

Ly AND Ia ANTIGEN PHENOTYPES OF T CELLS INVOLVED IN DELAYED-TYPE HYPERSENSITIVITY AND IN SUPPRESSION*

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T lymphocytes participate in many immunologic functions. For example, they may become cytotoxic cells (T_C),¹ exert helper (T_H) or suppressor (T_S) effects on antibody production, or trigger the inflammatory response of delayed-type hypersensitivity (DTH) (T_D). Recently, it has been demonstrated (1, 2) that some of the T cells involved in these functions may be characterized by their cell surface antigens. The Ly (2) and Ia (3) alloantigens have been especially useful in this regard. Thus cytotoxic and suppressor functions are diminished by treatment with anti-Ly-2 serum, but not by anti-Ly-1 serum (4, 5), whereas helper functions are sensitive to anti-Ly-1 serum, but not to anti-Ly-2 serum (2). Treatment with anti-Ia serum has been reported to diminish helper and suppressor functions, but not cytotoxic functions (3). In this paper we report that the cells responsible for DTH are similar to T_H in their Ly phenotypes but, unlike some primed helper cells, are Ia negative.

Materials and Methods

Mice. 2- to 3-mo-old specific pathogen-free CBA/CaHWehi, BALB/c An Bradley Wehi, and C57BL/6J Wehi mice were used. The mice used for antisera production were: AKR/J, A.TH*, A.TL*, B10.PL(7S)NS*, (B10.AKM \times 129)F₁*, C57BL/6-Ly-1^a*, and (C57BL/6 \times LP.RIII)F₁*. These mice were obtained from either The Jackson Laboratory, Bar Harbor, Maine or from Dr. D. Shreffler, Department of Human Genetics, University of Michigan, Ann Arbor, Mich. The strains marked with an asterisk were obtained from Dr. M. Cherry, The Jackson Laboratory.

Antigens. Erythrocytes from sheep (SRBC) and horse (HRBC) were obtained from the Commonwealth Serum Laboratories (CSL), Melbourne, Australia. Fowl gamma globulin (FGG) was obtained as described before (6) and human gamma globulin (HGG) (Cohn fraction no. 11) was purchased from CSL. These proteins were diluted in normal saline to the appropriate concentra-

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¹ *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; deHGG, deaggregated HGG; DTH, delayed-type hypersensitivity; FGG, fowl gamma globulin; GRF, genetically related factor; HGG, human gamma globulin; HRBC, horse erythrocyte; ¹²⁵I-UdR, ¹²⁵I-5-iodo-2'-deoxyuridine; L, left; PFC, plaque-forming cell; POL, polymerized flagellin; R, right; T_C , T cells responsible for cell-mediated lysis; T_D , T cells responsible for initiating DTH reactions; T_H , T cells responsible for helper function; T_S , T cells responsible for suppressor function.

tion before use. Deaggregated HGG (deHGG) was prepared according to the method of Basten et al. (7). DNP₅HGG and DNP₁₅FGG were prepared as described previously (8).

Sensitization Procedures. For the production of high levels of DTH, mice were treated with 200 mg/kg of cyclophosphamide (Endoxan, Asta; Mead Johnson, Crows Nest, Australia) (9) subcutaneously 2 days before giving 400 µg of FG (in 0.1 ml) emulsified in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.) into the two hind foot pads and subcutaneously into the abdomen. Mice were primed to HGG by injecting 500 µg alum-precipitated antigen (10) intraperitoneally with 2×10^8 *Bordetella pertussis* organisms (Pertussis vaccine, 4×10^{10} organisms/ml; CSL). In the case of dinitrophenylated antigens, 100 µg DNP-HGG or 30 µg DNP-FGG was given in the soluble form intraperitoneally. 10^8 HRBC were injected intravenously into each mouse. Mice were tolerized to HGG by the intravenous injection of 2.5 mg of deHGG (7).

Cell Suspensions. Lymph nodes were removed and cell suspensions prepared by teasing organs through a stainless steel wire mesh into Eisen's balanced salt solution. They were washed three times, counted, and their viability estimated by an eosin-dye exclusion method.

DTH Assay. This is described in detail elsewhere (11). Briefly this assay measures the influx of cells labeled with ¹²⁵I-5-iodo-2'-deoxyuridine (¹²⁵I-UdR) into DTH lesions. Conventionally, antigen is deposited into the left (L) pinna and the right (R) remains uninjected. 24 h later the ears are cut off and the ratio of the radioactivity in the L/R ears reflects the extent of DTH. To increase labeling efficiency 10^{-7} mol of 5-fluorodeoxyuridine is injected intraperitoneally 20 min before ¹²⁵I-UdR.

Assay for Plaque-Forming Cells (PFC's). PFC's were assayed by the technique of Cunningham and Szenberg (12). Indirect (IgG) PFC's were developed by the addition of rabbit-antimouse immunoglobulin serum to the cell mixture. This antiserum did not suppress direct (IgM) PFC's.

Cytotoxicity Assay. This method has been described in detail by Burton et al. (13). Briefly responder spleen cells are cultured in vitro with irradiated stimulator cells for 6 days. The cells are harvested and added to target cells labeled with ⁵¹Cr. The release of ⁵¹Cr into the supernate after a period of time is proportional to the number of cytotoxic lymphocytes added, and to the number of target cells lysed. Percent specific lysis is calculated by the formula:

$$\frac{{}^{51}\text{Cr release in experiment} - \text{background}}{\text{maximal releasable } {}^{51}\text{Cr} - \text{background}} \times 100;$$

where background is the release of ⁵¹Cr from target cells in the absence of immune lymphocytes and maximal releasable ⁵¹Cr is the amount released when labeled target cells are lysed with detergent.

Radioisotopes and Radiometry. ¹²⁵I-UdR, sp act 90-110 µCi/µg, ⁵¹Cr (sodium chromate, sp act 50-200 µCi/µg CEA, Gif-Sur-Yvette, France) was used as described before (13).

Production of Antisera

ANTI-Ly-1.1, ANTI-Ly-2.1 AND ANTI-Ia 1, 2, 3, 7. Thymus cells (1 donor per 10 recipients) were used for immunization for the Ly antisera, and thymus, lymph node, and spleen cells for the Ia antisera. Intraperitoneal injections were given weekly for 6-8 wk and thereafter every 2 wk. In the weeks between immunizations, the mice were bled and the sera collected and pooled. Sera were absorbed by mixing them with thymocytes (10 thymuses/ml serum) for 30 min at 4°C in order to remove autoantibody. The strains and sources of the mice used for the production and absorption of these sera are shown in Table I.

ANTI-Ly-1.2 AND 2.2. These sera were prepared by Dr. Shen as described in reference 14.

ANTI-Thy-1 SERUM. This was raised in AKR/J mice as described previously (15).

COMPLEMENT (c). Rabbits were bled, their serum separated and tested for C activity. Batches were selected for low cytotoxicity against mouse thymocytes and high C activity. When necessary the serum was absorbed with mouse thymocytes before use.

Treatment of Cells with Antisera

ANTI-Ly-1.1 AND 2.1 AND ANTI-Ia. To 1 ml of cells at a concentration of 1.5×10^6 /ml, 1 ml of antiserum was added to a final concentration of 1:10 for anti-Ly-1.1, 1:4 for anti Ly-2.1, and 1:100 for anti-Ia, and the cells left at room temperature for 30 min, washed twice, and resuspended in rabbit C at a final concentration of 5×10^6 /ml. They were then incubated for 30 min at 37°C and

washed three times before use. The concentrations of sera used were those which lysed the maximum number of cells as determined by previous cytotoxic assays.

ANTI-Ly-1.2, 2.2. Anti-Ly-1.2, 2.2 is described in detail elsewhere (14).

ANTI-Thy-1. Anti-Thy-1 was used as previously described (15).

Specificity of Ly Antisera. The Ly antisera were raised between strains of mice congenic for the *Ly-1* and *Ly-2* loci (Table I) and should contain no additional specificities other than autoantibody. This appeared to be the case as both sera reacted as expected: cells from CBA, C3H, DBA/1, and DBA/2 strains and the appropriate donors were positive when reacted with both antisera; C57BL/6, A, BALB/c, and 129 were negative.

Results

Effect of Anti-Ly and Anti-Ia Sera on the Transfer of DTH by Lymph Node Cells. Lymph node cells from cyclophosphamide-pretreated CBA mice were taken 5 days after immunization with FGG. The cells were divided into five aliquots of 10^8 viable cells each and were given the following treatment: (a) anti-Ly-1.1 serum and C; (b) anti-Ly-2.1 serum and C; (d) anti-Ia serum and C; (e) C only; and (f) no treatment. Each aliquot was then injected intravenously into five naive CBA mice which were ear-challenged immediately with FGG. The ability of these cells to transfer DTH was assessed by the radioisotopic ear assay. The results are shown in Table II. Incubation with C alone, anti-Ia serum and C, or anti-Ly-2.1 serum and C did not decrease the ability of the cells to transfer DTH, whereas anti-Ly-1.1 serum and C abolished it. The enhanced capacity of anti-Ly-2.1 serum-treated cells to transfer DTH (Table II) was observed in three out of six such experiments.

To check if anti-Ly-1.2 and anti-Ly-2.2 sera had similar effects we immunized C57BL mice with FGG as described above and treated the lymph node cells obtained 5 days later in the following ways: (a) anti-Ly-1.2 serum and C; (b) anti-Ly-2.2 serum and C; (c) C alone; and (d) no treatment. The results are shown in Table III. Again anti-Ly-1.2 serum but not anti-Ly-2.2 serum inhibited the transfer of DTH.

These results suggest that the Ly phenotype of T_D cells is the same as that reported for T_H (1, 2, 5). They do, however, differ in their sensitivity to anti-Ia serum (see below). We have previously found that lymph node cells from animals sensitized to give maximal DTH can also exert helper functions. Thus it was possible for us to compare directly the effect of antiserum treatment on the same population of lymph node cells that had the potential to transfer these two T-cell functions.

Effect of Anti-Ly and Anti-Ia Sera on the Helper Activity of Sensitized Lymph Node Cells. Lymph node cells were taken from the same CBA animals that were used for DTH experiments. Aliquots of 10^7 viable cells were treated in the following ways: (a) anti-Ly-1.1 serum and C; (b) anti-Ly-2.1 serum and C; (c) anti-Ia serum and C; (d) C alone; and (e) no treatment. Each aliquot was then transferred into six syngeneic, lethally irradiated mice together with 10^8 HRBC and 5×10^6 anti-Thy-1 serum-treated spleen cells from CBA mice primed with DNP-polymerized flagellin (POL) 2 mo previously. The animals were given 30 μ g of DNP-FGG intraperitoneally, and 7 days later the spleens were taken and assayed for anti-HRBC PFC and anti-DNP PFC (as DNP-Fab-SRBC). The results are shown in Table IV. Treatment with anti-Ly-1.1 serum and anti-Ia

TABLE I
Production, Absorption, and Cytotoxicity Titers of Anti-Ly-1.1, Anti-Ly-2.1, Anti-Ia,
and Anti-Thy-1 Sera

Specificity of antiserum	Donor of immunizing cells	Recipients	Origin of cells used for absorption	Cytotoxicity titers		
				Thymus	Lymph node	Spleen
Ly-1.1	C57BL/6-Ly-1 ^a	(C57BL/6 × LP.RIII)F ₁	C57BL/6 thymus	1/256	1/4,192	1/4,192
Ly-2.1	B6.PL(75NS)	(B10.AKM × 129)F ₁	C57BL/6 thymus	1/256	1/32	1/128
Ia-1,2,3,7	A.TL	A.TH	A.TH thymus	0	1/5,000	1/5,000
Thy-1.2	CBA	AKR	Krebs II ascites	ND	1/32	ND

TABLE II
Effect of Anti-Ly and Anti-Ia Sera on the Transfer of DTH by CBA Lymph Node Cells

No. of sensitized lymph node cells transferred before treatment	Antiserum treatment	L/R ¹²⁵ I-UdR uptake
0	—	1.25 ± 0.09*
15 × 10 ⁶	—	2.19 ± 0.12
15 × 10 ⁶	C	1.86 ± 0.13
15 × 10 ⁶	Anti-Ly-1.1 + C	1.21 ± 0.05
15 × 10 ⁶	Anti-Ly-2.1 + C	2.75 ± 0.13
15 × 10 ⁶	Anti-Ia + C	1.79 ± 0.15

* ixMean ± one SEM, five mice per group.

TABLE III
Effect of Anti-Ly Sera on the Transfer of DTH by C57BL Lymph Node Cells

No. of sensitized lymph node cells transferred (before treatment)	Antiserum treatment	L/R ¹²⁵ I-UdR uptake
0	—	1.31 ± 0.13*
12 × 10 ⁶	—	3.11 ± 0.21
12 × 10 ⁶	C	2.61 ± 0.26
12 × 10 ⁶	Anti-Ly-1.2 + C	1.30 ± 0.18
12 × 10 ⁶	Anti-Ly-2.2 + C	2.23 ± 0.08

*Mean ± one SEM. Four to five mice per group.

serum but not with anti-Ly-2.1 serum markedly inhibited the helper activity of these cells in terms of anti-DNP antibody production to DNP-FGG.

Before firm conclusions could be reached about the Ly and Ia phenotypes of T_D and T_H cells, it was important to demonstrate that our anti-Ly-1.1 serum did not delete all T-cell functions, and that our anti-Ly-2.1 and anti-Ia sera were indeed effective against some T-cell functions. These experiments are described below.

Effect of Anti-Ly Sera on the Activity of Cytotoxic T Lymphocytes In Vitro. To induce cytotoxic lymphocytes, CBA spleen cells were incubated with irradiated BALB/c spleen cells in vitro. After 6 days of incubation the cells were collected, washed three times, and divided into eight equal aliquots which were treated in the following ways: (a) no treatment; (b) C only; (c) anti-Ly-1.1

TABLE IV
Effect of Anti-Ly Serum on the Helper Activities of Sensitized Lymph Node Cells

No. of cells transferred into lethally irradiated* recipients together with 10 ⁸ HRBC‡		Antiserum treatment of transferred FGG-primed lymph node cells	PFC per spleen at 7 days		
			Anti-DNP		
DNP-POL-primed spleen cells treated with anti-Thy-1 serum	FGG-primed lymph node cells		Direct	Indirect	Indirect anti-HRBC
5 × 10 ⁶	None	—	30 (3.3)§	150 (1.6)	0
5 × 10 ⁶	2 × 10 ⁶	—	2,600 (1.2)	6,600 (1.3)	50 (2.7)
5 × 10 ⁶	2 × 10 ⁶	Anti-Ly-1.1 + C	170 (3.7)	290 (4.3)	450 (1.4)
5 × 10 ⁶	2 × 10 ⁶	Anti-Ly-2.1 + C	750 (1.3)	9,700 (1.1)	560 (1.4)
5 × 10 ⁶	2 × 10 ⁶	C alone	1,300 (1.5)	16,600 (1.2)	150 (2.9)
5 × 10 ⁶	2 × 10 ⁶	Anti-Ia + C	190 (2.4)	340 (1.7)	100 (3.1)

* 850 R 4 h before transfer.

‡ 30 µg DNP-FGG was given intraperitoneally at the same time. HRBC as 0.1 ml of 5% suspension.

§ Geometric means and SEM (number in parentheses denotes factor which multiplies and divides the mean to give the upper and lower limits of the SEM). Six mice per group. Numbers of direct HRBC PFC were less than 20 per spleen in all groups.

serum; (d) anti-Ly-2.1 serum; (e) anti-Thy-1 serum; (f) anti-Ly-1.1 serum plus C; (g) anti-Ly-2.1 serum plus C; and (h) anti-Thy-1 serum plus C. The cytotoxic activity of these various aliquots against WEHI-164 is shown in Table V. Anti-Thy-1 serum and C reduced this activity to 6%. Anti-Ly-2.1 serum and C and anti-Ly-1.1 serum and C reduced the cytotoxicity, but the reduction was more marked with anti-Ly-2.1 serum and C (16 vs. 26%). When BALB/c cytotoxic lymphocytes were treated in an identical manner there was no evidence of abrogation of cytotoxic activity with anti-Ly-1.1 and anti-Ly-2.1 sera (data not shown).

Effect of Anti-Ly and Anti-Ia Sera on the Suppressor Activity of Spleen Cells from HGG-Tolerant Mice. Spleen cells taken from CBA mice injected 14 days previously with 2.5 mg of deHGG provide a source of T-cell-dependent suppressor cells (7). When these cells were mixed with HGG-primed spleen cells and DNP-primed B cells and given to lethally irradiated syngeneic recipients challenged with DNP-HGG and HRBC, specific suppression can be demonstrated (see groups 1-4 in Table VI and reference 7). Groups 5-8 in Table VI show the effect of the treatment of the suppressor population with Ly and Ia antisera. Groups 9-12 show the PFC's resulting when the same antisera were used on the HGG-primed population. Three points can be made about the results obtained. Anti-Ia serum was effective in diminishing the suppressive effect of HGG-tolerant cells (group 7) but not the helper activity of HGG-primed cells (group

TABLE V
Effect of Treatment with Anti-Ly Sera on CBA Cytotoxic Cells

Antiserum treatment of cells*	% Cytotoxicity on WEHI-164‡
None	38 ± 2§
C	36 ± 0
Anti-Ly-1.1	37 ± 1
Anti-Ly-2.1	34 ± 1
Anti-Thy-1	35 ± 2
Anti-Ly-1.1 + C	26 ± 2
Anti-Ly-2.1 + C	16 ± 2
Anti-Thy-1 + C	6 ± 1

* Cytotoxic cells were incubated with target for 4 h at a cytotoxic lymphocyte:target ratio of 20:1. Cell numbers were not adjusted for differential viability resulting from antiserum treatment.

‡ WEHI-164 is a BALB/c fibrosarcoma.

§ Mean ± one SEM. Assay done in triplicate.

11). Anti-Ly-1.1 serum, in spite of its powerful capacity to abolish the helper activity of HGG-primed cells (group 9), had no significant effect on the suppressors (group 5). Anti-Ly-2.1 serum treatment partially removed the suppressor effect (group 6), while helper function was left intact (group 10). Suppressor cells induced in vivo in this system can therefore be characterized as Ly-1⁻, probably Ly-2⁺ and Ia⁺.

Discussion

Ly antisera have been very useful in characterizing the subsets of T cells required for helper (T_H) and cytotoxic (T_C) functions. When CBA lymph node cells were treated with anti-Ly-1.1 serum and C their ability to transfer DTH was abolished. Anti-Ly-2.1 serum and C did not however affect this function. The phenotype of T_D cells is therefore Ly-1⁺,2⁻. It was important to demonstrate that the anti-Ly-1 serum did not ablate all T-cell functions and, as a corollary, that anti-Ly-2.1 serum was effective against other T-cell types. This was done in two different ways. When cytotoxic cells were treated with antisera, anti-Ly-1.1 serum had only a mild effect, and anti-Ly-2.1 serum a stronger inhibitory effect (Table V). Similarly the suppressor effect of HGG-tolerant spleen cells was unaffected by anti-Ly-1.1 serum, but was partially inhibited by anti-Ly-2.1 serum.

Although the Ly-1.1 and 2.1 antisera were raised in congenic mice, and were always absorbed for autoantibody, their specificity for the appropriate allele was demonstrated in two ways. Firstly, when these sera were tested against BALB/c cytotoxic cells, there was no killing or diminution of function. Secondly, when the sera were absorbed with thymus cells from C57BL Ly congenic mice the appropriate anti-Ly activity was removed.

In order to make a general statement about the Ly phenotype of T_D cells we tested the effect of anti-Ly-1.2 and anti-Ly-2.2 sera on sensitized C57BL cells. In this system T_D cells were also Ly-1⁺,2⁻. This serum was raised by Shen et al. in the Memorial-Sloan Kettering Cancer Center, New York, and was confirmed to be specific for the allele in question (14).

TABLE VI
Effect of Ly and Ia Sera on Helper and Suppressor Function in CBA Mice

Group*	No. of cells injected into lethally irradiated syngeneic mice†				PFC per spleen at 7 days				
	Anti-Thy-1 serum-treated DNP-POL-primed spleen		HGG-primed spleen		DNP-SRBC		HRBC		
	HGG-tolerant spleen	HGG-tolerant spleen	Direct	Indirect	Direct	Indirect	Direct	Indirect	
1	5 × 10 ⁶	0	0	30 (0.6)§	10 (0.7)	100 (0.2)	30 (0.6)	100 (0.2)	30 (0.6)
2	5 × 10 ⁶	5 × 10 ⁶	0	33,200 (1.2)	140,800 (1.1)	1,300 (1.2)	1,900 (1.9)	1,300 (1.2)	1,900 (1.9)
3	5 × 10 ⁶	5 × 10 ⁶	25 × 10 ⁶	1,900 (1.2)	30,700 (1.2)	5,500 (1.1)	25,600 (1.2)	5,500 (1.1)	25,600 (1.2)
4	5 × 10 ⁶	0	25 × 10 ⁶	600 (1.7)	6,600 (1.3)	5,000 (1.2)	19,800 (1.4)	5,000 (1.2)	19,800 (1.4)
5	5 × 10 ⁶	5 × 10 ⁶	25 × 10 ⁶ anti Ly-1.1 + C	4,100 (1.6)	35,100 (1.2) [‡]	4,000 (1.1)	3,000 (1.5)	4,000 (1.1)	3,000 (1.5)
6	5 × 10 ⁶	5 × 10 ⁶	25 × 10 ⁶ anti Ly-2.1 + C	1,600 (1.6)	71,800 (1.4) ^{‡, 2}	5,900 (1.2)	1,400 (4.4)	5,900 (1.2)	1,400 (4.4)
7	5 × 10 ⁶	5 × 10 ⁶	25 × 10 ⁶ anti Ia + C	9,500 (1.3)	218,000 (1.1) [‡]	6,400 (1.2)	1,300 (4.4)	6,400 (1.2)	1,300 (4.4)
8	5 × 10 ⁶	5 × 10 ⁶	25 × 10 ⁶ + C	6,700 (1.3)	19,600 (1.2) ^{‡, 3, 4}	4,700 (1.2)	6,000 (1.4)	4,700 (1.2)	6,000 (1.4)
9	5 × 10 ⁶	5 × 10 ⁶ anti-Ly-1.1 + C	0	90 (2.5)	70 (4.1)	80 (2.5)	0	80 (2.5)	0
10	5 × 10 ⁶	5 × 10 ⁶ anti-Ly-2.1 + C	0	13,700 (1.3)	189,300 (1.1)	700 (1.2)	500 (1.6)	700 (1.2)	500 (1.6)
11	5 × 10 ⁶	5 × 10 ⁶ anti-Ia + C	0	40,000 (1.3)	243,600 (1.3)	1,300 (1.2)	400 (4.5)	1,300 (1.2)	400 (4.5)
12	5 × 10 ⁶	5 × 10 ⁶ + C	0	6,400 (1.3)	85,600 (1.2)	2,900 (1.3)	30 (4.5)	2,900 (1.3)	30 (4.5)

* Data of one experiment. Other experiments gave similar results.

† Recipients also received 10⁶ HRBC and 100 μg of DNP-HGG.

‡ Geometric means and SEM (number in parentheses denotes factor which multiplies and divides the mean to give the upper and lower limits of the SEM).

|| P values: 1, 4, <0.001; 2, <0.01; 3, <0.1.

We have provided evidence that Ly antisera are useful in characterizing functional subsets of CBA T cells and that effector cells mediating DTH in CBA mice have Ly-1⁺,2⁻ phenotypes. This is the same Ly phenotype which we and others (1, 2, 5) have found on T_H cells. There is other evidence for similarity between the T_D and T_H cells. Cells taken from mice which were pretreated with cyclophosphamide and immunized with FGG in CFA in order to exhibit maximal DTH, were as powerful helpers as cells taken from mice immunized with FGG in CFA only, which exhibit very weak or no DTH. In addition, the cells responsible for helper and DTH function, although easily separable from other lymphoid cells by sedimentation velocity,² cannot be separated from one another by this method. There are, however, several apparent differences between T_D and T_H cells. For example, using simple immunization regimes there is an inverse relationship between the ability of some antigens to immunize mice to generate helper cell function or DTH (16). Similarly these cells are different in their susceptibility to tolerogenesis (17). These differences may reflect functional restrictions affecting identical cells (e.g., an inhibition of rapid local antigen recognition). Alternatively, these cells may in fact belong to distinct subsets.

Ia antigens, although most densely represented on B cells (18), have been detected in small quantities on some T cells (19-21). Despite this meager representation, treatment with anti-Ia serum and C has been shown to inhibit certain T-cell functions. The T_S cell responsible for allotype suppression (3) and in some instances the T_H cell (3) have been inhibited by anti-Ia sera and C. These findings suggested that Ia antisera, like Ly, may be helpful in defining T-cell subsets. Our results confirmed the sensitivity of suppressor cells to Ia antisera with a different system. The suppressor activity in this system depends on two cell types: one, anti-Thy-1 sensitive; the other retained by an anti-Ig column (7). When these two populations of cells are mixed the suppressor activity is restored (7). Anti-Ia treatment of the purified T cells (tolerant spleen cells passed through an anti-Ig column) abolishes their ability to restore suppressor activity to the mixture (J. R. Gamble, M. A. Vadas, J. F. A. P. Miller, and A. Basten, unpublished observation). Thus it seems that T_S cells are Ia⁺. T_H cells could also be inhibited by anti-Ia serum, but only when limiting numbers of recently primed helper cells from cyclophosphamide-pretreated mice were used (Tables IV and VI). The T_D cells, by contrast, were not at all sensitive. This might suggest that T_H and T_D cells in fact belong to different cell populations. It is, however, not clear what Ia antigens represent on T cells. They may be passively absorbed onto a subset of T cells (22) or, as suggested by Parish et al. (23), T cells may secrete Ia antigens. Alternatively, it is possible that the macrophage is the source of Ia antigens [or Ia-antigen complex like the genetically related factor (GRF) of Erb et al. (24)] which are absorbed onto lymphoid cells. Interestingly, a very high proportion of recirculating lymphocytes from irradiated mice in which T cells had been activated to histocompatibility antigens are Ia positive (25). Cells activated to FGG in a similar manner have in our hands repeatedly failed to initiate DTH responses. This may imply that the

² Vadas, M. A., and J. M. Fidler. Manuscript in preparation.

surfaces of such cells are blocked by Ia-antigen complexes (e.g., GRF). The cells would thus be unable to recognize antigen in a site where this was deposited to elicit DTH.

Summary

The Ly and Ia phenotypes of T lymphocytes involved in different functions were characterized by the use of specific antisera. T cells responsible for delayed-type hypersensitivity (DTH) and for helper functions were found to be Ly-1⁺,2⁻ in contrast to cytotoxic T cells and T cells responsible for suppression of antibody responses which were Ly-1⁻,2⁺. Unlike some primed helper cells, T cells involved in DTH were Ia⁻. Suppressor cells in the system studied were Ia⁺.

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