

T-LYMPHOCYTE HETEROGENEITY IN THE RAT: SEPARATION OF FUNCTIONAL SUBPOPULATIONS USING A MONOCLONAL ANTIBODY

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In the mouse alloimmunization has revealed the Ly series of lymphocyte differentiation antigens which have been used to show the existence of T-cell subpopulations with distinct surface phenotypes (1, 2). Furthermore, cytotoxic analysis using anti-Ly antisera has shown that T cells exhibiting different functions, e.g. helper activity or suppressor activity, can be distinguished on the basis of their surface markers (3-5). In other species such alloantigenic markers are not available, and their identification would be time-consuming and haphazard since the existence of serologically detectable polymorphisms is unpredictable. A method (6, 7) is now available for producing monoclonal antibodies to cell surface antigens using xenogeneic immunization in which most cell surface molecules should be antigenic. Mice are immunized with cells or subcellular fragments from another species, and their spleen cells are subsequently fused with a mouse myeloma cell line. Hybrid cells are selected for and screened for the production of antibodies to cell surface antigens. Monoclonal antibodies can then be obtained by cloning the antibody-secreting hybrids.

The application of this system to lymphocytes was investigated using antibodies produced by hybrids formed with spleen cells from mice immunized with rat thymocyte membrane (7). One of these antibodies, W3/25, labeled 80% of rat thymocytes but only 52-58% of thoracic duct lymphocytes. This subset in thoracic duct lymphocytes (TDL)¹ was independent of the Ig⁺ cells, but contained only 71-75% of the remainder. W3/25 antibody thus appeared to be labeling a T-lymphocyte subset, and this could be clearly identified using the fluorescence-activated cell sorter (FACS II), thus allowing the separation of lymphoid cell populations into W3/25 positive and W3/25 negative fractions. The isolation of subsets of lymphocytes on the basis of antibody binding using the cell sorter rather than by complement-mediated cytolysis has the great advantage that both the labeled and unlabeled cell populations can be assayed for their immune functions.

In the present study, rat TDL labeled by W3/25 antibody were separated from the unlabeled cells, and both populations were assayed for graft-versus-host activity and helper or suppressor functions.

Materials and Methods

Rats. Inbred strains PVG.1-a (10th backcross) and PVG/c (1-b) congenic for the Ig-1 light chain allotype were used with F₁ hybrids between DA (1-a) and PVG/c. The strain PVG.1-a (8) was developed by Dr. S. V. Hunt (University of Oxford, England) who kindly provided the rats for these experiments. All rats were maintained at the MRC Cellular Immunology Unit,

¹ *Abbreviations used in this paper:* BGG, bovine gamma globulin; DAB, phosphate-buffered saline containing 0.1 g calcium chloride and magnesium chloride per liter; DNP, 2,4-dinitrophenol; FACS II, fluorescence-activated cell sorter model II; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; PFC, plaque-forming cell; TDL, thoracic duct lymphocytes.

at the University of Oxford. Recipients in adoptive transfer experiments were irradiated 24 h before transfer using a ^{137}Cs source (120 rads/min).

Antibodies. W3/25 antibody was used in the form of a tissue culture supernate (concentrated 5- to 10-fold) from uncloned W3/25 hybrid cells unless otherwise stated. From the uncloned W3/25 culture a clone was isolated (W3/25 HLK) by making multiple serial dilutions in agar and selecting a dilution at which only a few culture supernates were positive. One active clone was selected from these subcultures by removing individual clones from the agar, and it was then recloned at 100 cells/plate. This recloning procedure yielded 17 antibody-producing clones, 16 of which, designated HLK, expressed the antibody heavy and light chains with the kappa chain of the parental myeloma (this myeloma does not synthesize any heavy chain of its own), and one clone (HL) that failed to produce the myeloma kappa chain. In addition, two clones (HK) were obtained that lacked antibody activity but secreted the heavy chain of the specific antibody with the myeloma kappa chain. Chain compositions were established by sodium dodecyl sulphate polyacrylamide gel electrophoresis of reduced ^{14}C -labeled supernates. The evidence that the uncloned and cloned HLK supernates contained the same single antibody specificity is given in Results. The purified anti-Ig antibodies used were rabbit anti-rat F(ab')₂-fluorescein isothiocyanate (FITC) (9); rabbit F(ab')₂ anti-mouse IgG-FITC (10); ^{125}I -labeled rabbit anti-rat F(ab')₂ (10) and ^{125}I -labeled PVG/c anti-DA light chain (I-a) allotype (9).

Collection of TDL and Labeling with Antibody. TDL, obtained by cannulation (11), were collected on ice for 12 h into 5 ml phosphate-buffered saline containing 0.1g calcium chloride and magnesium chloride per liter (DAB) (Oxoid, London, England), containing 20 U/ml heparin. The cells were washed twice with DAB/10% fetal calf serum (FCS) before labeling.

TDL (1.5×10^8) were incubated for 60 min on ice with 500 μl of W3/25 antibody, and washed twice with DAB/10% FCS/10 mM sodium azide. Then they were incubated with 500 μl rabbit anti-mouse IgG-FITC at 30 $\mu\text{g}/\text{ml}$ plus 20 μl PVG/c serum (to absorb any cross-reacting antibody) for 60 min, and they were then washed again.

To label TDL with W3/25 antibody and anti-rat F(ab')₂ antibody, the first step was as described above, but in the second step, 500 μl of a mixture of rabbit anti-mouse IgG-FITC (30 $\mu\text{g}/\text{ml}$) plus rabbit anti-rat F(ab')₂-FITC (20 $\mu\text{g}/\text{ml}$) was used. To label TDL with rabbit anti-rat F(ab')₂-FITC antibody (20 $\mu\text{g}/\text{ml}$), one incubation and washing cycle were performed.

Separation of Cells on FACS II. The labeled TDL were sorted for ≈ 6 h and collected on ice. Erythrocytes and dead cells were excluded from the sorted populations by the appropriate scatter window gating (12). Machine settings were Photomultiplier voltage 680 V, Laser power 300 mW, Scatter Gain 2/1, Fluorescence Gain 8/1. Average cell recovery was 33%.

Priming. 2,4-Dinitrophenol bovine gammaglobulin (DNP₃₇BGG) was prepared by the method of Little and Eisen (13). Priming was by i.p. injection of 1 mg alum-precipitated DNP₃₇BGG with 10^9 killed *Bordetella pertussis* organisms (The Wellcome Research Laboratories, Beckenham, England). TDL donors were used 4–16 wk after priming.

Autoradiographic Plaque-Forming Cell Assay. This assay was carried out according to the method of Mason (14). Briefly, spleen cell suspensions were prepared by pressing the spleens through fine wire gauze, the cells were then washed twice in DAB/10% FCS, and incubated on DNP-gelatin-coated slides for 60 min at 37°C in an H₂O saturated atmosphere. The slides were then washed in phosphate-buffered saline (PBS)/1% Triton X-100 (British Drug Houses Ltd., Poole, England) followed by two washes in PBS, and they were then fixed in 95% ethanol for 30 min. After drying in air, the slides were incubated with ^{125}I -labeled anti-1-a allotype or ^{125}I -labeled anti-rat F(ab')₂ (4 $\mu\text{g}/\text{ml}$) for 60 min at 4°C. Then they were washed thoroughly in PBS, fixed in 95% ethanol for 30 min, dried, and dipped in Ilford K2 emulsion (Ilford Ltd., Ilford, England). Autoradiographs were developed 4–5 days later. Plaques were counted using transmitted light microscopy with $\times 128$ magnification and a graticule eyepiece.

Popliteal Lymph Node Assay. This was performed as described in Ford et al. (15). Recipient rats were injected with cell preparations in 100 μl PBS into both hind footpads. After 7 days, the popliteal lymph nodes were removed, cleaned of adherent fatty tissue, and weighed.

Results

W3/25 antibody was initially identified in a culture containing more than one clone of hybrid cells. The supernates of these uncloned cultures provided the source

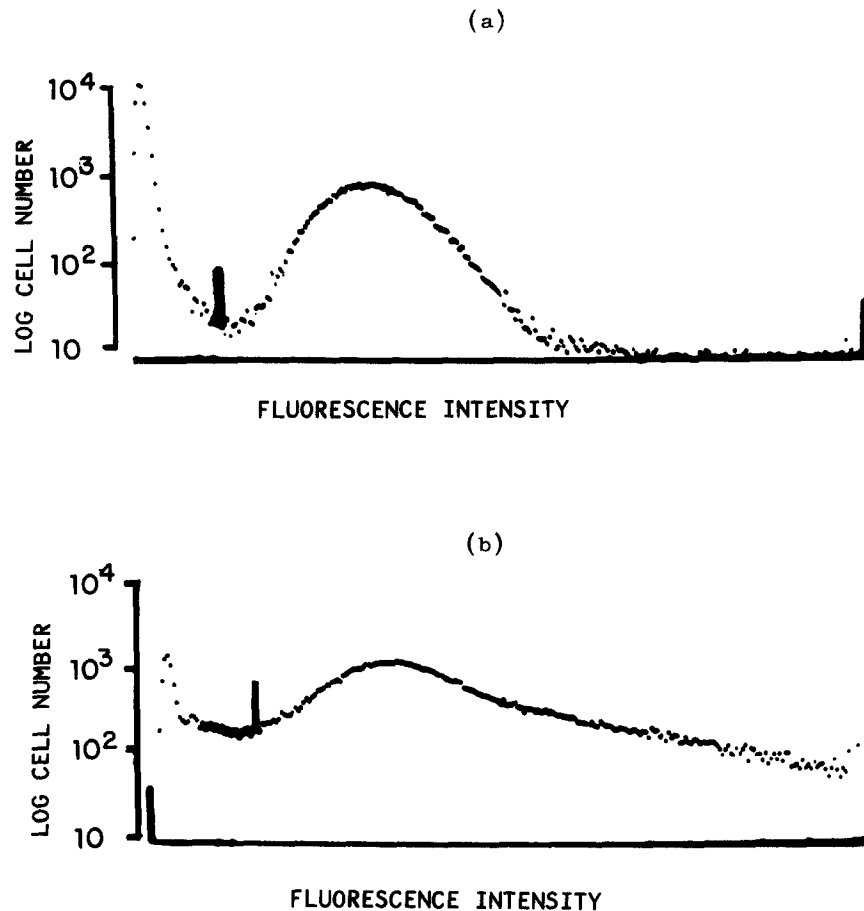


FIG. 1. (a), Fluorescence profile of PVG/c TDL after labeling with W3/25 antibody. Percentage of TDL above the marker = 37%. (b), Fluorescence profile of PVG/c TDL after simultaneous labeling with W3/25 antibody plus anti-rat $F(ab')_2$. Percentage of TDL above the marker = 88%. Total number of cells in each profile = 100,000.

of W3/25 antibody for both the tissue distribution analysis (7) and the present experiments. Recently, the hybrid secreting W3/25 antibody has been cloned by using multiple serial dilutions followed by cloning in soft agar (see Materials and Methods). The antibody is retained by immunoabsorbents specific for IgG₁ but not for IgG₂, and is therefore likely to be of the IgG₁ subclass. The specificity of the antibody from the W3/25 HL and HLK subclones was checked against that of the antibody from the uncloned cultures by incubating TDL with each antibody alone and also with two together, followed by labeling with rabbit anti-mouse IgG-FITC. The labeling was analyzed on the FACS II, and the profile is shown in Fig. 1 for W3/25 antibody from uncloned cultures. The percentage of labeled cells was found to be the same whether the antibodies were used separately or in mixtures, and the profiles were identical in form. Thus, the three preparations of W3/25 are identical with respect to specificity.

In Fig. 1 *a*, 37% of the TDL were labeled by W3/25 antibody, and the overall

TABLE I
Helper Cell Assay on TDL Separated on the FACS II after Labeling with W3/25 Antibody

| TDL transferred | Percentage of whole TDL | No. of recipients | PFC/Spleen (mean and range) | |
|--------------------------------------|-------------------------|-------------------|-----------------------------|---|
| | | | Anti-1-a (donor origin) | Anti-F(ab') ₂ (donor + host) |
| | % | | | |
| No cells | — | 3 | 42 (0-126) | 1,200 (1,000-1,600) |
| 6.5×10^6 W3/25 ⁺ | 48 | 2 | 17,100 (15,800-18,400) | 46,900 (34,000-60,000) |
| 6.5×10^6 W3/25 ⁻ | 48 | 2 | 57 (0-114) | 2,700 (900-4,570) |
| 1.3×10^7 Unseparated | 100 | 3 | 224,200 (180,000-290,700) | 229,000 (203,900-253,000) |

The TDL were collected from PVG.1-a rats primed with DNP₃₇BGG. PVG/c recipients were given TDL preparations and 1 mg DNP₃₇BGG or antigen alone i.v. after irradiation with 660 rads. The autoradiographic PFC assay was performed 7 days after the cell transfers. Purity of sorted cells: W3/25⁺, 91%; W3/25⁻, 99%.

range in the current experiments was 37-48%. These values are lower than those of 52-58% previously reported (7). This is almost certainly due to the fact that in the present experiments PVG/c rats were used, instead of the Wistars which were used previously. The proportion of T lymphocytes is about 55% and 70% of TDL, respectively, in these strains.

Helper Cell Assay. The assay for helper cell activity was carried out by adoptive transfer in an Ig-allotype congenic system. The donors of DNP₃₇BGG-primed TDL were PVG.1-a, and the irradiated recipients were of the congenic strain PVG/c (Ig 1-b). Thus, by using ¹²⁵I-labeled anti-1-a allotype or ¹²⁵I-labeled anti-rat F(ab')₂, both donor and total B-cell plaque-forming cell (PFC) responses could be measured. It has previously been shown that PVG.1-a helper T cells will effectively collaborate with PVG/c B cells (16). Table I shows the results of an experiment in which the DNP₃₇BGG-primed PVG.1-a TDL were separated on the FACS II on the basis of labeling with W3/25 antibody. The unlabeled cells contain both the unlabeled T cells and the B cells. The 1-a allotype PFC responses are particularly striking in that the unlabeled cell population failed to make a response, whereas the labeled cells produced a response \times 340 background wholly dependent upon the approximately 5% B-cell contamination of this fraction. In addition, the combined host plus donor PFC response in the recipients of labeled cells was increased by a factor of 40 over that of rats receiving antigen alone, whereas the unlabeled cells gave only twofold stimulation.

When the PVG.1-a TDL were separated on the FACS II on the basis of simultaneous labeling with both W3/25 antibody and anti-rat F(ab')₂, the B cells were isolated along with the labeled T cells (Fig. 1 b). In this system (Table II), unlabeled cells failed to produce a response above background of either host or donor allotype, whereas the labeled cells produced a total response of \times 350 background. The separated, labeled T cells performed at least as well as the equivalent total T-cell population in the unseparated TDL, and thus it appears that the T cells with helper activity are contained within the population labeled by W3/25 antibody.

Graft-Versus-Host Assay. In a preliminary study using TDL fractions separated on the FACS II on the basis of surface Ig labeling, it was shown that the popliteal lymph node enlargement of (PVG/c XDA) F₁ rats injected in the footpad with PVG/c TDL was dependent upon the T-cell dose, that the presence or absence of B cells had negligible influence, and that B cells alone were ineffective (unpublished observations). Table III shows the results obtained when the assay was performed using parental

TABLE II
Helper Cell Assay on TDL Separated on the FACS II after Simultaneous Labeling with W3/25 Antibody Plus Anti-Rat F(ab')₂

| TDL transferred | Percentage of whole TDL | No. of recipients | PFC/Spleen (mean and range) | |
|---|-------------------------|-------------------|-----------------------------|---|
| | | | Anti-I-a (donor origin) | Anti-F(ab') ₂ (donor + host) |
| | % | | | |
| No cells | — | 3 | 0 (—) | 660 (430-860) |
| 7.6×10^6 W3/25 ⁺ cells plus Ig ⁺ cells | 86 | 3 | 211,000 (170,600-263,000) | 235,000 (190,000-279,000) |
| 7.3×10^6 W3/25 ⁻ Ig ⁻ cells | 10 | 3 | 0 (—) | 950 (140-1,400) |
| 1.0×10^7 Unseparated cells | 100 | 3 | 102,500 (67,200-130,000) | 171,000 (144,000-195,000) |

The TDL were collected from PVG.1-a rats primed with DNP₃₇BGG. The PVG/c recipients were given TDL preparations and 1 mg DNP₃₇BGG or antigen alone i.v. 24 h after irradiation with 660 rads. The autoradiographic PFC assay was performed 7 days after the cell transfers. Purity of sorted cells: W3/25⁺ cells plus Ig⁺ cells, 98%; W3/25⁻ Ig⁻ cells, 94%.

TABLE III
Graft-Versus Host Assay on TDL Separated on the FACS II after Labeling with W3/25 Antibody

| Group | TDL injected | Percentage of whole TDL | No. of cells injected per footpad ($\times 10^{-6}$) | No. of footpads injected | Popliteal lymph node weight (mg) (mean and range) |
|-------|--------------------|-------------------------|--|--------------------------|---|
| | | % | | | |
| 1 | W3/25 ⁺ | 44 | 4.0 | 6 | 52.3 (29.0-69.1) |
| 2 | W3/25 ⁻ | 49 | 3.7 | 2 | 7.3 (7.0-7.5) |
| 3 | W3/25 ⁻ | 49 | 17.0* | 1 | 12.3 (—) |
| 4 | Unseparated | 100 | 2.2 | 5 | 37.6 (29.6-46.9) |
| 5 | Unseparated | 100 | 4.4 | 4 | 56.7 (35.7-69.6) |
| 6 | No cells | — | — | 4 | 5.0 (3.4-6.7) |

(PVG/c XDA) F₁ hybrids received PVG/c TDL preparations in 100 μ l PBS or 100 μ l PBS alone subcutaneously into each hind footpad. The popliteal lymph nodes were extracted and weighed 7 days afterwards. Purity of sorted cells: W3/25⁺ cells, 93%; W3/25⁻, 97%.

* Because the W3/25⁻ cells contained B cells as well as W3/25⁻ T cells, this cell dose was chosen so as to contain the same number of T cells as that used in group 1.

TDL sorted by W3/25 antibody labeling. It is clear that the labeled cell population is considerably more efficient at producing lymph node enlargement than the unlabeled cell population (groups 1 and 2), and this is apparently still true when approximately equal doses of W3/25 positive and W3/25 negative T cells were injected (groups 1 and 3).

A more detailed analysis is presented in Fig. 2 for which the parental TDL were sorted on the basis of simultaneous labeling with both W3/25 antibody and anti-rat F(ab')₂. The results indicate that 3.0×10^6 T cells from unseparated TDL produce the same lymph node enlargement as 2.2×10^6 labeled T cells. Thus, the labeled T cells produce a response as large as the corresponding whole T-cell population in unseparated TDL. As in the previous experiment (Table III), the W3/25 negative T cells produced little lymph node enlargement; 1.1×10^6 of these cells were equivalent to 0.5×10^6 T cells in unseparated TDL.

Allogeneic Suppression Effect. Modulation of both host and donor PFC responses by a concomitant graft-versus-host reaction in adoptive transfer experiments has been shown in several systems (17-19). In the rat, a suppression of both donor and host

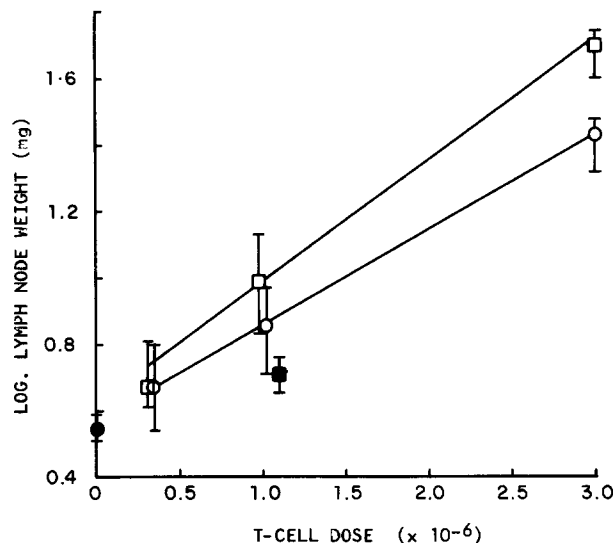


FIG. 2. Graft-versus-host assay on TDL separated on the FACS II after simultaneous labeling with W3/25 antibody plus anti-rat $F(ab')_2$. (PVG/c \times DA) F_1 hybrids received PVG/c TDL preparations in 100 μ l PBS, or 100 μ l PBS alone, subcutaneously into the hind foot-pads. The popliteal lymph nodes were extracted and weighed 7 days afterwards. (○) Unseparated TDL; (□), W3/25⁺ plus Ig⁺ cells, 88% of whole TDL, purity 99%; (■), W3/25⁻ cells, 9% of whole TDL, purity 89%; (●), PBS control. Mean and range values are shown with 3-4 nodes per dose in each group.

anti-DNP PFC responses was observed when DNP-BGG-primed parental cells were transferred to irradiated F_1 hybrid recipients and simultaneously challenged with DNP-BGG (20). Table IV shows the effect of depleting the DNP-BGG-primed parental TDL of the cells that remain unlabeled after incubation with W3/25 antibody and anti-rat $F(ab')_2$. The effects of this procedure on the congenic (PVG/c \rightarrow PVG.1-a) and the semi-allogeneic (PVG/c \rightarrow [PVG/c XDA] F_1) transfers are compared. In the syngeneic system, the removal of 15% of the transferred cells had little effect, which supports the earlier finding that the T cells with helper activity are included in the W3/25 positive population. The higher total PFC response of the depleted versus the unseparated TDL in experiment A was not seen in experiment B, and the addition of W3/25 negative cells to the unseparated TDL in experiment B had no significant effect. Thus, there was no consistent evidence to support a suppressor role for W3/25 negative cells in the congenic system.

In the semiallogeneic transfer, whereas the recipients of depleted TDL gave host and total PFC responses similar to those obtained in the congenic system, the recipients of unseparated TDL gave markedly reduced responses. The requirement for positive selection of those cells capable of producing the suppression was met by the reconstitution experiment shown in Table V. In group 3 of this experiment, the labeled and unlabeled populations were transferred together after separation on FACS II. The 1-a allotype PFC response produced when the labeled cells were transferred alone was reduced more than fivefold by the addition of the W3/25 negative population. The TDL donors in this experiment were used later after priming (14 wk) than those in the previous experiments, and this probably accounts for the lower total PFC response after transfer of the labeled TDL fraction.

These three experiments establish that cells that do not carry the antigenic

TABLE IV
The Allogeneic Suppression Effect with TDL Separated on the FACS II after Simultaneous Labeling with W3/25 Antibody and Anti-Rat F(ab')₂

| PVG/c TDL transferred | Recipient | No. of Recipients | PFC/Spleen (mean and range) | | |
|-----------------------|---|---------------------------|-----------------------------|---|---------------------------|
| | | | Anti-I-a (host origin) | Anti-F(ab') ₂ (donor + host) | |
| Experiment A | | | | | |
| 1.0 × 10 ⁷ | Unseparated cells | PVG.1-a | 3 | 57,900 (33,600-71,000) | 100,900 (80,400-116,700) |
| 1.1 × 10 ⁷ | W3/25 ⁺ cells plus Ig ⁺ cells | PVG.1-a | 2 | 49,800 (39,500-60,100) | 177,000 (169,300-184,700) |
| 1.0 × 10 ⁷ | Unseparated cells | (PVG/c XDA)F ₁ | 2 | 2,600 (2,500-2,800) | 15,300 (11,700-19,000) |
| 1.1 × 10 ⁷ | W3/25 ⁺ cells plus Ig ⁺ cells | (PVG/c XDA)F ₁ | 3 | 46,800 (33,300-61,800) | 61,700 (48,100-81,800) |
| Experiment B | | | | | |
| 1.0 × 10 ⁷ | Unseparated cells | PVG.1-a | 3 | 72,900 (62,400-88,900) | 309,200 (285,000-348,700) |
| 0.9 × 10 ⁷ | W3/25 ⁺ cells plus Ig ⁺ cells | PVG.1-a | 2 | 74,800 (73,500-76,100) | 265,000 (232,700-297,400) |
| 1.0 × 10 ⁷ | Unseparated cells plus 2.6 × 10 ⁶ W3/25 ⁻ Ig ⁻ cells | PVG.1-a | 2 | 73,100 (71,000-75,200) | 313,700 (279,000-347,800) |
| 1.0 × 10 ⁷ | Unseparated cells | (PVG/c XDA)F ₁ | 3 | 5,500 (2,700-10,800) | 56,000 (45,600-72,600) |
| 0.9 × 10 ⁷ | W3/25 ⁺ cells plus Ig ⁺ cells | (PVG/c XDA)F ₁ | 3 | 52,200 (33,700-74,400) | 190,000 (142,800-217,700) |

TDL were collected from PVG/c rats primed with DNP₃₇BGG. The recipients were given TDL preparations and 1 mg DNP₃₇BGG i.v. 24 h after irradiation with 660 rads. The autoradiographic PFC assay was performed 7 days after the cell transfers. Experiment A, W3/25⁺ cells plus Ig⁺ cells 85% of whole TDL, purity 96%. Experiment B, W3/25⁺ cells plus Ig⁺ cells 89% of whole TDL, purity 99%; W3/25⁻ Ig⁻ cells 6% of whole TDL, purity 92%.

TABLE V
The Allogeneic Suppression Effect: Reconstitution after Separation of TDL on the FACS II after Simultaneous Labeling with W3/25 Antibody Plus Anti-Rat F(ab')₂

| TDL transferred | No. of (PVG/c XDA)F ₁ recipients | PFC/Spleen (mean and range) | |
|---|---|-----------------------------|---|
| | | Anti-I-a (host origin) | Anti-F(ab') ₂ (donor + host) |
| 9.0 × 10 ⁶ unseparated cells | 3 | 1,900 (680-2,600) | 57,100 (30,000-66,700) |
| 8.0 × 10 ⁶ W3/25 ⁺ plus Ig ⁺ cells | 3 | 16,000 (11,200-21,000) | 68,900 (51,300-92,300) |
| 8.0 × 10 ⁶ W3/25 ⁺ plus Ig ⁺ cells with 1.3 × 10 ⁶ W3/25 ⁻ Ig ⁻ cells | 3 | 3,000 (1,400-5,100) | 38,900 (20,200-64,000) |

The TDL were collected from PVG/c rats primed with DNP₃₇BGG. (PVG/c XDA)F₁ recipients were given TDL preparations and 1 mg DNP₃₇BGG i.v. 24 h after irradiation with 660 rads. The autoradiographic PFC assay was performed 7 days after transfer of cells. W3/25⁺ cells plus Ig⁺ cells, 87% of whole TDL, purity 99%. W3/25⁻ Ig⁻ TDL, 10% of whole TDL, purity 95%.

determinant recognized by W3/25 antibody are required for the suppressive effect in a parental to F₁ adoptive transfer.

Discussion

The monoclonal antibody W3/25 recognizes a surface antigenic determinant that serves to distinguish functionally different subpopulations in rat TDL. This antigenic determinant is present on helper cells and cells that produce the lymph node enlargement seen in the popliteal lymph node assay.² However, it is absent from cells

² The significance of the small amount of lymph node enlargement obtained when W3/25 negative T cells were used in the assay is uncertain. Apparently, injection of syngeneic cells may produce enlargement of this magnitude (15). In any event, that the W3/25 positive cells were much more efficient, in terms of node enlargement per 10⁶ cells transferred, is clearly established by the experiments.

that are required for the suppression of the anti-hapten response of F_1 recipients of carrier-primed parental helper cells. Whereas helper and graft-versus-host activities are established T-cell functions, the allogeneic suppression effect is less well understood. In the absence of an established marker for rat T cells, the thymus dependency of the W3/25 negative cells mediating this effect remains to be formally demonstrated. In the mouse, however, a similar effect has been shown to be T-cell dependent (21).

If these results in the rat with W3/25 antibody are compared with those obtained in the mouse using anti-Ly antisera, a correlation is apparent between the monoclonal antibody and anti-Ly 1. First, the percentage of peripheral T cells recognized by anti-Ly 1, based on cytotoxicity, correlates well with the 71–75% of T cells labeled by W3/25 antibody as seen on the FACS II (2, 7). Second, helper cells have been shown to be Ly 1^+ (3–5), and finally, graft-versus-host activity in the mouse is sensitive to anti-Ly 1 antisera (22).

Suppressor cells in mice have been found to be Ly 1^{-2+} in several systems (4, 5, 23, 24), and this would appear to correlate with the finding in the present work that the allogeneic suppression effect required W3/25 negative cells. However, using the suppression system in the mouse that shows most procedural similarities to the one investigated here, Pickel and Hoffmann (19) showed a requirement for Ly 1^{+2+} cells for the suppression of antibody formation *in vitro* after induction of suppression *in vivo* by a graft-versus-host reaction. In a very similar system, Shand (25) has recently confirmed the Ly 1^{+2+} phenotype of the cells required for suppression. Since these experiments were performed using negative selection based on complement-mediated cytotoxicity, it has not been shown that only the Ly 1^{+2+} population is required for suppression, and so the possibility remains that collaboration between Ly 1^{+2+} and Ly 1^{-2+} cells is necessary. Thus, until more is known about the cellular basis of the suppression systems in both the rat and the mouse, the effect of these results on the correspondence between W3/25 antibody and anti-Ly 1 serum will not be clear. Investigations are in progress to determine whether or not collaboration between W3/25 negative and W3/25 positive subsets is required for the allogeneic suppression effect in the rat using positive selection on the FACS II.

The results obtained with the monoclonal antibody W3/25 clearly establish functional heterogeneity among rat T cells. By using xenogeneic immunization and the cell fusion technique for preparing monoclonal antibodies, in combination with cell sorting, it is in principle possible to identify all those functionally different T-cell subpopulations that bear unique combinations of xenogeneic membrane antigenic determinants. To what extent this potential may be realized in practice is yet to be explored.

Summary

W3/25 antibody is the monoclonal product of a hybrid cell resulting from the fusion of a mouse myeloma cell line with spleen cells from a mouse immunized with rat thymocytes. Pure clones have been derived, and segregants free of parental myeloma chains have been isolated. Previous studies have shown that this antibody recognizes a subpopulation of T cells among rat thoracic duct lymphocytes. In the work reported here, three T-cell functions were assayed after separating rat thoracic duct lymphocytes on the fluorescence-activated cell sorter on the basis of labeling with W3/25 antibody. Two of the functional activities appeared to be completely

segregated by this procedure. Thus, helper cell activity for an anti-hapten plaque-forming cell response was confined to the labeled population, whereas the allogeneic suppressive effect produced in a parental \rightarrow F₁ adoptive transfer was mediated by cells in the unlabeled fraction. The third function, graft-versus-host activity, was almost entirely contained within the labeled subpopulation. It is concluded that the antigenic determinant recognized by the monoclonal antibody W3/25 is a differentiation marker for T-cell functional subpopulations.

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