SPECIFIC ENRICHMENT OF THE SUPPRESSOR T CELL BEARING I-J DETERMINANTS

Parallel Functional and Serological Characterizations*

By KO OKUMURA, TOSHITADA TAKEMORI, TAKESHI TOKUHISA, AND TOMIO TADA

(From the Laboratories for Immunology, School of Medicine, Chiba University, Chiba, Japan)

Recent evidence indicates that determinants controlled by a locus (*Ia-4* locus) mapped in *I-J* subregion of mouse *H-2* major histocompatibility complex are selectively expressed on a functional subpopulation of peripheral T lymphocyte, which is endowed with a role to suppress the antibody and immunoglobulin production (1–3).[1] Unlike other *I*-region associated (Ia) antigens, which are primarily detectable on B cells, the products of *Ia-4* locus are not found on B cells, but are uniquely expressed on T cells (1, 4). In addition, the same locus in *I-J* subregion appears to control the determinants found on the antigen-specific suppressive T-cell factor (3), and thus *I-J* subregion products provide an important clue for studying the nature of both the Ia antigen and the antigen-receptor which are unique to T cells.

However, the presence of such *I*-region determinants on T cells has been mainly determined by functional analyses in which the activity of suppressor T-cell and suppressive T-cell factor is removed by anti-Ia antisera (1, 3, 5, 6), and no direct serological affirmation for the T-cell Ia antigen is yet available using standard cytotoxic assays. This is probably due to the fact that only a very small portion of T cells among total lymphoid cells express such Ia antigens, and this imposes a great limitation in determining the specificity, function, and biochemical structure of T-cell Ia antigens.

We have reported in a preliminary form a simple technique to enrich the antigen-specific suppressor T cell which carries *I-J* subregion determinants (7). We confirmed further that this method is highly effective in obtaining antigen-specific T cells and in analyzing their phenotypic expressions by the usual serological procedures. This report will describe the detailed method to enrich the *I-J*-bearing suppressor T cell, and the serological and functional analyses of the purified suppressor T cell and its products. We also present our recent results concerning the relationship between the I-J determinants, Lyt phenotype, and Fc receptor (FcR)[2] expressed on the specifically purified suppressor T cell.

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[1] Hämmerling, G. J. *In* Proceedings of the Third Ir Gene Workshop. H. O. McDevitt, editor. Academic Press, Inc., New York. In press.

[2] *Abbreviations used in this paper:* alum, aluminum hydroxide gel; BAT, brain associated T-cell antigen; C, complement; DNP, 2,4-dinitrophenyl; D'PBS, Dulbecco's phosphate-buffered saline; FACS, fluorescence-activated cell sorter; FcR, Fc receptor; FCS, fetal calf serum; Fr, fraction; KLH, keyhole limpet hemocyanin; MIg, mouse immunoglobulin; PFC, plaque-forming cells; SRBC, sheep erythrocyte; TsF, suppressive T-cell factor.

Materials and Methods

Animals. C3H/He and C57BL/6J mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu City, Shizuoka, Japan). Strains of B10.A(3R) and B10.A(5R) were kindly provided by Dr. C. S. David of the Department of Genetics, Washington University School of Medicine, St. Louis, Mo., and have been maintained in our animal facility.

Antigens. Keyhole limpet hemocyanin (KLH) was obtained from Calbiochem, San Diego, Calif. Dinitrophenylated KLH (DNP_{770}-KLH) was prepared by the method described previously (8). Egg albumin recrystallized five times was purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio.

Immunization of Mice. As the source of antigen-specific suppressor T cells, mice were immunized with two intraperitoneal injections of 100 μg soluble KLH at a 2-wk interval as described previously (8). Other mice were immunized with 100 μg of DNP-KLH or KLH alone in aluminum hydroxide gel (alum) together with 1×10^9 killed *Bordetella pertussis* vaccine. They were used as the source of DNP-primed B and KLH-primed T cells after appropriate treatments (see below).

Antisera. A polyvalent anti-mouse immunoglobulin antiserum (anti-MIg) was obtained by repeated immunizations of rabbits with normal mouse gamma globulin fraction in complete Freund's adjuvant. The rabbit anti-mouse brain-associated T-cell antigen (anti-BAT) was prepared by the method of Sato et al. (9). The anti-sheep erythrocyte (anti-SRBC) antibody for the Fc rosette assay is the 7S fraction of mouse antiserum against SRBC, which was obtained by gel filtration with Sephadex G-200.

Alloantisera directed at *I-J* subregion of $H\text{-}2^k$ and $H\text{-}2^b$ haplotypes (anti-$I\text{-}J^k$ and anti-$I\text{-}J^b$) were prepared by reciprocal immunization of B10.A(3R) and B10.A(5R) with their lymphoid cells. The antisera were absorbed with syngeneic spleen cells to remove auto-reactive antibodies before use. Anti-Lyt alloantisera were kindly provided by Dr. D. B. Murphy of Stanford University, Stanford, Calif., and anti-Thy-1.2 antiserum produced in Thy-1 congenic mice was the gift of Dr. H. Sato of the Asahikawa Medical School, Hokkaido, Japan.

Preparation of Antibody- and Antigen-Coated Columns. Rabbit anti-MIg antibody was specifically purified by adsorption to and elution from the immunoadsorbent composed of mouse gamma globulin fraction. The purified anti-MIg was then coupled to cyanogen bromide-activated Sephadex G-200 beads (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) according to the method described by Schlossman and Hudson (10). The conjugation of KLH to Sephadex G-200 was likewise performed by coupling 30 mg of KLH to 20 ml of activated Sephadex G-200. 20 ml of these materials was packed in 20-ml disposable syringes in which about 1 ml of Sephadex G-25 was layered at the bottom as a sieve. The columns were equilibrated with Dulbecco's phosphate-buffered saline (D'PBS) fortified with 5% heat-inactivated fetal calf serum (FCS; Grand Island Biological Co., Grand Island, N.Y.). Detailed methods to use these columns are described in results.

Adoptive Secondary Antibody Response. DNP-primed B cells were obtained by treating DNP-KLH-primed spleen cells with anti-BAT antiserum and guinea pig complement. KLH-specific helper T cells were separated with the nylon wool column according to the method of Julius et al. (11). The mixture of DNP-primed B and KLH-primed T cells was transferred intravenously into lethally (650R) irradiated syngeneic recipients that were subsequently immunized with 10 μg of DNP-KLH. The number of DNP-specific plaque-forming cells (PFC) in the spleen was measured 7 days after the immunization by the method of Cunningham and Szenberg (12).

Cell Culture Technique. A modified Mishell-Dutton culture system was utilized to induce hapten-specific in vitro secondary antibody response. 4×10^6 of DNP-KLH-primed spleen cells were cultured with 0.1 μg/ml of DNP-KLH in RPMI-1640 enriched with 10% FCS in Falcon No. 3008 tissue culture plates (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). The culture was maintained at 37°C for 5 days, and the anti-DNP antibody response was measured by the PFC assay.

Preparation of the Cell-Free Extract from Fractionated Cells. Cells fractionated by the antigen-coated column (see Results) were suspended in 0.15 M saline at a concentration of 1×10^6/ml. The suspension was subjected to sonication on ice with a Tomy UR-150 Sonicator (Tomy Seiko Co., Ltd., Tokyo, Japan) as described previously (8). The cell-free supernate was obtained by centrifugation at 40,000 *g* for 1 h, and then dialyzed against saline at 4°C.

Separation of FcR$^+$ and FcR$^-$ Cells. Rosetting of surface Fc receptor-positive (FcR$^+$) cells was performed by the method of Möller (13) except that a mouse 7S anti-SRBC fraction was used instead of rabbit antibody to sensitize SRBC. The rosetting (FcR$^+$) cells were separated from nonrosetting (FcR$^-$) cells by centrifugation on Isopaque/Ficoll gradient (Isopaque; Nyegaard and Co., Oslo, Norway, Ficoll; Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) according to the method of Parish et al. (14).

Cytotoxic Assay. The ^{51}Cr release assay was used for cytotoxic test (15). ^{51}Cr-labeled 1×10^6 cells/ml were incubated with diluted antiserum at room temperature for 30 min and further at 4°C for 5 min. They were washed and subjected to the further 30-min incubation at 37°C with diluted rabbit complement (C). After centrifugation, radioactivity in the supernate was assayed. Sequential cytotoxic treatments with the combination of alloantisera were performed by the method described by Cantor and Boyse (16).

Analysis of I-J-Bearing Cells by Fluorescence-Activated Cell Sorter (FACS). The cell fractions separated with antigen-coated columns were analyzed by FACS II (Becton, Dickinson Electronics Laboratory, Mountain View, Calif.). The cells were treated with anti-I-J antisera followed by staining with fluoresceinated anti-mouse IgG. The fluorescence profile was analyzed by FACS II after gating out dead cells by the size scatter analysis (17).

Results

Fractionation of KLH-Primed Spleen Cells with Antibody- and Antigen-Coated Columns. Spleen cells from KLH-immunized mice were first passed through a column of Sephadex G-200 coupled with anti-MIg at 4°C to deplete B cells. The medium used in these procedures was D'PBS containing 5% FCS. About 40% of the original spleen cells were harvested in the effluent, which consisted of more than 70% of Thy-1 antigen-positive cells. 3 to 5×10^8 of these enriched T cells were resuspended in 5 ml of warm (37°C) medium. The suspension was then applied to the column of KLH-coated Sephadex G-200 at 37°C. Cells were allowed to penetrate into the column, and were further incubated at 37°C for 30 min in the column. The column was then washed with a warm (37°C) medium by adjusting the flow rate to about 1 ml/min. The elution of nonadherent cells was completed by washing the column with 150–250 ml of warm medium. The column was then placed in the 4°C cold chamber for 30 min. The cells bound to the column were eluted by washing with cold (0–4°C) medium at a flow rate of 1 ml/min.

The elution pattern of cells from the column is depicted in Fig. 1. The total number of cells eluted with the cold medium fraction II (Fr. II) was usually about 0.5% of the original spleen cells. If the spleen cell suspension from the egg-albumin immunized or normal mice were applied to the same KLH-coated column, the recovery of Fr. II did not exceed 0.1% of the original cells under the identical condition.

Functional Analyses of Cell Fractions Separated by Antigen-Coated Column

SUPPRESSOR ACTIVITY. The suppressor activity of column-separated fractions from C57BL/6 mice was assayed by the ability of the mice to suppress specifically an adoptive secondary antibody response of primed syngeneic spleen cells against DNP-KLH. The cells were cotransferred with DNP-primed B cells and KLH-primed nylon-purified T cells into irradiated syngeneic recipients that were subsequently immunized with 10 μg of DNP-KLH. The data in Table I shows that even 0.2×10^6 of Fr. II cells could completely suppress the anti-DNP antibody response, whereas 1×10^7 of Fr. I cells

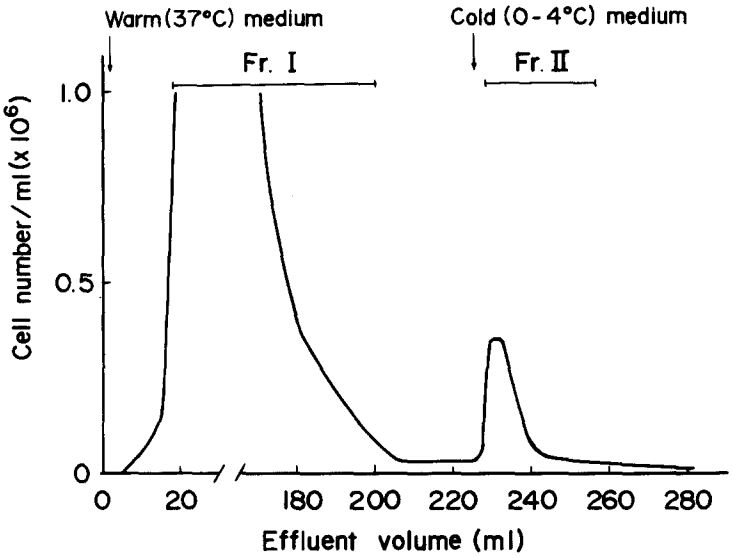

FIG. 1. Elution profile of KLH-primed T cells from the antigen-coated column. 5×10^8 cells were applied to a column of KLH-coated Sephadex G-200 at 37°C. After incubation at 37°C for 30 min, the cells were eluted with warm (37°C) medium (Fr. I), followed by elution at 4°C with cold (0–4°C) medium (Fr. II). Cells were washed and used for experiments. Normal as well as egg-albumin primed spleen cells did not yield the Fr. II peak under the same condition.

TABLE I

Enrichment of Suppressor T Cell with Antigen-Coated Column

KLH-primed suppressor T cell*		Anti-DNP IgG PFC/10^6‡
Fraction	Dose	
—§	—	1,516 (1.54)
Unfractionated	1×10^7	621 (1.68)
Fr. I	1×10^7	1,248 (1.13)
Fr. II	0.2×10^6	<10
B cell only‖		<10

* Suppressor T cells were cotransferred with 5×10^6 DNP-primed B cells and nylon column-purified KLH-primed helper T cells (1×10^6) into irradiated recipients.

‡ Direct PFC subtracted, geometric means and standard deviations calculated from four recipients.

§ Without suppressor T cells.

‖ 5×10^6 DNP-primed B cells without helper T cells.

produced no significant suppression. The T-cell fraction which was obtained by passage only through the anti-MIg column, produced moderate suppression at a dose of 1×10^7, and thus the suppressor activity was found almost exclusively concentrated in Fr. II which bound to the antigen-coated column at 37°C.

HELPER ACTIVITY. To assess whether the helper T cell could be separated by the same adsorption-elution procedure with the antigen-coated column, the spleen cells of C57BL/6 mice, which had been immunized by a single injection of KLH in alum 6 wk earlier to generate strong helper activity, were fraction-

TABLE II
Helper T Cell Does not Bind to the Antigen-Coated Column

KLH-primed helper T cell*		Anti-DNP IgG PFC/10^6‡
Fraction	Dose	
—§	—	351 (1.29)
Unfractionated	5×10^6	5,631 (1.67)
Fr. I	5×10^6	5,315 (1.67)
Fr. II	0.5×10^6	410 (1.47)
Fr. I + Fr. II	$5 \times 10^6 + 0.5 \times 10^6$	1,823 (1.89)

* KLH-primed helper T cells were cotransferred with 5×10^6 DNP-primed B cells into irradiated recipients.

‡ Direct PFC subtracted, geometric means and standard deviations calculated from four recipients.

§ Without helper T cells.

ated by exactly the same procedure as that used to enrich the suppressor T cell. The yield of Fr. II cells from the helper source was about the same as that from suppressor spleen cells. Each fraction was cotransferred with DNP-primed B cells into irradiated recipients that were then immunized with DNP-KLH. Table II shows that Fr. I cells, which did not bind to the KLH-column exerted a comparable helper effect to that of unseparated T-cell fraction at the same dose. On the contrary, even 0.5×10^6 Fr. II cells, which were obtained from more than 5×10^8 original spleen cells, exhibited very little helper activity. If the same number of Fr. II cells were admixed with 5×10^6 Fr. I cells and cotransferred with DNP-primed B cells to the recipient, the anti-DNP antibody response was much lower than that mounted by Fr. I cells and B cells. This indicates that the Fr. II cells which bind to the antigen-coated column are, in fact, suppressor T cells, even though the cells were derived from animals immunized to generate optimal helper activity. The results collectively indicate that the helper T cell does not bind to the antigen-coated column under the condition in which suppressor T cells are successfully removed. It is also shown that even the spleen cells, which exhibit strong helper activity as a whole, contain a significant number of suppressor T cells which can be separated by binding to the antigen-coated column.

The Suppressive T-Cell Factor Derives from Antigen-Binding T Cells. Since our previous studies indicated that the antigen-specific suppressive T-cell factor (TsF) is obtainable by sonication of suppressor T cells, we have attempted to extract TsF from fractionated cells. The cells of each fraction were disrupted by sonication, and the suppressive activity of the soluble supernate was assessed in the in vitro secondary antibody response. Table III shows the results of an experiment using DNP-KLH-primed C57BL/6 spleen cells as the responding cells to which varying doses of cell-free extracts obtained from syngeneic fractionated cells were added. It is clearly demonstrated that the extract from Fr. II cells produces a significant suppression of anti-DNP PFC response even at a dose corresponding to 10^3 live cells. With the extract corresponding to 10^4 Fr. II cells gave a comparable degree of suppression to that produced by the extract obtained from 100 times (1×10^6) as many unfractionated spleen cells. This

TABLE III
The Suppressive T-Cell Factor Derives from Antigen-Binding T Cells Lacking Fc Receptor

Extract from*	Corresponding cell number	Anti-DNP IgG PFC/culture‡	Suppression
			%
Without extract	—	3,217 ± 423	—
Unfractionated	1×10^6	813 ± 44	75
spleen	1×10^4	3,061 ± 114	5
Fr. I	1×10^6	2,873 ± 673	11
	1×10^4	3,228 ± 523	0
Fr. II	1×10^4	638 ± 365	80
	1×10^3	1,703 ± 356	47
Fr. II (FcR^+)§	1×10^5	3,413 ± 299	0
Fr. II (FcR^-)	1×10^5	420 ± 216	87

* Cell-free extract of each fraction corresponding to the given cell number was added to the cultured 4×10^6 DNP-KLH-primed spleen cells in modified Mishell-Dutton culture.
‡ Direct PFC subtracted, arithmetic means and standard deviations calculated from five cultures.
§ FcR^+ and FcR^- cells in Fr. II were separated by the rosetting with antibody-coated SRBC.

indicates that the fractionation procedure resulted in the 100-fold enrichment of suppressor T cell in Fr. II. On the other hand, almost no suppressive activity was observed with the extract from Fr. I cells.

Suppressive T-Cell Factor Derives from FcR^- T Cells. Fr. II cells were separated into two fractions by Fc-rosetting technique. About 25% of Fr. II cells were recovered as Fc rosette-forming (FcR^+) cells. After removal of SRBC by treating the cells with Gey's solution (18), both FcR^+ and FcR^- T-cell fractions were disrupted by sonication, and the supernates were likewise tested for their suppressive activity. As shown in the lower part of Table III, the suppression was induced only by the extract from FcR^- T cells, and hence, the producer of TsF was shown to lack surface Fc receptors.

Demonstration of I-J Determinants on Enriched Suppressor T Cells. Column-separated Fr. I and Fr. II cells were analyzed by cytotoxic ^{51}Cr release with various alloantisera and rabbit C. The results obtained with C57BL/6 spleen cells are depicted in Table IV. Both fractions contained 70–75% of Thy-1 antigen-positive cells. About 30% of Fr. II cells were constantly killed by anti-$I\text{-}J^b$ in repeated experiments, whereas no significant killing of Fr. I cells was observed with anti-$I\text{-}J^k$ antiserum. This killing was haplotype-specific, since the reciprocal antiserum (anti-$I\text{-}J^k$) did not lyse the cells from C57BL/6 mice, whereas it was able to kill Fr. II cells from C3H mice (see below). Another interesting fact is that Fr. II cells contained a larger proportion of cells that were killed by anti-Lyt-2.2, 3.2 antiserum than did Fr. I cells.

Cytotoxic curves with two anti-*I-J* antisera using Fr. II cells from C57BL/6 and C3H as targets are depicted in Fig. 2. The anti-$I\text{-}J^b$ antiserum specifically killed C57BL/6 Fr. II cells, and the anti-$I\text{-}J^k$ only killed C3H Fr. II cells. The titers of both antisera were relatively low showing the steep decline of cytotoxicity at dilutions beyond 1:20.

Relationship between the I-J Expression, Lyt-Phenotype, and Surface Fc Receptor on Enriched Suppressor T Cells. The above studies indicate that

TABLE IV
Cytotoxic Analysis of Cell Fraction of C57BL/6J Mice Separated by Antigen-Coated Column

Antiserum	Serum dilution	Cytotoxic ^{51}Cr release	
		Fr. I	Fr. II
		%	%
Anti-Thy 1.2*	1:40	70	75
Anti-*I-J*^{b}‡	1:10	<5	30
Anti-*I-J*^{k}§	1:10	<5	<5
Anti-Lyt-1.2	1:40	35	20
Anti-Lyt-2.2,3.2	1:40	20	40

* Congenic anti-Thy 1.2.
‡ B10.A(5R) anti-B10.A(3R).
§ B10.A(3R) anti-B10.A(5R).

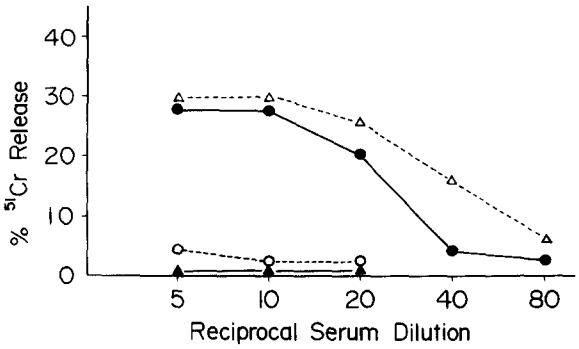

FIG. 2. Cytotoxic curves of Fr. II cells from C57BL/6J (solid lines) and C3H (broken lines) with anti-*I-J*^{b} (●——● ○- - -○) and anti-*I-J*^{k} (▲——▲ △- - -△) antisera. Cytotoxicity was undetectable with unfractionated cells and Fr. I cells (see text).

the enrichment of antigen-specific suppressor T cells by the antigen-coated column accompanied with the proportional increase of *I-J*$^+$, Lyt-2$^+$,3$^+$ cells. To examine whether the *I-J*$^+$ cells in fact belong to the Lyt-2$^+$,3$^+$ subclass, the phenotypic expressions of Fr. II cells were analyzed by the two-step sequential killing protocol according to the method used by Cantor and Boyse (16). The ^{51}Cr-labeled Fr. II cells from C57BL/6 mice were first treated with various alloantisera and rabbit C, and the residual live cells were then subjected to the second cytotoxic killing with different antisera. As shown in Table V, all the *I-J* determinant-bearing cells belong to the Thy-1 antigen-positive cell population. Only a small proportion of cells were killed by anti-Lyt-1.2 serum, while nearly 40% of cells were killed by anti-Lyt-2.2,3.2 serum. If the cells were first treated with anti-Lyt-2.2,3.2 antiserum, the residual cells were not killed by anti-Lyt-1.2 serum. This suggests that all the Lyt-1$^+$ cells in Fr. II are carrying also Lyt-2,3 determinants (Lyt-1$^+$,2$^+$,3$^+$ cells). The results of sequential killing with the combination of anti-Lyt and anti-*I-J* antisera (lower part of Table V) indicate that virtually all the I-J determinant-bearing cells possess Lyt-2,3 alloantigens, although the presence of a small number of Lyt-1$^+$,2$^+$,3$^+$ cells in this *I-J*$^+$ population is not excluded.

TABLE V
Phenotypic Expressions on Specifically Enriched Suppressor T Cells of C57BL/6J Mice

1st killing*		2nd killing‡	
Antiserum	^{51}Cr release	Antiserum	^{51}Cr release
	%		%
Anti-*I-J*^{b}	33	–	–
Anti-*I-J*^{k}	0	–	–
Anti-Thy 1.2	70	Anti-*I-J*^{b}	<5
Anti-Lyt-1.2	12	NMS§	0
Anti-Lyt-2.2,3.2	38	Anti-Lyt-1.2	<5
NMS§	0	Anti-Lyt-1.2	12
Anti-Lyt-1.2	12	Anti-*I-J*^{b}	25
Anti-Lyt-2.2,3.2	40	Anti-*I-J*^{b}	0

* ^{51}Cr-labeled Fr. II cells of C57BL/6J mice were treated with various alloantisera and rabbit C, and the radioactivity released in the medium was measured.

‡ Live cells after the 1st killing were then treated with antisera and C.

§ Normal C57BL/6J mouse serum absorbed with syngeneic spleen cells.

To learn the relationship between FcR and I-J determinants, the Fr. II cells were separated into FcR$^+$ and FcR$^-$ populations. As stated above, about 25% of Fr. II cells were FcR$^+$. No significant killing with anti-*I-J* antiserum was detectable in FcR$^+$ population, whereas about 30% of FcR$^-$ cells were killed by anti-*I-J* and C. Taken together, the I-J determinant-positive cells bear Lyt-2,3 alloantigens and lack the surface Fc receptor.

Analysis of I-J Determinant-Bearing Cells by Fluorescence-Activated Cell Sorter (FACS). Fr. II cells from C57BL/6 mice were reacted with anti-*I-J*^{b} or anti-*I-J*^{k} antiserum followed by staining with fluoresceinated anti-mouse IgG. The fluorescence distribution of cells was analyzed with FACS II after gating out dead cells by size scatter analysis. As can be seen in Fig. 3, the fluorescence profile of the Fr. II cells stained with anti-*I-J*^{b} showed a bimodal distribution, whereas the same cells treated with irrelevant (anti-*I-J*^{k}) antiserum showed a single fluorescence-negative peak. The computer analysis of the fluorescence-positive peak indicated that about 30% of the live Fr. II cells carry I-J determinants, the value being consistent with that obtained with cytotoxic ^{51}Cr release.

Discussion

The presence of unique *I* region gene products which are selectively expressed only on certain subsets of T cells has created new problems concerning the role of T-cell Ia antigen in the regulatory cell interactions in the immune response. The cell type which carries I-J determinants is involved in the allotype-, idiotype-, and antigen-specific suppressions (1–3, footnote 1), and in the initiation of T-cell response to concanavalin A (19). In addition, the determinants are found on the molecule which suppresses the antibody response in an antigen-specific fashion. Hence, the functional and biochemical analyses of *I-J* subregion gene products are of obvious importance for the further understand-

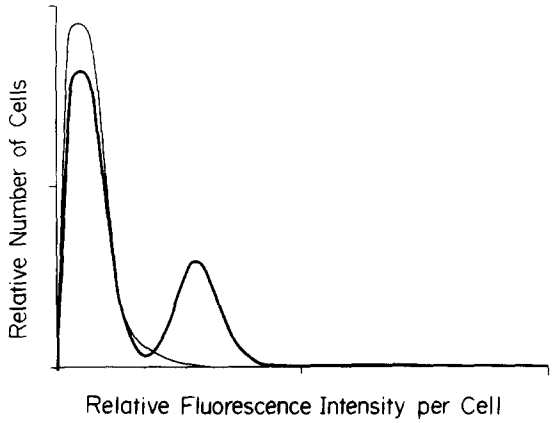

FIG. 3. Fluorescence distribution of Fr. II cells of C57BL/6J mice labeled with anti-$I\text{-}J^b$ (bold line) or anti-$I\text{-}J^k$ (light line) antiserum in FACS analysis. The cells were treated with alloantisera followed by staining with fluoresceinated rabbit anti-mouse IgG. Note the bimodal distribution of cells stained with anti-$I\text{-}J^b$ but not with anti-$I\text{-}J^k$. The cells stained with anti-$I\text{-}J^b$ comprise about 30% of total Fr. II cells.

ing of the molecular events in the regulatory cell interactions as well as of the nature of antigen receptor of T cells.

There was, however, a great limitation in proceeding with these studies, since the presence of *I-J*-bearing cells was only demonstrable by functional studies in which the activity of suppressor T cells and suppressor factor was removed by anti-*I-J* antisera, and since there was no way to directly detect the *I-J* subregion gene products by serological and immunochemical procedures. This is mainly due to the fact that the number of *I-J*-bearing cells in total spleen cells is too small to allow such an analysis. The present report described a simple method to enrich the antigen-specific suppressor T cell about 100-fold allowing us to study the phenotypic expressions on the cell type with respect to the I-J determinants, Lyt alloantigens, and Fc receptors. Results collectively indicate that I-J determinants are expressed mainly within the Lyt-2^+,3^+, FcR^- T-cell population. More recently, Taniguchi and Miller[3] were able to separate the antigen-specific suppressor T cell by adsorption to and elution from antigen-coated Petri dishes. Properties of the purified suppressor T cell are very similar to those of ours despite the fact that their suppressor T cell was derived from tolerant mice.

The present studies together with those reported previously (3, 20) indicate that the antigen-specific suppressor T cell indeed possesses antigen-binding sites together with I-J determinants. This is in sharp contrast to the helper T cell which does not bind to the antigen-coated column under the identical condition. These observations raise an important question as to whether or not the antigen recognition of helper and suppressor T cells are the same. It has been reported that macrophages are required to induce the helper T cell but not the suppressor T cell (21, 22). The optimal conditions to induce helper and

[3] Taniguchi, M., and J. F. A. P. Miller. Enrichment of specific suppressor T cells and characterization of their surface markers. Manuscript submitted for publication.

suppressor T cells are different with respect to the dose of antigen (23, 24), adjuvant vehicle (23), and the form of antigen (25, 26). One possible explanation is that the suppressor T cell recognizes antigen itself and can be stimulated by free antigen, while the helper T cell is only generated by modified antigen presented on macrophages. The other, and perhaps related explanation is that the affinity of antigen-binding sites of helper and suppressor T cells is different. Further biochemical and functional studies will give the final answer.

The method described in this paper appears to have several important applications to future studies on T-cell Ia antigens. In fact, the method allowed us to titrate the anti-*I-J* antibody by the direct cytotoxic assay, which was not possible with the total splenic T cells. We were also able to analyze and separate *I-J*-bearing cells with FACS. These will facilitate the biochemical analysis of the *I-J* subregion gene products with respect to both the possible constant part and antigen-binding site of the molecule, and the serological analysis of specificities of I-J determinants possessed by various haplotype strains. One of the most attractive applications may be to make hybrid cell lines expressing I-J determinants, which will allow the more precise biochemical and genetic studies of I-J molecules having antigen specificity and possibly an idiotype.

Summary

A simple procedure to enrich the antigen (keyhole limpet hemocyanin, KLH)-specific suppressor T cell was described. The suppressor T cell from KLH-immunized mice specifically bound to the KLH-coated Sephadex G-200 column at 37°C, and was eluted from the column by cold (0–4°C) medium. The helper T cell did not bind to the column under the identical condition. The suppressor T cell thus obtained had 100 times as potent suppressor activity as the original spleen cells in in vivo and in vitro secondary antibody responses against a hapten coupled to KLH. This procedure also enriched the cells bearing I-J determinants and Lyt-2,3 alloantigens, allowing us to study the phenotypic expressions on the suppressor T cell by direct serological procedures as well as by the use of the fluorescence activated cell sorter. Parallel functional and serological analyses indicated that the antigen-specific suppressor T cell belongs to a population of $I\text{-}J^+$, Lyt-2^+,3^+ and Fc R^- T cells.

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