

## THE Fc RECEPTOR ON THYMUS-DERIVED LYMPHOCYTES\*

### II. Mitogen Responsiveness of T Lymphocytes Bearing the Fc Receptor

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Recent studies have suggested that a high degree of functional heterogeneity exists in the thymus-derived (T) lymphocyte population. In addition to the maturational heterogeneity of the thymus cell population as a whole (1-3), "mature" T lymphocytes that participate in graft-vs.-host and cytotoxic responses display functional heterogeneity with respect to homing properties and sensitivity to the cytotoxic effects of antilymphocyte and anti-Thy 1.2 antibodies (4). Differences in relative responsiveness to the mitogens phytohemagglutinin (PHA)<sup>1</sup> and concanavalin A (Con A) have also been reported to exist in these T-cell subpopulations (5,6). It has been proposed that these differences in mitogen responsiveness may reflect differences in the state of maturation of the T cells (6).

In the preceding paper of this series (7), we reported the existence in normal mice of a subpopulation of peripheral T lymphocytes that bind antigen-antibody complexes, apparently via an Fc receptor. These T cells were predominantly small lymphocytes which did not cooperate with B cells to give an adoptive response to DNP-keyhole limpet hemocyanin (KLH) (7). The relationship of these subpopulations of T cells to those described by Cantor et al. (4), and Stobo and Paul (5,6) is not yet known. In the present report, we have examined the responsiveness of the purified Fc<sup>-</sup> and Fc<sup>+</sup> T lymphocytes to PHA and Con A and have found that, although both subpopulations respond to PHA, the response to Con A is a characteristic of the Fc<sup>+</sup> T lymphocytes and not of the Fc<sup>-</sup> T cells when separated from the Fc<sup>+</sup> T cells.

### Materials and Methods

Methods for preparations of antisera and cell suspensions, fluorescent-staining procedures, analysis, and separation of cells on nylon wool and the fluorescence activated cell sorter (FACS) have been described in detail in the previous paper (8). Complexes of fluoresceinated egg albumin and 7S BALB/cN anti-egg albumin were used throughout this study to label the Fc receptor.

*Mice.* Male and female mice from the inbred strain BALB/cN used in these experiments were obtained from our own colonies at Stanford University.

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<sup>1</sup>Abbreviations used in this paper: Con A, concanavalin A; D-PBS, Dulbecco's phosphate-buffered saline; FACS, fluorescence activated cell sorter; FCS, fetal calf serum; F\*(AgAb), fluoresceinated antigen-antibody complexes; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; PHA, phytohemagglutinin.

**Cell Culture.** For cell culture, lymphocytes were pelleted and resuspended to  $2 \times 10^6$  cells/ml of RPMI-1640 (H-18 powder, Grand Island Biological Co., Grand Island, N. Y.) supplemented to 5% vol/vol with fetal calf serum (FCS, GIBCO), 2 mM L-glutamine (GIBCO), 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin (GIBCO),  $5 \times 10^{-5}$  M 2-mercaptoethanol, and 10 mM HEPES buffer (no. 130440, GIBCO). The cells were dispensed in 100- $\mu$ l aliquots to the flat-bottom microwells of Microtest II culture plates (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). PHA-P (Wellcome Research Laboratories, Beckenham, England), Con A (A grade, Calbiochem, San Diego, Calif.), or *Escherichia coli* 011:B4 lipopolysaccharide-W (LPS, Difco Laboratories, Detroit, Mich.) were then added to the microwells in 100- $\mu$ l aliquots at the concentrations specified in the Results section. The plates were incubated in a humidified atmosphere of 95% air and 5% CO<sub>2</sub> for 40 h; 1  $\mu$ Ci of [<sup>3</sup>H]thymidine ([methyl-<sup>3</sup>H]thymidine, 6 Ci/mol, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) was added to each well and culture continued for 8 h. The cultures were then harvested on a multiple automated sample harvester (MASH-II, Microbiological Associates, Bethesda, Md.) and counted on a Packard Tri-Carb Liquid Scintillation Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

Adherent cells were obtained by dispensing  $5 \times 10^5$  normal spleen cells in 200- $\mu$ l aliquots of culture medium per microwell and incubating for 2 h at 37°C. The nonadherent cells were removed by washing the wells three times with warm culture medium, culturing an additional 24 h at 37°C, and washing one more time immediately before addition of lymphocyte populations and mitogen. The presence of cells adherent to the bottom of the microwells after the final wash was confirmed by light microscope examination.

Where indicated in the Results section, excess antigen-antibody complexes were eliminated from labeled lymphocytes by resuspending the cells to  $5 \times 10^6$ /ml of Dulbecco's phosphate-buffered saline (D-PBS) containing 0.1 mg/ml trypsin (Worthington Biochemical Corp., Freehold, N. J.) and incubating 10 min at 37°C. The cells were then pelleted through FCS and resuspended in culture medium.

## Results

In addition of 2.5  $\mu$ g/ml PHA, 1.5  $\mu$ g/ml Con A, or 25  $\mu$ g/ml LPS to cultures containing  $2 \times 10^5$  BALB/cN spleen cells resulted in a 280-fold, 200-fold, or 30-fold stimulation of [<sup>3</sup>H]thymidine incorporation, respectively, after a 48 h culture period (Table I). Incubation of the spleen cells on nylon wool (8) resulted in a significant enrichment of nonimmunoglobulin-bearing cells (<2% Ig<sup>+</sup>) and in an enrichment of PHA- and Con A-responsive cells while depleting the population of LPS-responsive cells (Table I).<sup>2</sup>

Treatment of the spleen cell population with 2 mg of antigen-antibody complexes/ $10^8$  lymphocytes/ml for 30 min at 37°C resulted in a substantial inhibition (>50%) of [<sup>3</sup>H]thymidine incorporation by the cells upon subsequent culture with Con A and LPS, but did not inhibit [<sup>3</sup>H]thymidine incorporation upon subsequent culture with PHA (Table I). A similar suppression of Con A responses but not PHA responses was observed upon treatment of nylon wool-purified splenic T cells with antigen-antibody complexes (Table I).

To determine whether the inhibition by antigen-antibody complexes was reversible by removal of the complexes from the surface of the cells, spleen cells or nylon wool-purified splenic T cells were treated with antigen-antibody complexes for 30 min at 37°C, pelleted, resuspended to  $5 \times 10^6$  cells/ml in 0.1 mg/ml

<sup>2</sup> The nylon wool-adherent population contains a substantial number of Fc<sup>+</sup> T cells in addition to the B lymphocytes. Use of excessive amounts of nylon wool (greater than 0.6 g/ $10^8$  spleen cells) for purification of splenic T cells or packing the nylon wool columns in a vol smaller than 6 ml/0.6 g nylon wool (7) results in a significant depletion of Fc<sup>+</sup> T lymphocytes from the nonadhering (splenic T) population (unpublished observation).

TABLE I  
Inhibition of Mitogenic Response to Con A and LPS by Antigen-Antibody Complexes

Cells*	(AgAb) treatment*	cpm ( $\times 10^{-3}$ ) [ $^3\text{H}$ ]thymidine/culture‡			
		RPMI	PHA	Con A	LPS
Spleen	-	0.25 $\pm$ 0.04	72.0 $\pm$ 4.3	49.6 $\pm$ 3.0	8.48 $\pm$ 0.98
Spleen	+	0.26 $\pm$ 0.05	65.4 $\pm$ 7.1	21.7 $\pm$ 3.3	2.4 $\pm$ 0.8
Splenic T§	-	0.19 $\pm$ 0.06	107.4 $\pm$ 8.4	53.6 $\pm$ 3.9	0.78 $\pm$ 0.09
Splenic T	+	0.21 $\pm$ 0.05	101.1 $\pm$ 12.2	28.7 $\pm$ 2.9	0.32 $\pm$ 0.05

\* Lymphocytes were incubated for 30 min at 37°C with or without preformed, washed AgAb complexes, washed, and plated at a concentration of  $10^6$  cells/ml ( $2 \times 10^5$  cells/well) in the presence of 2.5  $\mu\text{g/ml}$  PHA, 1.5  $\mu\text{g/ml}$  Con A, or 25  $\mu\text{g/ml}$  LPS. Cultures were harvested after 48 h of culture.

‡ Cultures were pulsed with 1  $\mu\text{Ci}$  [ $^3\text{H}$ ]thymidine 8 h before harvest. Numerical values represent average counts per minute of three cultures  $\pm$  one standard deviation.

§ Splenic T cells were purified by nylon wool filtration. The purified T-cell population contained less than 2%  $\text{Ig}^+$  cells.

TABLE II  
Restoration of Mitogen Responsiveness of (AgAb)-Labeled Spleen Cells by Trypsin Treatment

Cells*	(AgAb) treatment*	Trypsin treatment‡	cpm ( $\times 10^{-3}$ ) [ $^3\text{H}$ ]thymidine/culture§			
			RPMI	PHA	Con A	LPS
Spleen	-	-	0.46 $\pm$ 0.03	161.4 $\pm$ 9.3	111.1 $\pm$ 8.8	14.3 $\pm$ 0.7
Spleen	+	-	0.32 $\pm$ 0.08	111.3 $\pm$ 11.5	39.9 $\pm$ 5.5	1.9 $\pm$ 0.3
Spleen	+	+	0.48 $\pm$ 0.09	166.1 $\pm$ 12.0	94.3 $\pm$ 7.8	11.6 $\pm$ 0.8
Splenic T	-	-	0.38 $\pm$ 0.03	237.8 $\pm$ 19.4	108.8 $\pm$ 9.0	1.91 $\pm$ 0.47
Splenic T	+	-	0.37 $\pm$ 0.05	218.0 $\pm$ 20.1	55.7 $\pm$ 5.5	.75 $\pm$ 0.26
Splenic T	+	+	0.37 $\pm$ 0.06	224.6 $\pm$ 17.6	84.9 $\pm$ 10.2	1.10 $\pm$ 0.28

\* See footnote (\*) of Table I.

‡ After labeling with AgAb complexes, cells were incubated for 10 minutes at 37°C in 0.1 mg/ml trypsin in D-PBS.

§ See footnote (§) of Table I.

|| See footnote (§) of Table I.

trypsin, and incubated at 37°C for 10 min. The cells were then centrifuged through cold FCS, resuspended in culture medium and cultured for 48 h with PHA, Con A, or LPS. As can be seen in Table II, the responses of antigen-antibody complex-treated lymphocytes to Con A and LPS were restored almost to normal (untreated) levels by the trypsin treatment. This was true for both spleen cell- and nylon-purified splenic T-cell cultures. Neither the antigen-antibody complex treatment nor the trypsin treatment affected the response of the cells to PHA (Table II).

This selective suppression of Con A, but not PHA, responsiveness of splenic T cells by antigen-antibody complexes suggested that different cell populations of T lymphocytes were involved in the two mitogen responses. To examine this hypothesis, nylon wool-purified T cells were labeled with fluoresceinated antigen-antibody complexes [ $\text{F}^*(\text{AgAb})$ ] and separated on the FACS into  $\text{Fc}^-$  and  $\text{Fc}^+$

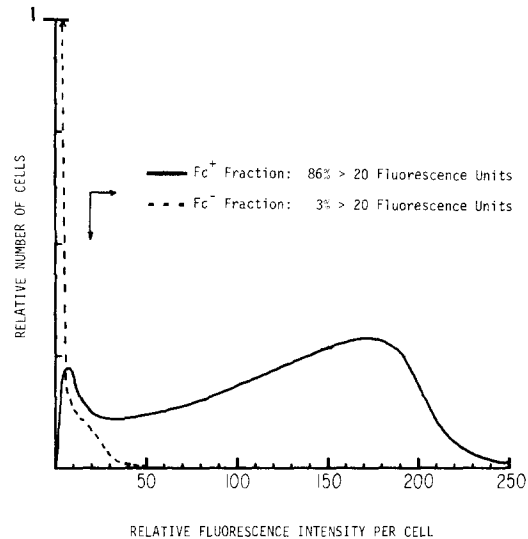


FIG. 1. Fluorescence distributions of  $Fc^+$ (AgAb)-labeled  $Fc^-$  and  $Fc^+$  T-cell fractions. Nylon-purified splenic T cells were labeled with complexes of fluoresceinated egg albumin and 7S antiegg albumin and separated into  $Fc^-$  and  $Fc^+$  fractions on the FACS. The collected fractions were then analyzed on the FACS. The distribution curves are based on cumulative analysis of 25,000 viable cells.

T-cell fractions. The fluorescence distributions of the  $Fc^+$  and  $Fc^-$  T-cell fractions are shown in Fig. 1. The  $Fc^+$  fraction contained at least 86%  $Fc^+$  T lymphocytes whereas the  $Fc^-$  fraction contained less than 5%  $Fc^+$  T lymphocytes (Fig. 1). These fractions, as well as the original labeled but unseparated T cells were pelleted and resuspended to  $5 \times 10^6$  cells/ml in 0.1 mg/ml trypsin in D-PBS, incubated for 10 min at 37°C, pelleted through cold FCS, and resuspended in supplemented RPMI-1640 for culture.

As can be seen in Table III, after 48 h of culture with 2.5  $\mu\text{g/ml}$  PHA or 1.5  $\mu\text{g/ml}$  Con A, no difference in [ $^3\text{H}$ ]thymidine incorporation was observed between unlabeled and antigen-antibody-labeled splenic T cells. The  $Fc^-$  T-cell population responded very well to PHA but very poorly to Con A. The  $Fc^+$  T-cell population, on the other hand, responded well to both Con A and PHA (Table III).

To determine whether the poor response of  $Fc^-$  T cells to Con A was due to a different concentration dependence for mitogen stimulation, the  $Fc^-$  and  $Fc^+$  T cells were cultured for 48 h with 0.5–5.0  $\mu\text{g/ml}$  PHA or 0.15–5.0  $\mu\text{g/ml}$  Con A. The  $Fc^-$  and  $Fc^+$  T-cell fractions displayed similar levels of responsiveness to PHA (Fig. 2 a). However, at optimal Con A concentration (1.5  $\mu\text{g/ml}$ ), the  $Fc^-$  T cells were, at best, only 20% as responsive as the unseparated T cells (Fig. 2 b). The  $Fc^+$  T cells displayed mitogenic responses equivalent to the unseparated T cells at concentrations of Con A ranging from 0.5–1.5  $\mu\text{g/ml}$  and displayed a response significantly greater than that of unseparated T cells to 5  $\mu\text{g/ml}$  Con A (Fig. 2 b).

To determine whether the poor response of the  $Fc^-$  T cells to Con A was due to a relative paucity of accessory cells (e.g., macrophages), unseparated, nylon

TABLE III  
Con A and PHA Responsiveness of  $Fc^+$  vs.  $Fc^-$  T Cells

Cells*	cpm ( $\times 10^{-3}$ ) [ $^3H$ ]thymidine/culture‡		
	RPMI	PHA	Con A
Unlabeled T cells	0.15 $\pm$ 0.01	138.5 $\pm$ 11.4	49.3 $\pm$ 3.1
(AgAb)-labeled T cells§	0.13 $\pm$ 0.01	121.3 $\pm$ 12.7	38.9 $\pm$ 2.6
$Fc^-$ T cells§	0.16 $\pm$ 0.03	124.3 $\pm$ 9.5	3.4 $\pm$ 0.5
$Fc^+$ T cells§	0.18 $\pm$ 0.05	80.5 $\pm$ 8.7	34.5 $\pm$ 5.7

\* See footnote (\*) of Table I.

‡ See footnote (‡) of Table I.

§ See footnote (§) of Table II.

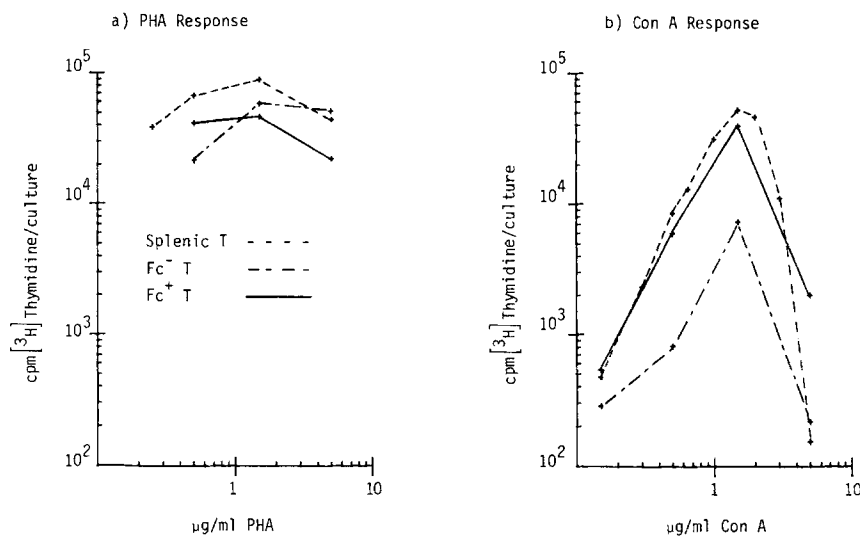


FIG. 2. Mitotic responses of  $Fc^-$  and  $Fc^+$  T cells as a function of mitogen concentration. Nylon purified splenic T cells were labeled with  $F^*(AgAb)$ , and separated into  $Fc^-$  and  $Fc^+$  T-cell fraction on the FACS. Excess  $F^*(AgAb)$  was removed from the surface of the labeled cells by incubating the cells in the presence of trypsin (0.1 mg/ml) for 10 min. The cells were then cultured at a concentration of  $10^6$  cells/ml for 48 h in the presence of 0.15-5.0  $\mu g/ml$  of either PHA and Con A. The cultures were pulsed for 8 h with 1  $\mu Ci$  [ $^3H$ ]thymidine before harvest.

wool-purified T cells and the FACS-purified  $Fc^-$  and  $Fc^+$  T cells were cultured for 48 h with 2.5  $\mu g/ml$  PHA or 1.5  $\mu g/ml$  Con A in the presence or absence of splenic adherent cells. The adherent cells cultured alone (without added T cells) did not respond to either Con A or PHA. The presence of adherent cells did not significantly effect the responses of either unseparated T cells,  $Fc^-$  T cells, or  $Fc^+$  T cells to either PHA or Con A (Table IV).

In all of the above experiments, the response of  $Fc^+$  T cells never exceeded the response of unseparated splenic T cells to 1.5  $\mu g/ml$  Con A. To determine whether a synergistic interaction between the  $Fc^+$  T cells and  $Fc^-$  T cells was occurring in the response of splenic T cells to Con A, mixtures of  $Fc^-$  and  $Fc^+$  T

TABLE IV  
*Effect of Adherent Cells on Mitogen Responses of Fc<sup>-</sup> and Fc<sup>+</sup> Cells*

Cells*	Adherent‡ cells added	cpm ( $\times 10^{-3}$ ) [ <sup>3</sup> H]thymidine/culture		
		RPMI	PHA	Con A
None (adherent cells alone)	+	0.44 $\pm$ 0.25	0.37 $\pm$ 0.19	0.43 $\pm$ 0.27
Splenic T cells§	-	0.27 $\pm$ 0.06	69.3 $\pm$ 9.3	53.8 $\pm$ 1.1
	+	0.27 $\pm$ 0.11	67.1 $\pm$ 5.9	55.3 $\pm$ 4.1
Fc <sup>-</sup> T cells§	-	0.28 $\pm$ 0.08	57.2 $\pm$ 1.9	8.3 $\pm$ 1.5
	+	0.17 $\pm$ 0.07	57.8 $\pm$ 3.4	11.8 $\pm$ 1.6
Fc <sup>+</sup> T cells§	-	0.41 $\pm$ 0.23	55.5 $\pm$ 3.1	39.4 $\pm$ 4.5
	+	0.48 $\pm$ 0.11	34.8 $\pm$ 3.5	41.2 $\pm$ 2.1

\* See footnote (\*) of Table I.

‡ Unfractionated spleen cells ( $4 \times 10^5$ /well) were incubated 2 h at 37°C to allow adherence of cells to the bottom of the well. The wells were washed with warm medium and the plates cultured for an additional 24 h and then washed again before addition of T cells and mitogen.

§ See footnote (§) of Table II.

cells were cultured for 48 or 72 h with 2.5  $\mu$ g/ml PHA or 1.5  $\mu$ g/ml Con A. The response to PHA of the Fc<sup>-</sup> T cells, the Fc<sup>+</sup> T cells, and any mixture of the two, was comparable after both 48 and 72 h of culture (Figs. 3 *a* and *b*). On the other hand, the response of the Fc<sup>-</sup> T cells to 1.5  $\mu$ g/ml Con A was significantly lower than that of the Fc<sup>+</sup> T cells at both 48 and 72 h (5-fold and 15-fold, respectively) (Figs. 3 *a* and *b*). The addition of Fc<sup>+</sup> T cells to the Fc<sup>-</sup> T cells resulted in an increased Con A response in excess of that which would be expected by simple addition of the two separate responses. This apparent synergy was even more striking after 72 h of culture, at which time the response of a 3:1 mixture of Fc<sup>-</sup>:Fc<sup>+</sup> T cells was more than 10-fold higher than Fc<sup>-</sup> T cells alone and not significantly different than that of Fc<sup>+</sup> T cells alone.

To determine whether the Fc<sup>-</sup> T cells were actively participating in the mitotic response to Con A in the presence of Fc<sup>+</sup> T cells or if the Fc<sup>-</sup> T cells were just providing a suitable culture environment and thus maximizing the response of the small number of Fc<sup>+</sup> T cells (50,000) added, mixtures of Fc<sup>+</sup> T cells and either Fc<sup>-</sup> T cells or X-irradiated (1,500 R) nylon wool-adherent cells (83% Ig<sup>+</sup>) were cultured for 72 h with 1.5  $\mu$ g/ml Con A. The reduction of the number of Fc<sup>+</sup> T cells per culture resulted in an essentially linear decrease in mitotic response to Con A when irradiated B cells were used to adjust total cell concentration to  $10^6$  cells/ml (Fig. 4). However, when Fc<sup>-</sup> T cells were used to adjust total cell concentration to  $10^6$  cells/ml no significant difference in Con A responsiveness was observed between  $0.5$ – $2.0 \times 10^5$  Fc<sup>+</sup> T cells/culture. The response of  $1 \times 10^5$  Fc<sup>+</sup> T cells mixed with  $1 \times 10^5$  Fc<sup>-</sup> T cells was 10-fold greater than either  $2 \times 10^5$  Fc<sup>-</sup> T cells or  $1 \times 10^5$  Fc<sup>+</sup> T cells mixed with  $1 \times 10^5$  irradiated B cells, suggesting an active participation in the mitotic response to Con A of the Fc<sup>-</sup> T cells in mixtures of Fc<sup>-</sup> and Fc<sup>+</sup> T cells.

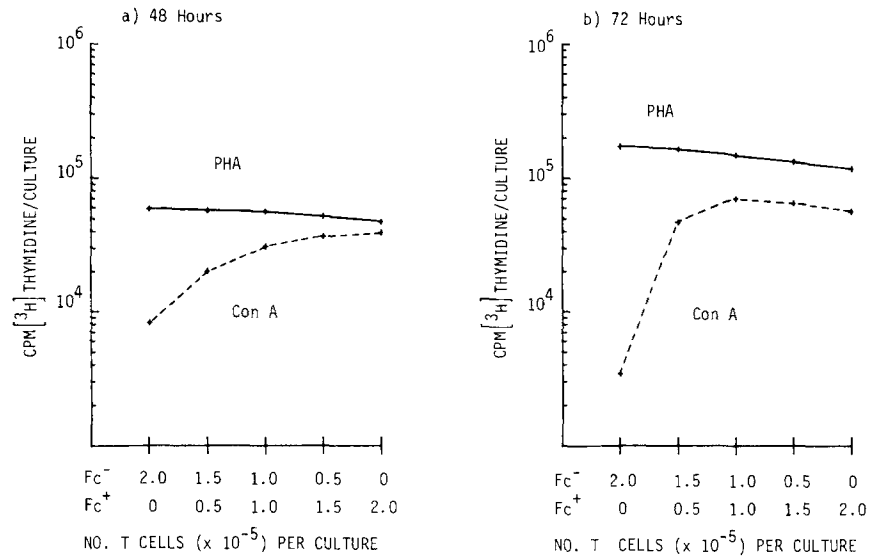


FIG. 3. Interaction of  $Fc^-$  and  $Fc^+$  T cells in the generation of a mitotic response to Con A. Nylon-purified splenic T cells were labeled with  $F^*(AgAb)$  and separated into  $Fc^-$  and  $Fc^+$  T-cell fractions on the FACS.  $F^*(AgAb)$  were removed from the surface of the labeled cells by incubating the cells in the presence of trypsin (0.1 mg/ml) for 10 min. Mixtures of the two fractions, at a total cell concentration of  $10^6$  cells/ml, were cultured for either 48 or 72 h in the presence of either 2.5  $\mu$ g/ml PHA or 1.5  $\mu$ g/ml Con A. The cultures were pulsed for 8 h with 1  $\mu$ Ci  $[^3H]$ thymidine before harvest.

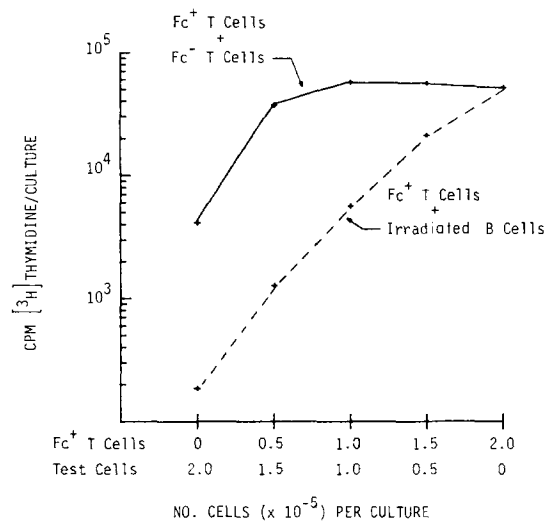


FIG. 4. Interaction of  $Fc^-$  and  $Fc^+$  T cells in the generation of a mitotic response to Con A. Nylon-purified splenic T cells were labeled with  $F^*(AgAb)$  and separated into  $Fc^-$  and  $Fc^+$  T-cell fractions on the FACS.  $F^*(AgAb)$  were removed from the surface of the labeled cells by incubating the cells in the presence of trypsin (0.1 mg/ml) for 10 min. Mixtures of  $Fc^+$  T cells with either  $Fc^-$  T cells (---) or irradiated (1,500 R) nylon-adherent (B-cell-enriched) lymphocytes, at a total cell concentration of  $10^6$  cells/ml, were cultured for 72 h in the presence of 1.5  $\mu$ g/ml Con A. The cultures were pulsed for 8 h with 1  $\mu$ Ci  $[^3H]$ thymidine before harvest.

### Discussion

Utilizing the FACS to separate T cells on the basis of their ability to bind antigen-antibody complexes, we have obtained two subpopulations of T cells — one which bears an Fc receptor (7) and responds well to both Con A and PHA, and one which does not bear an Fc receptor and which responds well to PHA but not to Con A. The poor responsiveness of the Fc<sup>-</sup> T cells to Con A was not due to a requirement for either different concentrations of Con A or for adherent cells.

The Fc<sup>+</sup> T-cell fraction responded both to Con A and to PHA. The response to Con A, however, was slightly less than or equal to the Con A response of unseparated T cells. Thus, on a per cell basis, only 20–30% of the Con A response was recovered in the Fc<sup>+</sup> T-cell fraction. This failure to enrich for Con A responsiveness could not be completely attributed to inhibition by antigen-antibody complexes since: (a) the recovery is based on the response of antigen-antibody complex-treated splenic T cells, and (b) both populations were treated with trypsin in a manner shown to reverse complex-mediated inhibition of Con A responsiveness.

The inability to recover 100% of Con A reactivity from fractions of separated T cells has been reported previously (6). In his review, Stobo (6) suggested that the loss of recoverable Con A reactivity upon T-cell fractionization compared to nonseparated cells might be due to the synergistic involvement of two distinct T-cell populations, one or both of which react with Con A specifically. This hypothesis was tested directly in our studies by mixing the Con A-unresponsive Fc<sup>-</sup> T cells with the Con A-responsive Fc<sup>+</sup> T cells in different ratios and determining the responsiveness of the mixtures to Con A. After 72 h, about the same Con A response was observed in all cultures containing from 25–100% Fc<sup>+</sup> T cells. The response of all of the mixtures was 10- to 20-fold higher than the Fc<sup>-</sup> T-cell fraction alone. Thus it appears that the Fc<sup>+</sup> T cells, upon reacting with Con A, can generate a mitotic response in the Fc<sup>-</sup> T-cell population. Whether this involves a specific interaction with Con A on the part of Fc<sup>-</sup> T cells is not yet known. The Fc<sup>-</sup> T cells may be reacting nonspecifically to changes in the culture environment induced by specific Con A activation of the Fc<sup>+</sup> T cells (e.g., elaboration of blastogenic factors) (6,9,10). On the other hand, the presence of Con A-activated Fc<sup>+</sup> T cells may result in the differentiation of Fc<sup>-</sup> T cells into a Con A-responsive (Fc<sup>+</sup>) state.

The latter hypothesis would suggest that the Fc<sup>-</sup> T-cell subset is a source of precursors for all or a subset of the Fc<sup>+</sup> T-cell population. The recent demonstration that the Fc<sup>-</sup> T-cell subpopulation acquires the Fc receptor upon *in vitro* allogeneic activation<sup>3</sup> would support this concept. There is, however, data which indicates that the Fc<sup>-</sup> and Fc<sup>+</sup> T-cell subpopulations may represent functionally distinct but parallel T-cell lines rather than different maturity stages of the same T-cell line. First, it has been suggested that the density of Thy 1.2 expressed on the surface of T cells decreases with maturation (1,4,11–13) and that the lymph node T-lymphocyte population, as opposed to the splenic T-lympho-

<sup>3</sup> Stout, R. D., S. D. Waksal, and L. A. Herzenberg. 1975. The Fc receptor on thymus-derived lymphocytes. III. Mixed lymphocyte reactivity and cell-mediated lympholytic activity of Fc<sup>-</sup> and Fc<sup>+</sup> T lymphocytes. Manuscript in preparation.



cyte population, is enriched for the T cells expressing a low density of Thy 1.2 on their surface. Separation of T lymphocytes into high and low Thy 1.2 density fractions has not, in our hands, resulted in an enrichment for Fc<sup>+</sup> T lymphocytes in either fraction (unpublished observation). In addition, the proportion of T lymphocytes bearing the Fc receptor appears to be the same in lymph node and spleen (7).

Secondly, the Fc<sup>-</sup> T-cell subpopulation expresses a function that the Fc<sup>+</sup> T-cell subpopulation does not express — “helper” T-cell activity in the antibody response to DNP-KLH (7). It therefore appears that the Fc<sup>-</sup> T-cell subpopulation either represents a functionally distinct subset of T cells, maturing along parallel lines to the Fc<sup>+</sup> T cells represents a mixture of T-cell subsets, one of which may be a precursor of a subset of T cells in the Fc<sup>+</sup> T-cell subpopulation.

The synergistic interaction of Fc<sup>-</sup> and Fc<sup>+</sup> T cells in Con A responsiveness reported here is strikingly similar to the synergy in *in vitro* generation of cytotoxic cells between T-cell subpopulations expressing either different tissue migratory patterns (11–14) or different Ly antigens (15,16). We have recently demonstrated that (a) the Fc<sup>-</sup> T-cell population contains the helper T-cell subset (7), (b) the Fc<sup>-</sup> T cells respond as well or better than Fc<sup>+</sup> T cells in mixed lymphocyte reactions, whereas the Fc<sup>+</sup> T cells respond better than Fc<sup>-</sup> T cells in the generation of cytotoxic effector cells<sup>3</sup> and (c) that the cytotoxic effector cells bear the Fc receptor.<sup>3</sup> Cantor and Boyse (15,16) have shown that (a) T cells bearing only the Ly-1 determinant function as helper T cells, (b) T cells bearing either the Ly-1 determinant only or both the Ly-2 and Ly-3 determinants respond in mixed lymphocyte reactions, whereas the subset bearing both the Ly-2 and Ly-3 determinants respond much better in the generation of cytotoxic effector cells, and (c) the cytotoxic effector cells bear both the Ly-2 and Ly-3 determinants. The functional similarity of the Fc<sup>-</sup>/Fc<sup>+</sup> T-cell subsets and the Ly-1<sup>+</sup>/Ly-1<sup>-</sup>3<sup>+</sup> subsets is striking. Preliminary data available at present indicate that both the Fc<sup>-</sup> and Fc<sup>+</sup> T-cell subpopulations are comprised of at least two subsets each, which may be distinguished on the basis of the Ly antigens they express (unpublished observation). A further comparison of the Fc<sup>+</sup> and Fc<sup>-</sup> T cells with the various Ly subsets is in progress in our lab.

In regard to this synergy, the observed inhibition of Con A responses by antigen-antibody complexes is of considerable interest. It is possible that the complexes are just sterically blocking the mitogen receptors. It has been shown that the binding of Con A to the surface of spleen cells does not inhibit subsequent binding of aggregated immunoglobulin to the Fc receptors of the B cells<sup>4</sup> but this does not rule out the possibility that the Con A receptors on T cells are physically associated with their Fc receptor. It is however unlikely that the antigen-antibody complexes are nonspecifically blocking all of the receptors on the cell surface since the complexes do not inhibit PHA responsiveness of the cells (Table I).

Ryan et al.<sup>4</sup> have proposed that the complexes, once bound to the Fc receptor, trigger a central “off” signal which prevents the response of the cells to the mitogens. But, if this were the case, one would expect the PHA response of the

<sup>4</sup> Ryan, J. L., R. D. Arbeit, H. B. Dickler, and P. A. Henkart. 1975. Inhibition of lymphocyte mitogenesis by immobilized antigen-antibody complexes. Manuscript submitted for publication.

Fc<sup>+</sup> T cells to be inhibited in addition to the Con A response, since the Fc<sup>+</sup> T cells also respond to PHA. Another, perhaps more simple, explanation would be that the antigen-antibody complexes are preventing the Fc<sup>+</sup> T cells from interacting with the Fc<sup>+</sup> T cells, thus substantially reducing the response mounted by a mixed population. The Fc<sup>+</sup> T cells have been shown to represent about 25% of the splenic T-cell population (7) and their responsiveness to Con A appears to be less sensitive to inhibition by antigen-antibody complexes than the responsiveness of the unseparated T-cell population (unpublished observation). This would explain why complexes, rather than completely inhibiting Con A responses, only reduce the response three- to fivefold (Table I and footnote 4). It would also suggest a role for antigen-antibody complexes in the feedback regulation of immune systems requiring cell cooperation. That is, if the Fc<sup>+</sup> T cell is involved in amplification of immune responses through precursor cell recruitment (6,17), the inhibition of Fc<sup>+</sup> T-cell function by antigen-antibody complexes would constitute a simple and effective mechanism for feedback control of an immune response by termination of cell recruitment.

In light of this proposed regulatory function for Fc<sup>+</sup> T cells, the demonstration that Con A stimulation of T lymphocytes results in a nonspecific suppression of antibody responses (18,19) is of considerable interest. The Fc<sup>+</sup> T cells, have been shown in the present report to be the cells responsible for the mitotic response of T cells to Con A and are, therefore, likely candidates for the cells involved in the Con A-induced nonspecific suppressor mechanism. The role of Fc<sup>+</sup> and Fc<sup>-</sup> T cells in immunoregulation is currently under investigation.

### Summary

The responsiveness of purified Fc<sup>-</sup> and Fc<sup>+</sup> T lymphocytes, isolated from normal spleen cell populations by cell sorting on the fluorescence activated cell sorter, has been examined. Although both Fc<sup>-</sup> and Fc<sup>+</sup> T cells responded to phytohemagglutinin, the response to concanavalin A (Con A) was found to be a characteristic of the Fc<sup>+</sup> T lymphocyte. The poor responsiveness of the Fc<sup>-</sup> T cells to Con A was shown not to be due to a requirement for either different concentrations of Con A or for adherent cells. The addition of Fc<sup>+</sup> T cells to the Fc<sup>-</sup> T cells in a ratio of 1:3 resulted in a mitotic response not significantly different from that observed with the purified Fc<sup>+</sup> T cells alone and up to 15-fold greater than that of Fc<sup>-</sup> T cells alone. It is suggested that the Fc<sup>-</sup> T cells can be recruited into mitosis as a result of Con A stimulation of the Fc<sup>+</sup> T cells.

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