

FC-RECEPTORS, Ia-ANTIGENS, AND IMMUNOGLOBULIN ON NORMAL AND ACTIVATED MOUSE T LYMPHOCYTES

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While it is generally accepted that most B lymphocytes express Fc-receptors (FcR) (1),¹ evidence for the expression of FcR by T cells is controversial. Thus, while some works have claimed that a high proportion of thymus cells display FcR (2), others have failed to demonstrate FcR on these cells (1). FcR, however, could be detected on several murine T cell lymphomas (2, 3, and footnote 2), and several groups have reported FcR on T cells activated by H-2-determinants in vivo (H-2-ATC) (2, 4). This might suggest, therefore, that T cells express FcR only at certain stages of differentiation.

The present paper reports that although a proportion of H-2-ATC recovered from spleen displayed FcR, those recovered from thoracic duct lymph (T.TDL) were FcR negative. The same observation has been made for T cells activated by *M*-locus determinants. In addition, Ia-antigens were found on ATC (spleen) and T.TDL. These findings are discussed in relation to T-cell differentiation.

Materials and Methods

Animals. AKR/J, CBA/J (H-2^k, Mls^d), C3H/J (H-2^k, Mls^c), SJL/J (H-2^s), and F₁ hybrid mice between CBA/J and C57BL/6 (C57BL) (H-2^b, Mls^b) and between SJL/J and C57BL/6 were used.

Cell Suspensions. Single cell suspensions were prepared from thymus, lymph node, and spleen by teasing the tissue through a 60-mesh stainless steel screen into cold tissue culture (TC) Medium 199 (Difco Laboratories, Detroit, Mich.) plus 10% fetal calf serum (FCS) (Flow Laboratories, Rockville, Md.) and gently pipetting the cells through a 1-ml pipette. Cells were washed three

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¹ *Abbreviations used in this paper:* ATC, activated T cells; H-2-ATC, allogeneically- activated T cells; CFTD, complement fixation test diluent; Con A, concanavalin A; CRL, lymphocytes forming complement rosettes; CRT, cortisone-resistant thymocytes; E-rosette, erythrocyte rosette; EA(IgG), ORBC sensitized with IgG anti-ORBC serum; EA(IgM), ORBC sensitized with IgM anti-ORBC serum; FCS, fetal calf serum; FDA, fluorescein diacetate; FcR, Fc-receptors; GPC, guinea pig complement; Thy 1.2, theta C3H; MC, mouse (AKR/J serum) complement; MIgG, EA rosettes with ORBC sensitized with DBA anti-ORBC; MIgM, EA rosettes with ORBC sensitized with mouse anti-ORBC IgM; ORBC, ox (bovine) red blood cells; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; RC, rabbit C; RIgG, EA rosettes with ORBC sensitized with serum 224; RIgM, EA rosettes with ORBC sensitized with rabbit anti-ORBC IgM; TC, tissue culture; TDL, thoracic duct lymphocytes; T.TDL, thoracic duct T cells activated by GvH reaction.

² Krammer, P. H., R. Citronbaum, S. E. Read, L. Forni, and R. Lang. 1975. Murine thymic lymphomas as model tumors for T cell studies. T cell markers, immunoglobulin, and Fc-receptors on AKR thymomas. *Cell Immunol.* In press.

times in cold medium, and clumps were removed by letting the cell suspension settle for 5 min. Viability of cells in the supernatant fluid was determined by trypan blue dye exclusion.

Thoracic duct lymphocytes (TDL) were collected as previously described (5). T cells activated to H-2-determinants were prepared by injecting 2×10^8 CBA thymus cells intravenously into X-irradiated (800R) (CBA/J \times C57BL)F₁ hybrid mice. The activated cells were then obtained 4–6 days later either from the spleen (ATC-spleen) or from thoracic duct lymph (T.TDL) (5). In some experiments, lymph node cells were used to prepare the ATC. ATC-spleen (SJL/J) were prepared by injection of 5×10^6 nylon wool column purified SJL/J lymph node T cells into X-irradiated (800 R) (SJL/J \times C57BL)F₁ hybrid mice. To obtain T cells activated by *M*-locus determinants, C3H/J thymus cells were transferred into irradiated CBA/J mice.

Dead cells were removed from ATC-spleen by the method of v. Boehmer et al. (6), and lymphocytes were isolated over a Ficoll gradient (recovery of viable ATC-spleen was 30–40%). Suspensions of thymus cells were depleted of B cells by filtration through Sephadex G-100 columns conjugated with rabbit antimouse Ig antibody (7). In other experiments lymph node cells were freed of B cells by passage through nylon wool columns (8). The relative proportions of T and B cells in each population was determined by indirect immunofluorescence and cytotoxicity with anti-Thy 1.2 (theta C3H) serum. These methods have been described elsewhere (9,10).

For preparation of cortisone resistant thymocytes, (CRT) mice were injected with 0.125 mg hydrocortisoneacetate/g and the thymus removed 2 days later. Normal thymocytes or CRT were stimulated by concanavalin A (Con A) or phytohemagglutinin (PHA). Stimulation of cultured cells was measured by incorporation of [³H]thymidine as described elsewhere (11). 5 μ g Con A and 10 μ g PHA were optimal for stimulation. Stimulation indices were: normal thymus cells Con A 196, PHA 5; CRT Con A 230, PHA 30.

Antisera. Anti-Thy 1.2 serum was raised by the method of Raff (12). Anti-C57, anti-CBA, and anti-SJL sera were produced in CBA, C57, and C3H, respectively, as described previously (13). These sera were specific for their targets. The preparation and specificity of ATH anti-ATL serum has extensively been described elsewhere (14).

Fc- and C-Receptors. A rosette test with relatively nonagglutinable bovine erythrocytes (ox (bovine) red blood cells, ORBC) as indicator cells was used to detect FcR (EA rosettes) and C-receptors (EAC rosettes).

Rabbit (gift from Dr. M. Ferrarini, Genova) as well as mouse (DBA) anti-ORBC sera were prepared. Rabbit-anti-ORBC serum 224 (rich in IgG), rabbit-anti-ORBC (IgM) (purified IgM fraction in a dilution of 1:50 in complement fixation test diluent [CFTD]), and Balb/cM anti-ORBC serum (Sephadex G-200 column purified IgM) were used for sensitization of indicator cells. Neither Balb/cM anti-ORBC IgM nor DBA anti-ORBC serum showed any agglutination of ORBC, although the hemolytic titer (with guinea pig complement [GPC] diluted 1:10) was 1:64 for Balb/cM anti-ORBC (IgM) and 1:1,024 for DBA anti-ORBC serum. For sensitization of ORBC, Balb/cM anti-ORBC IgM was diluted 1:2 or 1:4 in CFTD, and DBA anti-ORBC serum was diluted 1:5 in phosphate-buffered saline (PBS).

Preparation of Sensitized Red Cells for EA-Rosettes. Equal volumes of a 2% ORBC suspension in PBS and either rabbit or mouse anti-ORBC antisera (serum 224 or DBA anti-ORBC antiserum), at the dilutions indicated above, were incubated for 30 min at room temperature. After incubation, the red cells were washed twice in PBS and resuspended to 0.5% (vol/vol) in TC Medium 199 plus 10% FCS (modified from [15]).

Preparation of Sensitized Red Cells for EAC-Rosettes. ORBC, thoroughly washed in CFTD (16), were sensitized as above with the indicated dilutions of either rabbit anti-ORBC IgM or Balb/cM anti-ORBC IgM, and washed twice in CFTD. The EA complex was then treated with mouse complement (AKR serum previously absorbed at 4°C for 30 min with an equal volume of washed, packed ORBC and diluted 1:2 in CFTD), washed twice in CFTD, and adjusted to a concentration of 0.5% in Medium 199 plus 10% FCS (modified from [17]).

Rosette Test. 20 μ l of the 0.5% suspension of sensitized red cells were added to 20 μ l of the test cell suspension (3×10^6 cells/ml) in plastic tubes (Luckhan Ltd., LP2). Tubes were centrifuged at 400g for 5 min at 4°C. EAC-rosettes were immediately resuspended by gentle pipetting with a Pasteur pipette; EA-rosettes were resuspended after 30 min at 0°C. A drop of suspension was placed on a siliconized slide and overlaid with a siliconized coverslip. The edges of the coverslip were sealed with wax. Each test was performed in triplicate and the percentage of rosettes

determined (a minimum of 300 cells per preparation was counted). A cell with more than four adherent erythrocytes was regarded as a rosette.

Each test included erythrocyte rosettes (E-rosettes) as simultaneous controls for EA(IgG)-rosettes and EA(IgM)-rosettes as controls for EAC-rosettes. Fluorochromasia with fluorescein diacetate (FDA) (18) was used to assess the percentage of live cells forming rosettes.

Inhibition of Fc-Rosettes. Purified myeloma proteins (gifts from Drs. G. Torrigiani, R. Pink, and F. Melchers), at a final concentration of 500 µg/ml, were used to inhibit rosette formation and were added to the cells at the same time as the IgG coated ORBC.

Results

FcR and C-Receptors on Normal Lymphocyte Populations. Table I shows the proportion of Fc- and C-receptor positive lymphocytes in various cell preparations. It is evident that ORBC coupled with either MIgG or RIgG [EA(IgG)] gave roughly the same number of rosettes (Fc- and C-rosettes) at optimal dilution of the antiserum. Rosettes were not detected with ORBC coated with IgM[EA(IgM)].

The proportion of cells forming Fc-rosettes found in thymus, lymph node, spleen, and TDL agrees with other reports (1). The proportion of FcR positive cells was very low (1%) in normal thymus and in lymph node cells depleted of B cells by anti-Ig column filtration (<1% B cells by indirect immunofluorescence) (Table I).

A variety of T-cell enriched populations were tested for their capacity to form Fc-rosettes [EA(IgG) or EA(IgM)]. These included: thymus cells stimulated with either Con A or PHA, CRT, and CRT stimulated with Con A or PHA. Within each of these populations only a few (<1%) formed Fc-rosettes [EA(IgG)] (data not shown). It is important to emphasize that these experiments were always

TABLE I
FcR and C-Receptors on Normal Lymphocyte Populations

Cell population*	% Rosettes (range)						
	E	EA(IgG)		EA(IgM)		EAC(MC)	
		MIgG	RIgG	MIgM	RIgM	MIgM	RIgM
Thymus cells	0	0.75(0.6-1.6)	ND	ND	ND	ND	ND
CRT‡	0	3.5(3-4)	ND	ND	ND	ND	ND
Lymph node cells	0	17(7-23)	20(10-28)	0	0	18(16-20)	17(13-19)
Lymph node cells anti-Ig column ef- fluent popula- tion§·	0	1.5(1-2)	1.5(1-2)	0	0	2(2-3)	1.8(1.3-2.3)
Spleen cells	0	41(40-42)	42(40-44)	0	0	ND	ND
TDL¶**	0	14(12-16)	ND	ND	ND	ND	ND

ND, not determined; Results are expressed as mean and range of at least two experiments.

* CBA/J cells (a pool from at least two mice) were used for cell preparation.

‡ 3.5% of CRT Ig positive by direct immunofluorescence.

§ Anti-Ig column modified from Schlossmann and Hudson (8).

|| Ig positive cells by indirect immunofluorescence <1%.

¶ TDL were prepared as described by Sprent (6).

** No difference in numbers of rosettes with three or nine washes before rosette formation.

run in parallel with tests on normal spleen and lymph node cells as positive controls; the percentage of Fc-rosettes in each positive control was comparable to the data shown in Table I.

FcR on Activated T Cells. 40% of activated T cells from the spleen of irradiated F₁ mice (ATC-spleen) given H-2 or *M*-locus incompatible thymus cells 4 or 5 days previously formed Fc-rosettes (Table II). More than 90% of the ATC-spleen suspensions were viable after dead cell removal; in addition >95% of these viable cells were lysed by anti-Thy 1.2 serum plus C. Only 1–2% of ATC-spleen, however, formed EAC-rosettes (Table II).

In another series of experiments, nylon-wool-purified T cells (Ig⁺ 0%, Thy 1.2⁺ > 99%) derived from lymph nodes were used to prepare ATC-spleen. As seen in Table III lymph node T cells, though FcR-negative before transfer (Table I), developed FcR after *in vivo* activation by H-2- or *M*-locus differences. In both populations, 80–90% of the Fc-rosette-forming cells were blasts. Again, the number of EAC-rosettes was negligible.

95% of the *M*-locus-activated ATC-spleen (*M*-ATC-spleen) were lysed by anti-Thy 1.2 serum and C. This serum lysed 100% of H-2-ATC-spleen (Fig. 1); by indirect immunofluorescence, 100% of these cells expressed Thy 1.2, and 0% were Ig⁺. Fig. 1 also shows that virtually all of the isolated H-2-ATC-spleen were donor-derived, i.e. 100% of the cells were killed with C57BL anti-CBA serum, whereas only background lysis was obtained with CBA anti-C57BL (antihost) serum; the latter killed almost 100% of normal C57BL or (CBA × C57BL)F₁ cells.

Inhibition of ATC FcR by Purified Myeloma Proteins. The degree of inhibition of Fc-rosette formation by purified myeloma proteins is shown in Table IV. At the protein concentration used, only MOPC 141 (IgG2b) gave strong inhibition of rosette formation, using both *M*-ATC-spleen and H-2-ATC-spleen. No inhibition was observed with either cellular or secreted IgM from MOPC 104.

Failure to Detect FcR on T.TDL. Almost 100% of T.TDL are Thy 1.2 positive and of donor origin (Fig. 2). In direct contrast to ATC-spleen, T.TDL contained

TABLE II
FcR and C-Receptor on Activated T Cells Prepared by Injection of Thymus Cells into Irradiated H-2- or M-Locus Different Hosts

Activated* T cells‡ (spleen)	% Rosettes (range)§							% Ig positive cells
	E	EA(IgG)		EA(IgM)		EAC(MC)		
		MIgG	RIgG	MIgG	RIgM	MIgM	RIgM	
Activation by H-2 difference¶	0	37(32–48)	44(43–48)	0	0	1(0–3)	2(1–4)	4.1
Activation by <i>M</i> -lo- cus difference**	0	15(13– 17)‡‡	11(6–16)‡‡	0	0	ND	ND	ND

* H-2-ATC-spleen collected 5 days and *M*-ATC-spleen 4 days after thymocyte transfer.

‡ Both cell populations were >95% T cells by cytotoxicity test with anti-Thy 1.2 serum and GPC.

§ Numbers are expressed as mean and range of two experiments.

|| Mean ± SE.

¶ 2 × 10⁶ CBA thymocytes injected i.v. into 800 R irradiated (CBA/J × C57BL)F₁ mice.

** 2 × 10⁶ C3H thymocytes injected i.v. into 800 R irradiated CBA/J mice.

‡‡ More than 50% of EA-rosettes were blast cells.

TABLE III
Fc- and C-Receptors on Activated T Cells Prepared by Injection of Nylon Wool Purified Lymph Node T Cells into Irradiated H-2- or M-Locus Different Hosts

Activated T cells (spleen)	Irradiated recipient	Donor (nylon wool purified lymph node cells)*	% Rosettes (range) on activated T cells (spleen)			
			E	EA (MIgG)	EA (MIgM)	EAC (MIgM, MC)
Activation by H-2-difference‡	(CBA/J × C57BL)F ₁	CBA/J§	0	27(25-29)	0	0.5(0-1)
Activation by M-locus difference¶	CBA/J	C3H/J§	0	15(14-16)**	0	0

* 5×10^6 viable nylon wool purified T cells injected i.v.
 ‡ 5-day activation; pool of three mice; Ig⁺ 0%, 100% Thy 1.2 positive by indirect immunofluorescence (200 cells counted per preparation). See also Fig. 1.
 § Ig positive: 0%, Thy 1.2 positive: >99% (200 cells counted per preparation).
 || 88% of the rosetting cells were blast cells (200 rosettes counted).
 ¶ 4-day activation; pool of four mice; more than 95% T cells by cytotoxicity with anti-Thy 1.2 serum and RC.
 ** 78% of the rosetting cells were blast cells (200 rosettes counted).

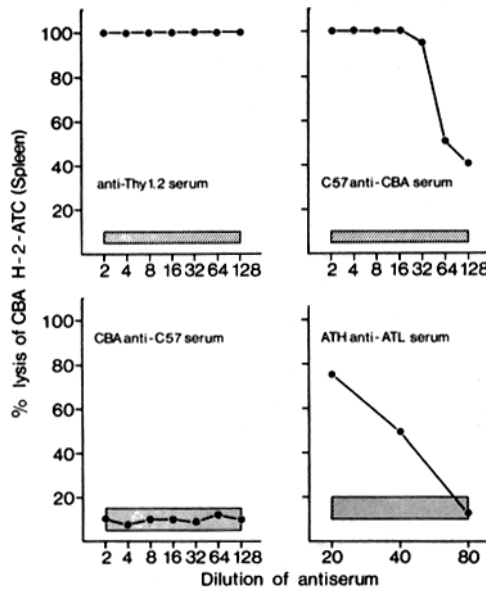


FIG. 1. Percent lysis of CBA/J H-2-ATC-spleen (derived from X-irradiated (CBA/J × C57BL)F₁ hybrid mice given nylon wool column purified CBA/J lymph node T cells) by various antisera plus complement. Hatched bars represent controls with cells alone, cells and complement, and cells and antiserum alone.

TABLE IV
Inhibition of Fc-Rosettes with H-2-ATC-Spleen and M-ATC-Spleen by Purified Myeloma Proteins

Myeloma	Ig class*	% Inhibition of Fc-rosettes‡	
		H-2-ATC-spleen§	M-ATC-spleen§
MOPC 104	Secreted IgM	9	13
MOPC 104	Cellular IgM	0	0
MOPC 315	IgA	0	7
RPC 23	IgG1	0	0
SS 63	IgG2a	2	3
MOPC 141	IgG2b	81	83

* Purified myeloma proteins at final concentration of 500 $\mu\text{g/ml}$.

‡ Myeloma proteins were added to the lymphocytes at the same time as the IgG-coated ORBC.

Inhibition calculated from the mean percent of rosettes without addition of myeloma proteins.

§ Same cell populations as in Table III.

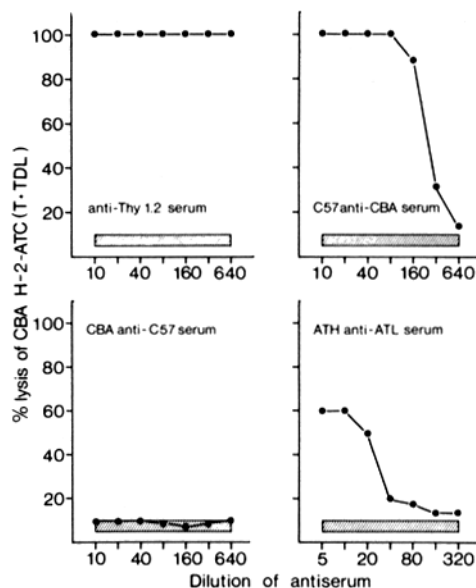


FIG. 2. Percent lysis of CBA/J H-2-T.TDL (derived from X-irradiated (CBA/J \times C57BL) F_1 hybrid mice given CBA/J lymph node cells) by various antisera and rabbit complement. Controls as in Fig. 1.

no Fc-rosette-forming cells (Table V), using ORBC coated with MIgG, RIgG, MIgM, or RIgM. EAC-(MIgM, RIgM, MC) rosettes were also absent.

No rosette-forming cells were detected when T.TDL were incubated with ORBC AT 37°C instead of at 4°C or if the T.TDL were preincubated at 37°C for 6–8 h in RPMI 199 and 10% FCS (5% CO₂ incubator).

A high proportion of T.TDL raised against H-2 differences (H-2-T.TDL) express surface Ig (Table V) most of which appears to be specific IgM antibody of donor B-cell origin (19). To determine whether the presence of this material

TABLE V
Assay for Fc and C-Receptors and Ig on T.TDL

T.TDL*	% Rosettes‡							Ig positive cells§
	E	EA(IgG)		EA(IgM)		EAC(MC)		
		MIgG	RIgG	MIgM	RIgM	MIgM	RIgM	
Activation by H-2-difference	0	0	0	0	0	0	0	67 ± 6
Activation of B-depleted thymocytes by H-2-difference¶	0	0	0	0	0	0	0	9 ± 1
Activation by <i>M</i> -locus difference**	0	0	ND	0	ND	ND	ND	2 ± 0.5

* Cells collected from thoracic duct lymph at 4 to 5 days after thymocyte transfer.

‡ Data derived from three experiments.

§ Mean ± SE.

|| 2 × 10⁸ CBA/J thymocytes injected i.v. into 800R irradiated (CBA/J-C57BL)F₁ mice.

¶ Thymocytes were B-cell depleted by filtration through anti-Ig columns.

** 2 × 10⁸ C3H thymocytes injected i.v. into 800R irradiated CBA/J mice.

could have contributed to the failure of T.TDL to form Fc-rosettes, tests were carried out with two populations of T.TDL which lack surface Ig. These populations were (a) H-2-T.TDL prepared from T cells depleted of B cells before injection and (b) T.TDL raised between strains differing by determinants recognized by T cells but not B cells, i.e., *M*-locus determinants (*M*-T.TDL). As shown in Table V, neither of these populations formed Fc-rosettes.

Previous work³ showed that when T.TDL were reexposed to the activating antigens, i.e. by transfer to secondary hosts syngeneic with the primary hosts, the cells synthesized DNA in the spleens of their new hosts. Irradiated F₁ mice receiving H-2-T.TDL had twice as many Fc-rosette-forming cells per spleen compared to unreconstituted X-irradiated mice (Table VI). This would suggest that at least a proportion of T.TDL had the potential to express FcR after reactivation.

Ia-Antigens on ATC. *Ia*-antigens can easily be demonstrated on B cells where they are closely associated with the B cell FcR (20). In agreement with other authors (10), *Ia* antigens were demonstrated in a microcytotoxicity assay with ATH anti-ATL serum and C on a low number of thymus cells and a proportion of cortisone resistant and lymph node T cells (Fig. 3).

Fig. 1 and 4 show that *Ia*-antigens were also expressed on H-2-activated T cells (ATC-spleen and H-2-T.TDL). As ATH anti-ATL serum is specific for *Ia*^k determinants, i.e. determinants present on cells from H-2^k (e.g. CBA/J) strains but not on H-2^s (e.g. SJL/J) strains, the antiserum lysed only H-2-ATC-spleen of CBA/J origin (Fig. 1), but not of SJL/J origin (Fig. 4). Virtually all H-2-ATC of

³ Sprent, J. 1975. Recirculating lymphocytes. In *The Lymphocyte Structure and Function*. J. J. Marchalonis, editor. Marcel Dekker, Inc., New York. Manuscript in preparation.

TABLE VI
Assay for Fc-Receptors on T.TDL Reinjected into Secondary, Irradiated Recipients

Cells tested*	Total number of EA(MIgG)-rosettes per spleen	Ratio of total numbers of EA(MIgG)-rosettes between cell populations A, B and C, D
Spleen cells from X-irradiated (800 R) (CBA/J × C57BL) _F ₁ mice injected with C57BL-activated CBA/J T.TDL‡ (A)	116 × 10 ⁴	$\frac{A}{B} = 2\frac{1}{2}\S$
Spleen cells from X-irradiated (800 R) (CBA/J × C57BL) _F ₁ mice given no cells (B)	58 × 10 ⁴	
Spleen cells from X-irradiated (800 R) CBA/J mice injected with CBA/J-activated C3H/J T.TDL‡ (C)	140 × 10 ⁴	$\frac{C}{D} = 2.6\frac{1}{2}\S$
Spleen cells from X-irradiated (800 R) CBA/J mice given no cells (D)	54 × 10 ⁴	

* Spleen cells taken 2 days after injection of T.TDL.

‡ T.TDL activated by H-2- and M-locus difference as in Table V. 5×10^6 of T.TDL transferred into secondary recipients within 2 h of irradiation of the recipient mice.

§ Data in table from a single experiment and one mouse in each group. Four other experiments gave similar results (range of ratios 1.7–2.1 (4) and 1.3–2.6 (5)).

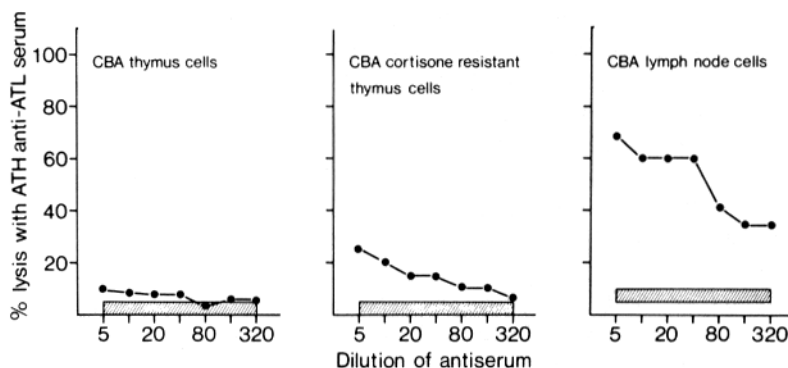


FIG. 3. Percent lysis of thymus, cortisone resistant thymus, and lymph node cells (CBA) by ATH anti-ATL serum. Assay conditions as in Fig. 1.

SJL origin were killed by anti-Thy 1.2 serum as well as by C57 anti-SJL serum. The titer of the anti-Ia serum on CBA H-2-ATC (spleen and TTDL), however, was much lower than on CBA normal spleen cells (10).

Discussion

In using antibody-coated erythrocytes for detecting FcR, selected samples of ORBC have the advantage that, unlike sheep RBC, they do not agglutinate

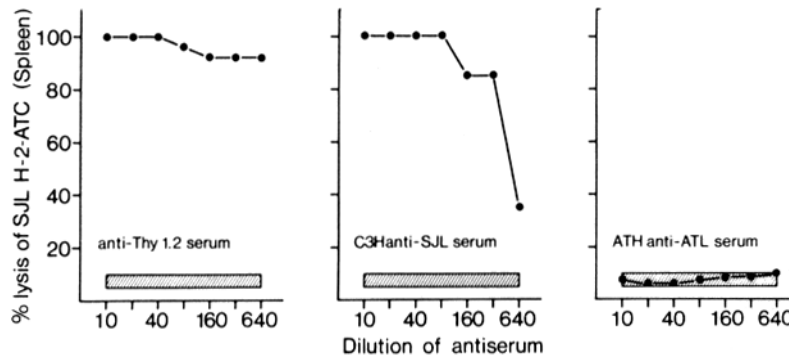


FIG. 4. Percent lysis of SJL/J H-2-ATC spleen (derived from X-irradiated (SJL/J \times C57BL/F₁ hybrid mice given SJL/J nylon wool column purified lymph node T cells) by various antisera. Conditions as in Fig. 1.

when treated with high concentrations of antierythrocyte antibody (23). Antibody-coated ORBC should therefore present a greater density of Fc-determinants to FcR-bearing cells. Thus, antibody-coated ORBC are sensitive indicator cells for detecting FcR. Furthermore, preference for the ORBC rosetting technique is based on the ease with which a fractionation of rosetting and nonrosetting cells can eventually be accomplished.

FcR were detected on 20% of normal lymph node cells, but not on normal cortisone-resistant thymus cells or on lymph node cells depleted of Ig-bearing cells. These data are consistent with the evidence of other workers that the majority of B lymphocytes, but not T lymphocytes, express easily detectable FcR (1).

Of the various T-cell populations studied, FcR were detected only on ATC-spleen. As with B cells, rosette formation with ATC-spleen was demonstrable with EA(IgG), but not with EA(IgM); furthermore, as observed by other workers (2), rosette formation was blocked in the presence of myeloma IgG (IgG2b) but not with IgM. In contrast to B cells, ATC-spleen did not express detectable C-receptors. Significantly, FcR were not detected on other activated T-cell populations, i.e., T.TDL, Con A blasts, or PHA blasts. H-2-T.TDL derived from normal thymus or lymph node cells carry surface IgM which appears to be donor-B-cell-derived alloantibody (19). It was possible, therefore, that the failure to detect FcR on T.TDL was the result of blockade by passively absorbed surface Ig. This seemed unlikely, however, since FcR were also not detectable on two populations of T.TDL which lacked surface Ig: (a) H-2-T.TDL derived from T-cell populations depleted of B cells and (b) T.TDL raised against *M*-locus determinants, i.e., determinants which stimulate T cells but do not induce alloantibody formation by B cells (22) (Table VII).

Inasmuch as the production of T.TDL is associated with extensive T-cell proliferation in the lymphoid organs, particularly the spleen (23), it is probable that T.TDL are largely derived from ATC-spleen. The present data might suggest, therefore, that in response to major transplantation antigens or *M*-locus determinants in vivo, T cells change from being FcR-negative at the time of injection to FcR-positive during proliferation in the spleen and then revert to an FcR-negative state during migration into the central lymph. Can one con-

TABLE VII

Summary of Results. Immunoglobulin, Fc-Receptors, and Ia-Antigens on the Membrane of Various T-Cell Populations

Cell population	% Ig positive	% Fc-receptor positive	% Ia positive
Thymocytes	0.9	0.75	10
Cortisone resistant thymocytes	3.5	3.5	25
H-2-ATC-spleen	4.1	44	75
H-2-T.TDL	67	0	60
M-ATC-spleen	ND*	15	ND
M-T.TDL	2	0	ND

* ND, not determined.

clude, therefore, that T cells express FcR only at a precise stage of differentiation? Several points are of relevance to this question.

(a) It has been mentioned that most T.TDL are probably derived from ATC-spleen. There is no evidence, however, that the progenitors in the spleen are FcR-positive. Since the proportion of FcR-positive cells in ATC-spleen did not exceed 50%, it is equally possible that T.TDL were derived from the FcR-negative population. It is nevertheless of interest that T.TDL appeared to express FcR when they were restimulated upon secondary transfer (Table VI).

(b) Since FcR were not demonstrable on T cells stimulated with PHA or Con A, the expression of FcR did not appear to be simply a nonspecific event upon blast transformation.

(c) The rosetting technique is only one of several techniques available for detecting FcR. In this respect it is reported that FcR can be demonstrated with heat-aggregated Ig on normal thymus cells (2). It could be argued, therefore, that the failure to find FcR on T.TDL reflected inadequacies in the sensitivity or the specificity of the technique employed. On this point it may be mentioned that in a separate study, Basten et al.⁴ have been unable to demonstrate FcR on T.TDL with a variety of techniques, viz. rosetting with SRBC, heat-aggregated human gamma globulin, and radiolabeled immune complexes. Despite these and our negative findings, Ig can be detected on a large proportion of H-2-T.TDL (Table V and (19)), but not on H-2-ATC-spleen (Table II). These findings will be discussed in detail elsewhere.⁵

In view of the above points it is equally possible that there is no clear-cut division of T cells into FcR-positive and FcR-negative cells, but that at certain stages of differentiation there is either a quantitative or qualitative change in the expression of these receptors.

No evidence was found that IgM was cytophilic for either T cells or B cells. Nevertheless, uptake of IgM has been demonstrated on T cells by a variety of techniques and in several species, e.g. rats (24), chickens (25), and man (26).

⁴ Basten, A., J. F. A. P. Miller, N. L. Warner, R. Abraham, E. Chico, and J. Gamble. 1975. A subpopulation of T cells bearing Fc receptors. *J. Immunol.* 115:1159.

⁵ Hudson, L., and J. Sprent. 1975. The specific adsorption of IgM antibody onto activated mouse T lymphocytes. Manuscript in preparation.

Although in some of these studies it is not clear that the IgM detected was indeed Fc-bound, it is possible nevertheless that T cells do have a limited capacity to absorb IgM via FcR.

In view of the evidence of Dickler and Sachs (20) that FcR on B cells are closely associated with Ia-antigens, the expression of these antigens was studied on various FcR-positive and FcR-negative T cells. In agreement with other authors (10) Ia-antigens were demonstrated on a proportion of CRT and lymph node T cells. In addition it was observed that 80% of H-2-ATC-spleen and 60% of H-2-T.TDL were Ia-positive.

It has been shown that several anti-Ia sera contain strong activity against C-type viruses.⁶ It is also known that such viruses can be activated by graft-vs.-host reactions (27). It was conceivable, therefore, that the lytic activity of the anti-Ia serum on ATC (i.e., cells proliferating as the result of a graft vs. host reaction) was directed against viral antigens. The fact that the antiserum used (ATH anti-ATL (anti-Ia^k)) lysed CBA/J ATC (Ia^k) but not SJL/J ATC (Ia^s) makes this possibility unlikely. Experiments are currently in progress to exclude the possibility that the Ia-antigens were passively absorbed.

In conclusion, the distribution of Ia-antigens, FcR, and Ig on the various T-cell populations studied, as shown in Table VII, does not provide any evidence for a discernible interrelationship between these three cell surface structures. For Ia-antigens and FcR, however, biochemical studies on the molecular level are necessary to determine an association of these cell surface components, especially when they are present on the membrane of the same cell like on H-2-ATC-spleen.

Summary

Using antibody coated bovine erythrocytes we were unable to demonstrate Fc-receptors on either thymus cells or T cells prepared from lymph node cell suspensions by anti-Ig column filtration. However, if parental thymus or lymph node T cells were transferred to X-irradiated F₁ hybrids, activated donor T cells recovered from the recipient's spleen (ATC-spleen) were shown to express Fc-receptors. Fc-receptors were also demonstrable on ATC-spleen prepared between strain combinations differing at the *M*-locus. In marked contrast, Fc-receptors were not detected on ATC recovered from thoracic duct lymph (T.TDL). This applied to (a) H-2-activated T.TDL derived from normal thymus cells, (b) H-2-activated T.TDL derived from thymus cells depleted of B cells, and (c) *M*-locus-activated T.TDL. Of these three populations, surface Ig (of B cell origin) was detected on a large proportion of the first but not on the second and third populations. Thus, the failure to detect Fc-receptors on any of these populations could not be attributed to blocking by adsorbed surface Ig.

In addition, various T-cell populations were examined by a microcytotoxicity assay for the presence of cell surface Ia-antigens. 5–10% of the thymus cells, 20–30% of cortisone-resistant thymus cells, 60–70% of lymph node cells, and 60–80% of ATC-spleen and T.TDL showed Ia.

⁶ Wettstein, P. J., P. H. Krammer, C. S. David, J. A. Frelinger, and D. C. Shreffler. 1975. A cautionary note regarding Ia and H-2 typing of murine lymphoid tumors. Manuscript in preparation.

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