activity, which resulted in the apparent abrogation of suppressor function. Another cause might be the difference in the PWM-induced assay culture system. Although it cannot be ruled out that Con A-induced suppressor activity is, at least in part, an IL2-dependent inhibition, much of the suppressive effect is likely an inhibition caused by “genuine” suppressor cells.

We should investigate further in detail how and at which stage of the expression of suppressor activity by Con A-induced autorosetting cells IL2 affects the activity. Moreover, if monoclonal antibodies directed at these activated T cell subpopulations could be established, such antibodies could represent attractive candidates for in vivo use as immunomodulating agents. Although OKT3 antibody, the anti-human pan T cell reagent, has been used to produce prompt reversal of rejection in recipients of renal allografts (32), monoclonal antibodies to the specific functional, and activated T cell subsets may be far better suited for certain clinical situations in which the goal of treatment would be to diminish or enhance an ongoing response. Such studies are currently in progress.

Previous studies demonstrated that patients with active systemic lupus erythematosus (SLE) have a loss of suppressor T cell function (10, 33–37). In several studies, the importance of anti-T cell antibodies in altering T cell number and function in patients with active SLE was emphasized (38, 39). Indeed, SLE anti-T cell antibodies found in the sera of active SLE patients have been shown to preferentially react to T cells that bear the OKT8⁺ phenotype characteristic of suppressor cells (40, 41). Moreover, such active patients in fact have a decreased proportion of OKT8⁺ subsets (42). When in the inactive state, the patients did not have anti-T cell antibodies (6, 40), a reduction in the suppressor T cell function or in proportion of OKT8⁺ cells was found to be largely corrected (6, 42, 43). It can therefore be postulated that the defect in suppressor function mediated by OKT8⁺ suppressor T cell subsets in active SLE patients may result from the action of anti-T cell antibodies produced by B cells. On the other hand, it has been shown that patients with active SLE have heterogeneous patterns of T cell phenotypes identified by monoclonal antibodies (44). More recently, we have also demonstrated that SLE patients have an intrinsic defect in the suppressor T cell function mediated by OKT4⁺ cells, which occurs independently of B cell abnormalities (41). In addition, in preliminary experiments we have observed that in patients with active

SLE the autorosette levels are profoundly decreased in both Con A-activated OKT4⁺ and OKT8⁺ cell populations. The failure of Con A-activated SLE OKT4⁺ cells to form autorosettes is still observed during disease quiescence. Since there exists a heterogeneity among suppressor cells, dissection of the defective suppressor cell subsets in various diseases with the use of autorosette technique and monoclonal antibodies may lead to a better understanding of the cellular basis for immune abnormalities in these disorders.

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REFERENCES

- Reinherz, E. L., and S. F. Schlossman. 1980. Regulation of the immune response: inducer and suppressor T-lymphocyte subsets in human beings. *N. Engl. J. Med.* 303:370-373.
- Reinherz, E. L., and S. F. Schlossman. 1981. The characterization and function of human immunoregulatory T lymphocyte subsets. *Immunology Today*. 3:69-74.
- Friedman, S. M., S. B. Hunter, O. H. Irigoyen, P. C. Kung, G. Goldstein, and L. Chess. 1981. Functional analysis of human T cell subsets defined by monoclonal antibodies. II. Collaborative T-T interactions in the generation of TNP-altered-self-reactive cytotoxic T lymphocytes. *J. Immunol.* 126:1702-1705.
- Sakane, T., M. Honda, Y. Taniguchi, and H. Kotani. 1981. Separation of concanavalin A-induced human suppressor and helper T cells by the autologous erythrocyte rosette technique. *J. Clin. Invest.* 68:447-453.
- Sakane, T., and I. Green. 1978. Protein A from *Staphylococcus aureus*—a mitogen for human T lymphocytes and B lymphocytes but not L lymphocytes. *J. Immunol.* 120:302-311.
- Sakane, T., A. D. Steinberg, J. P. Reeves, and I. Green. 1979. Studies of immune functions of patients with systemic lupus erythematosus. Complement-dependent immunoglobulin M anti-thymus-derived cell antibodies preferentially inactivate suppressor cells. *J. Clin. Invest.* 63:954-965.
- Chatenoud, L., M. F. Baudrihay, H. Kreis, G. Goldstein, J. Schindler, and J.-F. Bach. 1982. Human *in vivo* antigenic modulation induced by the anti-T cell OKT3 monoclonal antibody. *Eur. J. Immunol.* 12:979-982.
- Thomas, Y., L. Rogozinski, O. H. Irigoyen, S. M. Friedman, P. C. Kung, G. Goldstein, and L. Chess. 1981. Functional analysis of human T cell subsets defined by monoclonal antibodies. IV. Induction of suppressor cells within the OKT4⁺ population. *J. Exp. Med.* 154:459-467.
- Reinherz, E. L., P. C. Kung, G. Goldstein, and S. F. Schlossman. 1979. Separation of functional subsets of human T cells by a monoclonal antibody. *Proc. Natl. Acad. Sci. USA.* 76:4061-4065.
- Sakane, T., A. D. Steinberg, and I. Green. 1978. Studies of immune functions of patients with systemic lupus erythematosus. I. Dysfunction of suppressor T-cell activity related to impaired generation of rather than response to suppressor cells. *Arthritis Rheum.* 21:657-664.
- Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting Ig of a given type or class. *Eur. J. Immunol.* 6:588-590.
- Sakane, T., S. Takada, H. Kotani, and T. Tsunematsu. 1982. Effects of methyl-B₁₂ on the *in vitro* immune functions of human T lymphocytes. *J. Clin. Immunol.* 2:101-109.
- Morimoto, C., E. L. Reinherz, Y. Borel, E. Mantzouranis, A. D. Steinberg, and S. F. Schlossman. 1981. Autoantibody to an immunoregulatory inducer population in patients with juvenile rheumatoid arthritis. *J. Clin. Invest.* 67:753-761.
- Morimoto, C., J. A. Distaso, Y. Borel, S. F. Schlossman, and E. L. Reinherz. 1982. Communicative interactions between subpopulations of human T lymphocytes required for generation of suppressor effector function in a primary antibody response. *J. Immunol.* 128:1645-1650.
- Larsson, E. L., and A. Coutinho. 1979. The role of lectins in T cell triggering. *Nature (Lond.)* 280:239-241.
- Palacios, R., and G. Möller. 1981. T cell growth factor abrogates concanavalin A-induced suppressor cell function. *J. Exp. Med.* 153:1360-1365.
- Leonard, W. J., J. M. Depper, T. Uchiyama, T. A. Waldmann, and W. C. Greene. 1982. Monoclonal anti-TAC antibody blocks the action of human T cell growth factor (TCGF). *Clin. Res.* 30:352a. (Abstr.)
- Miyawaki, T., A. Yachie, N. Uwadana, S. Ohzeki, T. Nagaoki, and N. Taniguchi. 1982. Functional significance of Tac antigen expressed on activated human T lymphocytes: Tac antigen interacts with T cell growth factor in cellular proliferation. *J. Immunol.* 129:2474-2478.
- Uchiyama, T., S. Broder, and T. A. Waldman. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J. Immunol.* 126:1393-1397.
- Uchiyama, T., D. L. Nelson, T. A. Fleisher, and T. A. Waldman. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. II. Expression of Tac antigen on activated cytotoxic killer T cells, suppressor cells, and one of two types of helper T cells. *J. Immunol.* 126:1398-1403.
- Thomas, Y., L. Rogozinski, O. H. Irigoyen, H. H. Shen, M. A. Talle, G. Goldstein, and L. Chess. 1982. Functional analysis of human T cell subsets defined by monoclonal antibodies. V. Suppressor cells within the activated OKT4⁺ population belong to a distinct subset. *J. Immunol.* 128:1386-1390.
- Meuer, S., S. F. Schlossman, and E. L. Reinherz. 1982. Influence of monoclonal antibodies on cloned functional human T cell subsets. *Fed. Proc.* 4:1291a. (Abstr.)
- Krensky, A. M., C. S. Reiss, J. W. Mier, J. L. Strominger, and S. J. Burakoff. 1982. Long-term human cytolytic T-cell lines allospecific for HLA-DR6 antigen are OKT4⁺. *Proc. Natl. Acad. Sci. USA.* 79:2365-2369.
- Biddison, W. E., P. E. Rao, M. A. Talle, G. Goldstein, and S. Shaw. 1982. Possible involvement of the OKT4 molecule in T cell recognition of class II HLA antigens.

- Evidence from studies of cytotoxic T lymphocytes specific for SB antigens. *J. Exp. Med.* 156:1065-1076.
25. Damle, N. K., and S. Gupta. 1982. Heterogeneity of concanavalin A-induced suppressor T cells in man defined with monoclonal antibodies. *Clin. Exp. Immunol.* 48:581-588.
 26. Palacios, R., L. Llorente, D. Alarcón-Segovia, A. Ruíz-Arguelles, and E. Díaz-Jouanen. 1980. Autologous rosette-forming T cells as responding cells in human autologous mixed lymphocyte reaction. *J. Clin. Invest.* 65:1527-1530.
 27. Fournier, C., and J. Charreire. 1978. Activation of a human T cell subpopulation bearing receptors for autologous erythrocytes by concanavalin A. *J. Immunol.* 121:771-776.
 28. Kimura, A. K., and M. Wigzell. 1978. Cell surface glycoprotein of murine cytotoxic T lymphocytes. I. T145, a new cell surface glycoprotein selectively expressed on Lyl⁻2⁺ cytotoxic lymphocytes. *J. Exp. Med.* 147:1418-1434.
 29. Corte, G., L. Moretta, G. Damiani, M. C. Mingari, and A. Bargellesi. 1981. Surface antigens specifically expressed by activated T cells in humans. *Eur. J. Immunol.* 11:162-164.
 30. Moretta, A., M. L. Mingari, B. F. Haynes, R. P. Sekaly, L. Moretta, and A. S. Fauci. 1981. Phenotypic characterization of human cytolytic T lymphocytes in mixed lymphocyte culture. *J. Exp. Med.* 153:213-218.
 31. Muraguchi, A., J. L. Butler, J. H. Kerl, and A. S. Fauci. 1983. Differential sensitivity of human B cell subsets to activation signals delivered by anti- μ antibody and proliferative signals delivered by a monoclonal B cell growth factor. *J. Exp. Med.* 157:530-546.
 32. Cosimi, A. B., R. B. Colvin, R. C. Burton, R. H. Rubin, G. Goldstein, P. C. Kung, W. P. Hansen, F. L. Delmonico, and P. S. Russell. 1981. Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. *N. Engl. J. Med.* 305:308-314.
 33. Abdou, N. I., A. Sagawa, E. Pascual, J. Hebert, and S. Sadeghee. 1976. Suppressor T-cell abnormality in idiopathic systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* 6:192-199.
 34. Bresnihan, B., and H. E. Jasin. 1977. Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosus. *J. Clin. Invest.* 59:106-116.
 35. Horowitz, S., W. Borchering, A. V. Moorthy, R. Chesney, H. Schulte-Wissermann, R. Hong, and A. Goldstein. 1977. Induction of suppressor T cells in systemic lupus erythematosus by thymosin and cultured thymic epithelium. *Science (Wash. DC)*. 197:999-1001.
 36. Sagawa, A., and N. I. Abdou. 1978. Suppressor-cell dysfunction in systemic lupus erythematosus: cells involved and in vitro correction. *J. Clin. Invest.* 62:789-796.
 37. Fauci, A. S., A. D. Steinberg, B. F. Haynes, and G. Whalen. 1978. Immunoregulatory aberration in systemic lupus erythematosus. *J. Immunol.* 121:1473-1479.
 38. Terasaki, P. I., V. D. Mottironi, and E. V. Barnett. 1970. Cytotoxin in disease. *N. Engl. J. Med.* 283:724-728.
 39. Stastney, P., and M. Ziff. 1971. Antibodies against cell membrane constituents in systemic lupus erythematosus and related diseases. I. Cytotoxic effect of serum from patients with systemic lupus erythematosus (SLE) for allogeneic and for autologous lymphocytes. *Clin. Exp. Immunol.* 8:543-550.
 40. Morimoto, C., E. L. Reinherz, T. Abe, M. Homma, and S. F. Schlossman. 1980. Characteristics of anti-T cell antibodies in systemic lupus erythematosus: evidence for selective reactivity with normal suppressor cells defined by monoclonal antibodies. *Clin. Immunol. Immunopathol.* 16:474-484.
 41. Sakane, T., H. Kotani, S. Takada, Y. Murakawa, and Y. Ueda. 1983. A defect in the suppressor circuits among OKT4⁺ cell populations in patients with systemic lupus erythematosus occurs independently of a defect in the OKT8⁺ suppressor T cell function. *J. Immunol.* 131:753-761.
 42. Morimoto, C., E. L. Reinherz, A. D. Steinberg, P. H. Schur, A. Mills, and S. F. Schlossman. 1980. Alterations of immunoregulatory T cell subsets in active systemic lupus erythematosus. *J. Clin. Invest.* 66:1171-1174.
 43. Sakane, T., A. D. Steinberg, and I. Green. 1980. Studies of immune functions of patients with systemic lupus erythematosus. V. T cell suppressor function and autologous mixed lymphocyte reaction during active and inactive phases of disease. *Arthritis Rheum.* 23:225-231.